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Assessment of the genetic and clinical determinants of fracture risk: genome wide association and mendelian randomisation study

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Additional material is published online only. To view please visit the journal online.

Cite this as: *BMJ* 2018;362:k3225 <http://dx.doi.org/10.1136/bmj.k3225>

Accepted: 2 July 2018

ABSTRACT OBJECTIVE

To identify the genetic determinants of fracture risk and assess the role of 15 clinical risk factors on osteoporotic fracture risk.

DESIGN

Meta-analysis of genome wide association studies (GWAS) and a two-sample mendelian randomisation approach.

SETTING

25 cohorts from Europe, United States, east Asia, and Australia with genome wide genotyping and fracture data.

PARTICIPANTS

A discovery set of 37 857 fracture cases and 227 116 controls; with replication in up to 147 200 fracture cases and 150 085 controls. Fracture cases were defined as individuals (>18 years old) who had fractures at any skeletal site confirmed by medical, radiological, or questionnaire reports. Instrumental variable analyses were performed to estimate effects of 15 selected clinical risk factors for fracture in a two-sample mendelian randomisation framework, using the largest previously published GWAS meta-analysis of each risk factor.

RESULTS

Of 15 fracture associated loci identified, all were also associated with bone mineral density and mapped to genes clustering in pathways known to be critical to bone biology (eg, *SOST*, *WNT16*, and *ESR1*) or novel pathways (*FAM210A*, *GRB10*, and *ETS2*). Mendelian

randomisation analyses showed a clear effect of bone mineral density on fracture risk. One standard deviation decrease in genetically determined bone mineral density of the femoral neck was associated with a 55% increase in fracture risk (odds ratio 1.55 (95% confidence interval 1.48 to 1.63; $P=1.5 \times 10^{-68}$). Hand grip strength was inversely associated with fracture risk, but this result was not significant after multiple testing correction. The remaining clinical risk factors (including vitamin D levels) showed no evidence for an effect on fracture.

CONCLUSIONS

This large scale GWAS meta-analysis for fracture identified 15 genetic determinants of fracture, all of which also influenced bone mineral density. Among the clinical risk factors for fracture assessed, only bone mineral density showed a major causal effect on fracture. Genetic predisposition to lower levels of vitamin D and estimated calcium intake from dairy sources were not associated with fracture risk.

Introduction

The United Nations recently predicted that the ratio of people aged 65 years and older to those aged 15-64 years will triple globally by 2100.¹ Musculoskeletal conditions are the most common causes of severe pain and physical disability, and their prevalence will increase with the ageing of society.² One of the largest musculoskeletal burdens is attributable to osteoporotic fractures, the incidence of which increases exponentially with age.³ Therefore, the prevention of fractures is an important public health goal.

The causes of multifactorial common diseases, such as osteoporotic fractures, include genetic and environmental influences, as well as their interactions (gene by environment, or G×E). Clinically useful risk factors for the prediction of osteoporotic fracture risk need not be necessarily causal and have been implemented by well validated risk score algorithms such as FRAX⁴ and the Garvan^{5 6} fracture risk calculator. Yet, the extent to which modification of predictive clinical risk factors reduces fracture risk is not generally known. A better understanding of causal mechanisms will enable prevention strategies, direct the launch of proper clinical trials, and provide targets for effective lifestyle and pharmacological interventions. Acquiring this knowledge is particularly timely and relevant considering the increasing recognition that many individuals at high fracture risk often do not receive fracture prevention interventions.⁷

WHAT IS ALREADY KNOWN ON THIS TOPIC

The genetic determinants of fracture risk are not well described, and whether commonly used clinical risk factors for fracture are causal is not known. For example, the effect of vitamin D supplementation in the general population on fracture risk is under debate; although such supplementation is part of clinical guidelines, recent randomised controlled trials have failed to consistently show a beneficial effect.

WHAT THIS STUDY ADDS

This mendelian randomisation study provides evidence against a causal effect of several proposed clinical risk factors for fractures (eg, diabetes, glucose, rheumatoid arthritis, and vitamin D).

Genetic predisposition to lower vitamin D levels and estimated calcium intake from dairy sources were not associated with fracture risk.

However, these results highlight the central causal role of low bone mineral density in the pathophysiology of fracture risk.

Fracture risk is a moderately heritable trait (whereby h^2 is roughly 30%),^{8,9} for which no large scale, genome wide association studies (GWAS) have been undertaken so far. Large GWAS meta-analyses can also be used to perform mendelian randomisation analyses to explore the causal effects of heritable risk factors on disease in people, while reducing bias due to confounding (because genetic variation is essentially randomly assigned at conception) or reverse causation (because allele assignment always precedes disease onset).¹⁰ Conceptually similar to a randomised controlled trial, the mendelian randomisation approach enables an assessment of the cumulative effect of a genetically determined exposure on fracture risk, minimising the biases that frequently weaken observational studies.

Understanding whether interventions aimed at clinical risk factors would reduce fracture risk is important, because clinicians often ensure that such risk factors are optimised in individuals at high risk of fracture. If the risk factors are not causal, then such optimisation would not decrease fracture risk. Therefore, to better understand genetic and clinical risk factors for fracture, we undertook a large scale GWAS for fracture risk in up to 264 973 participants (37 857 fracture cases) in the discovery stage and in conjunction with the largest available GWAS for clinical risk factors, determined the genetic correlation (shared heritability) of key clinical risk factors and fracture. We then performed mendelian randomisation studies to explore the causal effect of these risk factors on fracture.

Methods

Study populations

A total of 23 cohorts with genome wide genotyping and fracture data were recruited globally through the GENetic Factors for Osteoporosis consortium (GEFOS; <http://www.gefos.org/>). These cohorts were predominantly of European descent and from Europe (n=13), North America (n=8), Australia (n=1), and east Asia (n=1; tables S1A and S2A), and included 20 439 fracture cases and 78 843 controls. After meta-analysis, replication of promising findings was performed initially in the GENOMOS consortium (18 779 cases and 32 078 controls from 29 additional studies, tables S1B and S2B). Two additional large GWAS (UK Biobank, 14 492 cases and 130 563 controls; EPIC-Norfolk study, 2926 cases and 17 710 controls) were then included in the discovery set, comprising in total 37 857 cases and 227 116 controls (aged 18-106 years, including 69% women). Genetic markers reaching genome wide significance in this expanded meta-analysis and previously reported bone mineral density markers associated with fracture¹¹ were additionally replicated in 147 200 cases and 150 085 controls from 23andMe, a personal genetic company (23andMe GWAS participants were customers who consented to participate in research with self reported fracture data). Figure S1 shows the overall study design. To enable two-sample mendelian randomisation studies, we compiled summary level results from the largest

Table 1 | Fracture risk factors assessed and number of samples in each genome wide association study

Disease or trait	Total sample size
Femoral neck bone mineral density ¹¹	32 961
Lumbar spine bone mineral density ¹¹	31 800
Age at menopause ¹²	69 360
Rheumatoid arthritis ¹³	58 284 (14 361 cases)
Inflammatory bowel disease ¹⁴	34 652 (12 882 cases)
Type 1 diabetes ¹⁵	26 890 (9934 cases)
Thyroid stimulating hormone ¹⁶	26 523
Homocysteine ¹⁷	44 147
Grip strength ¹⁸	142 035
Age of puberty ¹⁹	182 416
Fasting glucose ^{20,21}	58 074
Coronary heart disease ²²	107 432 (41 513 cases)
Type 2 diabetes ²³	56 862 (12 171 cases)
Vitamin D levels ^{24,25}	33 996
Dairy calcium intake ^{26*}	171 213†

*Lactase intolerance (MCM6-rs4988235) was used as a proxy for dairy consumption.

†Effect estimates were derived from reference 26.

available GWAS meta-analyses performed so far on a large set of clinical risk factors for fracture (table 1). All studies were approved by their respective institutional ethics review committees and all participants provided written informed consent.

Study endpoint (fracture definition)

To maximise the statistical power to detect genetic loci, we used an inclusive definition of fracture, which was successfully used in previous efforts to test bone mineral density associated variants for association with fracture^{11,27} and allowed us to undertake the largest GWAS on fracture risk so far. Fracture cases were defined as those individuals (>18 years old) who had fractures at any skeletal site confirmed by medical, radiological, or questionnaire reports (table S3). Fractures of the fingers, toes, and skull as well as high trauma fractures were excluded whenever possible, although there have been some reports that even high trauma fractures are also predicted by low bone mineral density and are predictive of future low trauma fracture.^{28,29} Controls were defined as individuals (>18 years old) from the same cohorts, without a history of fracture.

Fracture GWAS meta-analysis and replication

Genome wide genotyping was performed in each cohort by use of Illumina or Affymetrix genome wide genotyping chips (table S4A) and was imputed to ensure accurate ascertainment of nearly all common genetic variation above a minor allele frequency threshold of 1%. After strict quality control criteria were applied to samples and single nucleotide polymorphisms (SNPs), we followed a consortium wide standardised analytical plan to assess the association of SNPs with risk of fracture. We used logistic regression adjusted for sex, age (simple and quadratic terms), height, and weight, testing additive (per allele) genetic effects. Before performing meta-analysis, three separate meta-analytical centres checked the data independently. All individual GWAS were corrected by genomic control

before we performed a fixed effects meta-analysis using METAL software. A total of 2 539 801 autosomal SNPs present in more than two studies were meta-analysed. We took forward for replication a set of promising SNPs for de novo genotyping in 26 studies at LGC Genomics (UK), using KASP genotyping as described previously²⁷ (table S4B) and tested them in three more studies (table S4C), for a total of 29 studies. Allele and genotype frequencies of all genotyped variants followed Hardy-Weinberg equilibrium proportions. To obtain unbiased estimates of effect size, all SNPs associated at a genome wide significant level (that is, $P < 5 \times 10^{-8}$) and previously known bone mineral density fracture loci¹¹ were tested for replication in the 23andMe cohort (table S4C).

Genetic determinants of risk factors for fracture

We used the genetic determinants of 15 available clinical risk factors from the largest GWAS datasets available. Genome wide association analyses have been published for bone mineral density (femoral neck and lumbar spine),¹¹ age of puberty,¹⁹ age at menopause,¹² grip strength,¹⁸ vitamin D,^{24 25} homocysteine,¹⁷ thyroid stimulating hormone level,¹⁶ fasting glucose,^{20 21} type 1 diabetes,¹⁵ type 2 diabetes,²³ rheumatoid arthritis,¹³ inflammatory bowel disease,¹⁴ and coronary artery disease.²² The well established lactose intolerance marker (LCT_(C/T_13910) polymorphism; rs4988235)³⁰ was used as a surrogate to assess long term differences in dairy derived calcium intake.³¹ Additional risk factors were considered for inclusion; however, at the time of analyses, well powered GWAS were not available for some risk factors of interest including alcohol intake,³² smoking³⁴ and plasma calcium levels.³⁵ Body mass index³⁶ was not evaluated given that the fracture discovery analysis was adjusted for body weight and height.

Genetic correlation

We used LD score regression to estimate the genetic correlation of the selected clinical risk factors and fracture (table 1).³⁷ This method estimates the degree of shared genetic risk factors between two diseases or traits, and was applied to 11 of the 15 selected risk factors for fracture (since genome wide association results were not publicly available for type 1 diabetes and thyroid stimulating hormone and dairy calcium intake). We accounted for multiple testing by using a conservative Bonferroni correction for 12 tests (that is, $\alpha = 4.2 \times 10^{-3}$). We also tested whether the above mentioned risk factors were genetically correlated with bone mineral density.

Mendelian randomisation

Next, we undertook mendelian randomisation analyses to estimate effects of 15 selected clinical risk factors in a two-sample mendelian randomisation framework. The mendelian randomisation approach was based on the following assumptions:

- The genetic variants used as instrumental variables are associated with the clinical risk factors.

- The genetic variants are not associated with any confounders of the exposure-outcome relation.
- The genetic variants are associated with fracture only through the clinical risk factors—that is, a lack of pleiotropy (fig 1).

We used the largest previously published GWAS meta-analyses of the risk factors, at the time of analyses, to maximise statistical power (table S5A).³⁸⁻⁴¹ To reduce potential bias due to population stratification, we restricted the analyses to studies with participants of European descent. To ensure independence between the SNPs used to evaluate the association of the risk factor and fracture risk, we grouped by LD ($r^2 > 0.05$) those SNPs achieving genome wide significance, keeping only the SNP with the lowest P value per group. Next, we recorded the effect size and standard error attributed to each allele's effect on the risk factor (table S5B). Finally, for age of menopause, we performed sex specific mendelian randomisation analysis in women only.

The resulting individual SNP effect estimates were pooled by use of the Wald type ratio estimator, which is formally analogous to an inverse weighted meta-analysis.⁴² Again, we applied a conservative Bonferroni corrected threshold (that is, $\alpha = 3.3 \times 10^{-3}$, because 15 risk factors were assessed) to account for the multiple risk factors tested. We also tested the assumptions underlying the mendelian randomisation approach (fig 1). To test the third assumption (a lack of pleiotropic effects of the SNPs on the outcome, independent of the exposure), we used mendelian randomisation-Egger regression.⁴³ Moreover, as sensitivity analyses for robust causal inference, we additionally performed mendelian randomisation analyses using a weighted median estimator and penalised weighted median estimator. We also tested the effect of the same clinical risk factors on bone mineral density²⁷ using the same methods. For the binary exposures, the odds ratios were converted (by multiplying log-odds ratios by 0.693 and then exponentiating) in order to represent the odds ratio per doubling of the odds

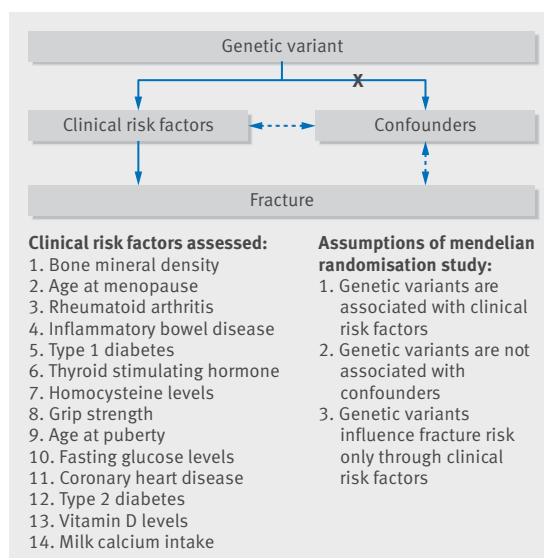


Fig 1 | Mendelian randomisation study design

of susceptibility to disease.⁴⁴ Finally, we undertook mendelian randomisation power calculations⁴⁵ for all such analyses.

Patient involvement

No patients were directly involved in the design, recruitment, or conduct of the study. Nevertheless, several of the participating studies comprised collections of patients who were made aware of their contribution of medical data to research through their informed consents signed by all study participants. After publication, dissemination of the results will be sought across different countries involving respective patient organisations, the general public, and other stakeholders; typically, across social media, scientific meetings and media interviews. Finally, some studies sent newsletters informing their participants about important findings and their implications.

Results

Genetic loci associated with fracture

We saw no evidence of excessive genomic inflation ($\lambda=1.02$, LD score intercept=0.99) in the GWAS meta-analysis, suggesting that the results were not biased because of population stratification, genotyping artefacts, or cryptic family relationships (fig 2). As shown in table 2 and figure 2, 15 genomic loci were associated at a genome wide significant level with fracture risk after meta-analysis of the discovery (table S6A) and replication (tables S6B, S6C, and S6D) stages. All loci were at, or near, loci previously shown to be associated with bone mineral density,^{11 27 46-52} a major determinant of fracture risk (table S6E and figure S2). The effect sizes of these common SNPs on fracture risk was modest (odds ratios ranging from 1.03 to

1.10), which is consistent with GWAS findings for other complex diseases.⁵³

Genetic correlations with clinical risk factors

SNPs influencing bone mineral density were strongly and inversely correlated with odds of fracture (table 3; genetic correlation -0.59 , $P=2\times 10^{-24}$ for femoral neck bone mineral density, with similar results for lumbar spine bone mineral density, -0.53 , $P=1\times 10^{-20}$). By contrast, none of the remaining clinical risk factors evaluated was strongly genetically correlated with risk of fracture with the exception of homocysteine (table 3). Genetically increased risk of type 2 diabetes was positively correlated with femoral neck bone mineral density, while genetically increased grip strength had positive correlations with bone mineral density of both the femoral neck and lumbar spine (table S7).

Mendelian randomisation

Using mendelian randomisation analyses to assess the effect of the 15 risk factors on fracture, we saw evidence for a major effect of genetically decreased bone mineral density on fracture risk (fig 3 and table 4; odds ratio per standard deviation decrease in femoral neck bone mineral density=1.55, 95% confidence interval 1.48 to 1.63, $P=1.5\times 10^{-68}$). We also observed a large effect of grip strength on fracture risk (2.14, 1.13 to 4.04, $P=0.01$), but these results had wide confidence intervals and were not significant after multiple testing correction.

Vitamin D levels assessed by use of 25-hydroxy-vitamin D variants were not found to be linearly associated with increased fracture risk (odds ratio per standard deviation decrease=0.84, 95% confidence interval 0.70 to 1.02, $P=0.07$). Most of these mendelian randomisation effects did not seem to be

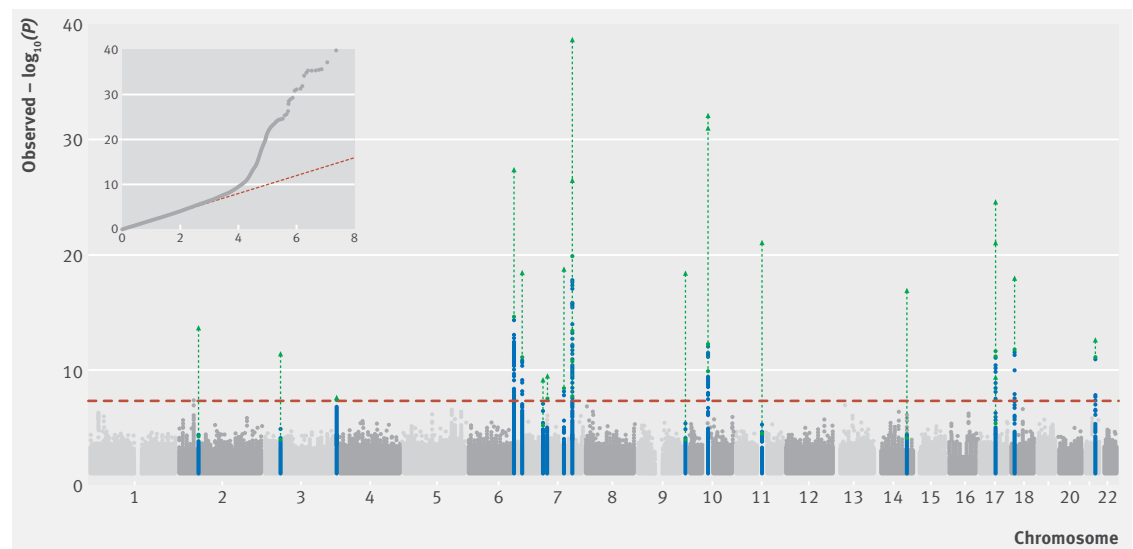


Fig 2 | Manhattan plot of $-\log_{10}$ association P values for discovery meta-analysis, and quantile-quantile plot (QQ plot) of the distribution of observed $-\log_{10}$ association P values against the expected null distribution for discovery meta-analysis. Dashed horizontal red line=genome wide significant (GWS) threshold ($P<5\times 10^{-8}$); blue dots=SNPs at GWS loci that are within 500kb of leading SNPs in previous genome wide association studies with different bone traits. Green lines and triangles=combined $-\log_{10}$ association P values after replication in the 23andMe cohort

Table 2 | Genome wide significant single nucleotide polymorphisms (SNPs) for fracture

Locus	Candidate gene	SNP	Distance to gene (kb)	EA	EAF	Discovery stage*			Replication stage*			Combined*		
						EA	Odds ratio (95% CI)	P	EA	Odds ratio (95% CI)	P	EA	Odds ratio (95% CI)	P
2p16.2	SPTBN1	rs4233949	-23.21	G	0.61	1.03 (1.02 to 1.05)	6.9×10 ⁻⁵	1.04 (1.05 to 1.05)	8.9×10 ⁻¹¹	1.03 (1.02 to 1.04)	2.8×10 ⁻¹⁴	185 057	22.4	
3p22.1	CTNMB1	rs430727	107.2	T	0.45	1.03 (1.02 to 1.05)	1.0×10 ⁻⁴	1.03 (1.02 to 1.04)	1.1×10 ⁻⁸	1.03 (1.02 to 1.04)	5.0×10 ⁻¹²	185 057	0	
6q22.33	RSPO3	rs10457487	0	C	0.51	1.06 (1.05 to 1.08)	2.3×10 ⁻¹⁵	1.04 (1.03 to 1.05)	1.7×10 ⁻¹⁵	1.05 (1.04 to 1.06)	4.8×10 ⁻²⁸	185 057	5	
6q25.1	ESR1	rs2982570	0	C	0.58	1.05 (1.04 to 1.07)	8.1×10 ⁻¹²	1.03 (1.02 to 1.04)	5.2×10 ⁻¹⁰	1.04 (1.03 to 1.05)	4.3×10 ⁻¹⁹	185 057	23	
7q31.31	WNT16, CPED1	rs2908007	-3.25, 24.67	A	0.60	1.08 (1.06 to 1.10)	1.2×10 ⁻²⁰	1.05 (1.04 to 1.06)	5.6×10 ⁻²²	1.06 (1.05 to 1.07)	2.3×10 ⁻³⁹	185 055	0	
7q21.3	C7orf76, SHFM1	rs6465508	0, 0	G	0.34	1.05 (1.03 to 1.07)	4.0×10 ⁻⁹	1.04 (1.03 to 1.05)	4.1×10 ⁻¹²	1.04 (1.03 to 1.05)	2.0×10 ⁻¹⁹	185 056	35	
7p14.1	STARD3NL	rs6959212	-89.01	T	0.34	1.04 (1.02 to 1.06)	6.9×10 ⁻⁶	1.02 (1.01 to 1.04)	1.1×10 ⁻⁵	1.03 (1.02 to 1.04)	8.8×10 ⁻¹⁰	185 057	15.6	
7p12.1	GRB10, COBL	rs1548607	40.33, -182.4	G	0.32	1.05 (1.03 to 1.07)	3.2×10 ⁻⁸	1.02 (1.01 to 1.04)	2.1×10 ⁻⁴	1.03 (1.02 to 1.05)	4.7×10 ⁻¹⁰	185 052	40	
9q34.11	FUBP3	rs7851693	0	G	0.35	1.03 (1.01 to 1.06)	1.3×10 ⁻⁴	1.05 (1.06 to 1.06)	4.8×10 ⁻¹⁶	1.04 (1.03 to 1.05)	5.0×10 ⁻¹⁹	185 057	23.5	
10q21.1	MBL2/DKK1	rs11003047	-90.63	G	0.11	0.99 (1.07 to 1.12)	6.2×10 ⁻¹²	1.08 (1.07 to 1.10)	1.4×10 ⁻²¹	1.09 (1.07 to 1.10)	9.5×10 ⁻³³	185 05	0	
11q13.2	LRP5	rs3736228	0	T	0.15	1.05 (1.03 to 1.07)	3.0×10 ⁻⁵	1.07 (1.05 to 1.08)	2.8×10 ⁻¹⁸	1.06 (1.05 to 1.08)	1.0×10 ⁻²¹	185 056	24.6	
14q32.12	RPS6KA5	rs1286083	0	T	0.82	1.04 (1.02 to 1.06)	8.8×10 ⁻⁵	1.05 (1.04 to 1.07)	3.0×10 ⁻¹⁴	1.05 (1.04 to 1.07)	1.6×10 ⁻¹⁷	185 085	43.3	
17q21.31	SOST, DUSP3, MEOX1	rs2741856	-4.26, -16.65, 88.02	G	0.92	1.11 (1.08 to 1.14)	2.4×10 ⁻¹²	1.08 (1.06 to 1.11)	5.3×10 ⁻¹⁵	1.10 (1.07 to 1.11)	3.1×10 ⁻²⁵	184 977	0	
18p11.21	FAM210A, RNMT	rs4635400	0, -7.149	A	0.36	1.06 (1.04 to 1.07)	1.5×10 ⁻¹²	1.03 (1.02 to 1.04)	2.7×10 ⁻⁹	1.04 (1.03 to 1.05)	1.1×10 ⁻¹⁸	185 057	22	
21q22.2	ETS2	rs9980072	141.9	G	0.73	1.06 (1.04 to 1.08)	8.4×10 ⁻¹²	1.03 (1.01 to 1.04)	1.8×10 ⁻⁵	1.04 (1.03 to 1.05)	3.4×10 ⁻¹³	185 057	36	

EA=effect allele; EAF=effect allele frequency; I²=index of heterogeneity.

*Discovery stage (37 857 cases; 227 116 controls); replication stage (147 200 cases; 150 085 controls); combined (185 057 cases; 377 201 controls).

Table 3 | Estimated genetic correlation between fracture and other clinical risk factors

Disease or trait	Genetic correlation (95%CI)	P
Femoral neck bone mineral density	-0.59 (-0.70 to -0.48)	2×10 ⁻²⁴
Lumbar spine bone mineral density	-0.53 (-0.64 to -0.42)	1×10 ⁻²⁰
Age at menopause	-0.12 (-0.23 to -0.003)	0.04
Rheumatoid arthritis	0.02 (-0.10 to 0.14)	0.74
Inflammatory bowel disease	-0.01 (-0.13 to 0.11)	0.90
Homocysteine levels	0.22 (0.07 to 0.37)	0.004
Grip strength	-0.10 (-0.21 to 0.01)	0.07
Age of puberty	0.03 (-0.05 to 0.11)	0.43
Fasting glucose	-0.05 (-0.19 to 0.09)	0.46
Coronary heart disease	-0.05 (-0.09 to 0.19)	0.48
Type 2 diabetes	-0.07 (-0.22 to 0.08)	0.35
Vitamin D levels	0.23 (-0.52 to 0.98)	0.56

strongly influenced by directional pleiotropy, because the intercepts of the mendelian randomisation-Egger test were tightly centred around the null, except for rheumatoid arthritis, type 2 diabetes, grip strength, glucose, and homocysteine levels (table 4). The estimates from the inverse variance weighted fixed meta-analysis were very similar to the estimates from the weighted median and penalised weighted median method (table S8). However, despite some indication of causality of fasting glucose levels on fracture risk (table S8) in the median weighted analyses, it did not surpass the multiple testing threshold.

Consistent with the results of the genetic correlation analyses, we found that none of the other evaluated clinical risk factors had evidence of a causal effect on risk of fracture, despite adequate statistical power (mean=98% (range 56-100%), table 4).⁴⁵ When evaluating the effect of genetically increased risk factors on bone mineral density (table S9), only age of puberty had an effect on bone mineral density after accounting for multiple testing; fasting glucose, type 2 diabetes, and age at menopause had marginal effects, consistent with a recent mendelian randomisation study of type 2 diabetes and glycaemic traits on bone mineral density.⁵⁴

We next undertook careful evaluation of the three mendelian randomisation assumptions. The first assumption was verified by the selection of only common variants (minor allele frequency >5%) strongly associated with the clinical risk factor (P<5×10⁻⁸). After performing a thorough literature search, we can exclude reported associations between the genetic variants and potential confounding factors (second assumption). Finally, by using mendelian randomisation-Egger regression, we found no evidence of the presence of pleiotropy between the instruments and the outcomes (table 4).

Discussion

Principal findings and interpretation

In this large GWAS for fracture, we identified genetic determinants (at 15 loci) of fracture and tested the role of 15 selected clinical risk factors on fracture risk and

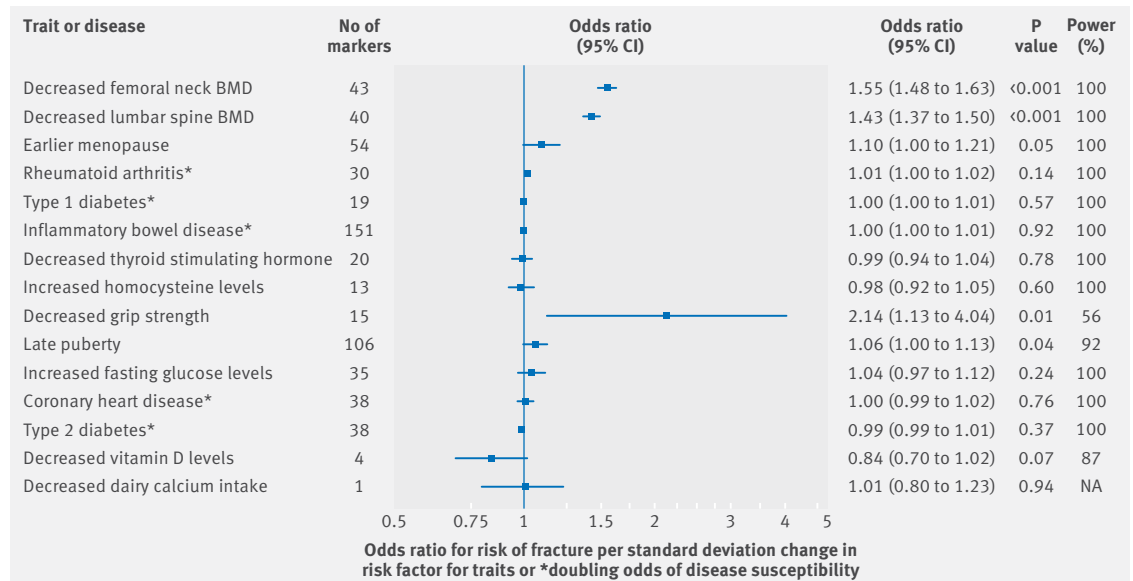


Fig 3 | Forest plot showing effect of 15 genetically determined risk factors on fracture risk. Power=statistical power to detect an odds ratio of 1.15 at $\alpha \leq 3.3 \times 10^{-3}$; NA=not applicable; BMD=bone mineral density

bone mineral density. Using mendelian randomisation analyses, we demonstrated that genetically decreased bone mineral density (and, to a lesser extent, hand grip strength) was the only clinical risk factor among those tested, with evidence for an effect on fracture risk. By contrast, despite high statistical power, none of the other tested and well accepted risk factors (eg, rheumatoid arthritis and other causes of secondary osteoporosis) or any of the other clinically relevant risk factors (vitamin D levels, dairy food derived calcium intake, fasting glucose, type 2 diabetes, and coronary heart disease) had evidence of a major causal effect on fracture risk. Furthermore, all identified genetic determinants of fracture also influenced bone mineral density.

In our previous work,¹¹ we tested 96 bone mineral density markers for association with fracture. In the meta-analysis, 14 bone mineral density loci were associated with fracture risk ($P < 5 \times 10^{-4}$), of which six surpassed genome wide significance ($P < 5 \times 10^{-8}$). In our current project, we began with GWAS meta-analysis for fracture risk. We confirmed the 2p16.2 (*SPTBN1*), 7q21.3 (*SHFM1*), 10q21.1 (*MBL2/DKK1*), 11q13.2 (*LRP5*), and 18p11.21 (*FAM210A*) loci, and observed an increased signal at *SOST*, *CPED1/WNT16*, *FUPB3*, *DCDC5*, *RPS6KA5*, *STARD3NL*, and *CTNNT1*. Lastly, we added the 6q22.33 (*RSPO3*), 6q25.1 (*ESR1*), 7p12.1 (*GRB10/COBL*), and 21q22.2 (*ETS2*) loci to the list of novel fracture loci. Among the genome wide significant loci associated with fracture, several

Table 4 | Estimated effects of 15 genetically determined risk factors on fracture risk

Trait or disease	No of markers	Inverse variance weighted meta-analysis			Mendelian randomisation-Egger regression§	
		Odds ratio (95% CI)*	P	Power (%)‡	Intercept (95% CI)	P
Decreased femoral neck BMD¶	43	1.55 (1.48 to 1.63)	1.5×10^{-68}	100	-0.0010 (-0.011 to 0.008)	0.83
Decreased lumbar spine BMD¶	40	1.43 (1.37 to 1.50)	2.3×10^{-55}	100	0.0050 (-0.006 to 0.014)	0.93
Earlier menopause	54	1.10 (1.00 to 1.21)	0.05	100	0.0007 (-0.006 to 0.007)	0.83
Rheumatoid arthritis†	30	1.01 (1.00 to 1.02)	0.14	100	0.0099 (0.003 to 0.017)	0.005
Type 1 diabetes†	19	1.00 (1.00 to 1.01)	0.57	100	0.0028 (-0.004 to 0.010)	0.39
Inflammatory bowel disease†	151	1.00 (1.00 to 1.01)	0.92	100	0.0003 (-0.003 to 0.004)	0.86
Decreased thyroid stimulating hormone	20	0.99 (0.94 to 1.04)	0.78	100	0.0050 (-0.019 to 0.009)	0.47
Increased homocysteine levels	13	0.98 (0.92 to 1.05)	0.60	100	0.0134 (0.001 to 0.026)	0.03
Decreased grip strength	15	2.14 (1.13 to 4.04)	0.01	56	0.1070 (0.011 to 0.203)	0.03
Late puberty	106	1.06 (1.00 to 1.13)	0.04	92	0.0036 (-0.002 to 0.009)	0.21
Increased fasting glucose levels	35	1.04 (0.97 to 1.12)	0.24	100	-0.0083 (-0.014 to -0.002)	0.01
Coronary heart disease†	38	1.00 (0.99 to 1.02)	0.76	100	0.0028 (-0.007 to 0.013)	0.57
Type 2 diabetes†	38	0.99 (0.99 to 1.01)	0.37	100	-0.0089 (-0.016 to -0.002)	0.02
Decreased vitamin D levels	4	0.84 (0.70 to 1.02)	0.07	87	-0.0143 (-0.103 to 0.074)	0.56
Decreased dairy calcium intake	1	1.01 (0.80 to 1.23)	0.94	NA	NA	NA

NA=not applicable; BMD=bone mineral density.

*Odds ratio is for the risk of fracture per standard deviation change in the risk factor for traits (1 standard deviation change=0.13 g femoral neck BMD, 0.18 g lumbar spine BMD, 3.9 years earlier menopause, 0.76 mIU/L thyroid stimulating hormone, 11.3 kg grip strength, 1.42 years late puberty, 0.62 mmol/L fasting glucose, 25.2 nmol/L vitamin D), or risk of fracture per doubling of odds of disease susceptibility; dairy calcium intake units are servings/day. Estimates obtained using a fixed effects model.

‡Statistical power to detect an odds ratio of 1.15 at $\alpha \leq 3.3 \times 10^{-3}$.

§Egger regression analyses can be performed if the number of genetic variants is more than two; Egger effect estimates are presented in table S7.

¶Findings that remain associated (that is, $\alpha \leq 3.3 \times 10^{-3}$) after correction for multiple testing.

contain well established causal proteins for fracture risk that are targets for clinically useful osteoporotic fracture treatments, such as *ESR1*, which encodes the oestrogen receptor, and *SOST*, which encodes sclerostin.⁵⁵ These discoveries highlight known and novel factors in pathways critical to bone biology (that is, Wnt, for mesenchymal stem cell differentiation) as well as potential new factors and biological pathways that might constitute future drug targets.⁵⁶

All the discovered fracture loci are also associated with bone mineral density, implying that skeletal fragility characterised by reduced bone mineral density is central to the pathophysiology of osteoporotic fracture. This contention is in line with the significant genetic correlation we identified between bone mineral density and fracture. Our mendelian randomisation analyses also indicate that the suggestive effect of late puberty and earlier age at menopause on fracture risk is, at least partly, mediated through reduced bone mineral density. By contrast, hand grip strength was not found to be a determinant of bone mineral density, and vice versa. Still, grip strength could be a proxy for overall muscle strength and risk of falling, and could be involved in a pathway leading to fracture independently of bone mineral density.⁵⁶ However, the hand grip estimates holds wide confidence intervals in our analyses expressed in standard deviations to allow comparison to other risk factors. We believe that these large standard deviations can be attributed to multiple factors, including effort and encouragement of the participants, posture, position, and intrinsic measurement variability between individuals. A recent effort using UK Biobank data also showed through mendelian randomisation that higher grip strength is associated with decreased fracture risk.¹⁸ As such, inclusion of grip strength (or a different assessment of muscle function such as leg strength) could improve the predictive performance of risk prediction calculators that already contain bone mineral density, just as has been reported for a history of falls.⁵⁶

Older individuals at high risk of fractures often have low levels of vitamin D (owing to low dietary intake and sun exposure). Therefore, fracture prevention guidelines have suggested the use of vitamin D supplementation in the general population.^{57 58} These recommendations have contributed to the marked increase in vitamin D use in older populations worldwide, where in the United States alone the proportion of individuals aged 70 years and older who use at least 1000 IU of vitamin D daily increased about 100-fold from 2000 to 2014.⁵⁹ Despite these guideline recommendations, it is unclear whether modestly low levels of vitamin D, rather than profoundly low values, are causally associated with a higher risk of fracture. Our mendelian randomisation work examined a linear relation between vitamin D levels and fracture risk. We did not test for the possibility of a threshold dependent relation—that is, effects that could be present only at very low levels of vitamin D. Nevertheless, our analyses showed that vitamin D levels had no protective linear effect on fracture in community dwelling individuals,

despite adequate statistical power. We also show, in line with other previous reports,⁶⁰⁻⁶² no evidence for a causal effect of vitamin D levels on bone mineral density. Although a threshold effect is likely to be present, where profoundly lowered vitamin D levels do increase risk of fracture, our mendelian randomisation results strongly suggest that increasing levels of vitamin D in the (non-deficient) general population is unlikely to decrease risk of fracture.

Likewise, calcium supplementation has been called into question recently. A mendelian randomisation study found that higher levels of serum calcium are a risk factor for coronary heart disease,⁶³ supporting the current recommendation of not exceeding total calcium intake of 1200 mg/day in older individuals.⁶⁴ Further, our study assessed the lactose persistence variant as a surrogate of long term intake of dairy calcium (used previously as an instrument for dairy consumption in association with blood pressure²⁶ and other traits), and found no evidence for a protective effect of sustained intake of dairy derived calcium on fracture risk.

Comparison with other studies

Previous observational studies and clinical trials have reported the beneficial effect of vitamin D^{65 66} or calcium⁶⁷ supplementation on fracture risk reduction, findings which are not supported by our results. These discrepant findings can be due to inadequate methods or high heterogeneity induced, for example, by combining community dwelling participants and inpatients in the same analysis. Consistent with our findings, a recent meta-analysis of 33 randomised trials⁶⁸ (n=51 145) found that supplementation with calcium, vitamin D, or both did not decrease the incidence of fractures in community dwelling older adults. Findings such as these should be interpreted with caution, because they do not necessarily apply to patients undergoing osteoporosis treatment, considering that trials evaluating osteoporosis treatment are carried out concomitant with vitamin D and calcium supplementation.

Studies seeking to show whether these supplements do increase the efficacy of osteoporotic treatment or decrease adverse events (that is, hypocalcaemia) are lacking. In either case, screening for vitamin D deficiency and seeking its correction should be warranted before the initiation of anti-resorptive treatment. Moreover, in a recent mendelian randomisation study investigating the role of 25-hydroxy-vitamin D in maintaining bone mineral density,⁶² increased levels of 25-hydroxy-vitamin D had no effect on bone mineral density measured by dual energy x-ray absorptiometry (n=32 965; 0.02 g/cm² change in femoral neck bone mineral density per standard deviation increase in 25-hydroxy-vitamin D). However, increased 25-hydroxy-vitamin D was associated with a slight reduction in heel bone mineral density estimated by ultrasonography (n=142 487; -0.03 g/cm² change in estimated bone mineral density per standard deviation increase in 25-hydroxy-vitamin

D). These results are consistent with our mendelian randomisation findings of no causal effect of vitamin D levels on fracture.

Implications for clinicians

Our mendelian randomisation findings are relevant to clinical care. Although clinical risk factors, when used jointly in well validated prediction algorithms, predict fracture risk, our findings are a reminder that clinically relevant changes in most of these risk factors are unlikely to result in large differences in fracture risk. These findings also suggest clinical outcomes such as fracture risk can be subject to bias owing to uncontrolled confounding in observational epidemiological studies. A strength of our study design is that mendelian randomisation limits this potential confounding, because alleles are essentially assigned randomly at conception, and are therefore not generally affected by confounders. Furthermore, because allele assignment must precede fracture, mendelian randomisation is not prone to bias due to reverse causation. These findings provide guidance for the design of future clinical trials on interventions that are more likely to be successful in reducing fracture risk.

Epidemiological studies have shown that older people with a fracture will have abnormal bone mineral density in the osteoporotic range (that is, T score lower than -2.5 standard deviations), but most will have a fracture will be osteopenic (T score between -1 and -2.5 standard deviations). In fact, about 87% of women and 82% of men with a non-vertebral fracture have a T score lower than -1.0 .⁶⁹ Our findings suggest that low bone mineral density (not only after reaching the osteoporotic range) constitutes a risk factor that captures a substantial and causal part of the influences that increase risk for all types of fracture. Therefore, interventions targeting an increase in bone mineral density (presuming this is associated with improvements in bone structure or quality) are likely to have pivotal roles in reducing fracture risk.

The interpretation of our findings merits careful consideration for some of the risk factors. Hyperthyroidism is an established risk factor for fracture, and we have not used genetic determinants of hyperthyroidism risk, but rather genetic determinants of thyroid stimulating hormone level, which are likely to be different. Moreover, our study described the effect of clinical risk factors on fracture in the general population, and is therefore not generalisable to states or conditions of extreme circumstances known to cause fracture (eg, sustained vitamin D deficiency, rickets, or osteomalacia). Furthermore, our results apply only to 25-hydroxy-vitamin D, and might not necessarily reflect effects of its active form, 1,25-dihydroxy-vitamin D. However, vitamin D supplementation, as is commonly used, acts by influencing 25-hydroxy-vitamin D. Altogether, the course of action for effective fracture prevention relies on establishing vitamin D deficiency and seeking its correction, rather than the widespread use of non-indicated ineffective supplementation.

Strengths and weaknesses of the study

To our knowledge, we have generated the largest and most comprehensive assessment of the genetic determinants of fracture risk so far. Moreover, use of the largest GWAS datasets available enabled adequate power to estimate the relation between genetically modified risk factors and fracture. Our study also had limitations. In our mendelian randomisation approach, we were unable to account for the sample overlap between the exposure and outcome GWAS datasets. However, we used powerful instruments to estimate the relation³⁹ between the risk factors and the outcomes. Therefore, any sample overlap should not significantly bias our findings. Another potential limitation was that the first release of the UK Biobank selected some individuals based on a nested case-control study of smoking and lung function,⁷⁰ and is therefore subject to selection bias.⁷¹ But after excluding the UK Biobank from our analyses, we observed no significant differences. Furthermore, the majority of our cohorts were imputed to HapMap (instead of more comprehensive reference panels), which could have affected the number of identified loci. However, given our power setting, our focus was mainly on common variants (which are well characterised in the HapMap imputation panel). In addition, analysis of large cohorts (UK Biobank and EPIC-Norfolk) imputed to more recent reference panels did not yield additional genome wide significant loci.

Moreover, we could not assess several relevant clinical risk factors. For example, body mass index⁷²⁻⁷⁴ could not be assessed in our mendelian randomisation framework because all GWAS analyses of fracture have been adjusted for body weight, preventing any inference assessment on causality. We also lacked power to estimate the casual relation between smoking and alcohol consumption: two potentially key risk factors for fracture. Similarly, we did not evaluate other risk factors that were not modifiable (such as age, sex, parental fracture history and body height), or those that have not been assessed by GWAS to yield genetic instruments for mendelian randomisation studies (such as falls, which are likely to be an important modifiable risk factor for fracture).⁷⁵

Furthermore, factors unlikely to be predominantly genetic in origin (eg, occupation) might still have a role in the pathogenesis of fracture but could not be readily assessed through our mendelian randomisation approach. Nevertheless, proxy phenotypes for such risk factors are increasingly being investigated by GWAS (eg, education for occupation), and can be used as robust instruments in future research. In addition, because information on bone mineral density was not available for all study participants who were investigated for fracture, we could not determine directly the degree to which bone mineral density mediated the effect of genetic determinants on fracture risk. Mendelian randomisation is a helpful method to minimise several biases in observational studies, but the possibility of residual pleiotropy could bias estimates in this study. However, the likelihood of this bias is reduced because

the mendelian randomisation-Egger regression test showed no clear directional pleiotropy for most of the factors. Lastly, because most of the study population was of European ancestry, results should not be directly generalised to other ethnicities.

Similarly, null results of a mendelian randomisation study could be influenced by canalisation, which is defined as compensatory feedback mechanisms that cannot be taken into account.⁷⁶ The possible influence of the risk factors on fracture risk might be specifically linked to their complications or management of the disease, which we also could not take into account in mendelian randomisation. As in most epidemiological studies, mendelian randomisation also assumes a linear relation between the risk factor and the outcome, which might not invariably be the case for all risk factors of fracture. Some risk factors, such as vitamin D and estimated calcium intake, could have non-linear threshold associations, as discussed above. Furthermore, we could not account for the dose-response association (eg, between the lactose variant rs4988235 and dairy intake) within our design, or differences in biological effects across different types of grouped exposures (that is, fermented v non-fermented types of dairy products). Finally, the non-significant trend observed for vitamin D towards having increased risk of fracture could be attributed to the selection of healthy people (that is, participants with very low levels of vitamin D and fracture, as well as those who are older, frail, and physically impaired, could have been under-represented in the studies included in the GWAS meta-analyses). Therefore, the vitamin D estimates of the current study cannot be generalised to these groups of older people.

Conclusion

From a study of over 500 000 individuals (about 185 000 fracture cases), we provide evidence that the main genetic determinants of osteoporotic fracture also influence bone mineral density, which was the only clinical risk factor to have shown a major effect on fracture risk among the study population assessed. By contrast, we found that other genetically estimated clinical risk factors for fracture, had either a very modest or no effect on fracture risk in the general population. Notably, genetic predisposition to lower levels of vitamin D and estimated calcium intake from dairy sources were not associated with fracture risk. Our study confirms bone mineral density as a pivotal cause of osteoporotic fracture and postulates that, among all the clinical risk factors we evaluated, interventions aimed at increasing bone mineral density are likely to have the most clinically relevant effect on fracture risk reduction.

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We thank all study participants for making this work possible; the many colleagues who contributed to collection and phenotypic characterisation of the clinical samples, as well as genotyping and analysis of the GWAS data; and the 23andMe research team. Part of this work was conducted using the UK Biobank resource. Full list of acknowledgments, funding organisations, and grants are listed per cohort in the supplemental material.

The following members of the GEFOS/GENOMOS consortium and the 23andMe research team are coauthors of this manuscript: Vincenzo Forgetta, Aaron Leong, Omar S Ahmad, Charles Laurin, Lauren E Mokry, Stephanie Ross, Cathy E Elks, Jack Bowden, Nicole M Warrington, Aaron Kleinman, Sara M Willems, Daniel Wright, Felix R Day, Anna Murray, Katherine S Ruth, Konstantinos K Tsilidis, Cheryl L Ackert-Bicknell, J H Duncan Bassett, Bram C J van der Eerden, Kaare M Gautvik, Sjur Reppe, Graham R Williams, Carolina Medina-Gómez, Karol Estrada, Najaf Amin, Joshua C Bis, Stephan Breda, Daniel I Chasman, Serkalem Demissie, Anke W Enneman, Yi-Hsiang Hsu, Thorvaldur Ingvarsson, Mika Kähönen, Candace Kammerer, Andrea Z Lacroix, Guo Li, Ching-Ti Liu, Yongmei Liu, Mattias Lorentzon, Reedik Mägi, Evelin Mihailov, Lili Mlani, Alireza Moayyeri, Carrie M Nielson, Nerea Alonso, Pack Chung Sham, Kristin Siggeirsdottir, Gunnar Sigurdsson, Unnur Thorsteinsdottir, Stella Trompet, Gudmar Thorleifsson, Liesbeth Vandenput, Nathalie van der Velde, Jorma Viikari, Su-Mei Xiao, Jing Hua Zhao, Kristina E Akesson, Marianne Andersen, Biljana Atanasovska, Susana Balcells, Joel Eriksson, Melissa M Formosa, Carmen Garcia-Ibarbia, Jesús Gonzalez-Macias, Natalia Garcia-Giralt, Goran Hallmans, Magnus Karlsson, Rita Khusainova, Beom-Jun Kim, Timothy C Y Kwok, Seung Hun Lee, Ping C Leung, Hans Mallmin, Laura Masi, Beatrice S Melin, Simona Mencej-Bedrac, Maria Nethander, José M Olmos, Panagoula Kollia, Janez Prezelj, Natasja M van Schoor, Olle Svensson, Pawel Szulc, Carmen Valero, Jean Woo, Maria Brandi, Sulin Cheng, Roland Chapurlat, Claus Christiansen, Cyrus Cooper, George Dedoussis, John A Eisman, Morten Frost, Sylvie Giroux, Daniel Grinberg, David Goltzman, Lynne J Hocking, Wim Van Hul, Jung-Min Koh, Lars Rejnmark, Jens-Erik B Jensen, Bente Langdahl, Joshua R Lewis, Roman S Lorenc, Elza Khusnutdinova, Janja Marc, Fiona E McGuigan, Dan Mellström, Karl Michaelsson, Xavier Nogue, Peter Nordström, Barbara Obermayer-Pietsch, Ulrika Pettersson-Kymmer, Richard L Prince, Jonathan Reeve, David M Reid, Jose A Riancho, Francois Rousseau, Nelson L S Tang, Angela Xuereb-Anastasi, William D Leslie, Daniel S Evans, Steven R Cummings, Jane Cauley, Cornelia M van Duijn, Matt Brown, Emma L Duncan, Lisette CPGM de Groot, Tonu Esko, Vilundur Gudnason, Tamara B Harris, Rebecca D Jackson, J Wouter Jukema, M Arfan Ikram, David Karasik, Stephen Kaptoge, Kay-Tea Khaw, Annie Wai-Chee Kung, Terho Lehtimäki, Leo-Pekka Lytikäinen, Paul Lips, Robert Luben, Andres Metspalu, Joyce B J van Meurs, Ryan L Minster, Eric Orwoll, Edwin Oei, Bruce M Psaty, Olli T Raitakari, Stuart H Ralston, Paul M Ridker, John A Robbins, Albert V Smith, Tim D Spector, Unnur Stykarsdottir, Joseph Zmuda, Gregory J Tranah, Kari Stefansson, Andre G Uitterlinden, M Carola Zillikens, Evangelia E Ntzani, Evangelos Evangelou, John P A Ioannidis, John R B Perry, Joyce Y Tung, David A Hinds, Robert A Scott. Full details of the authorship, including job titles and affiliations, are included in the supplemental material.

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Funding: This research and the Genetic Factors for Osteoporosis (GEFOS) consortium have been funded by the European Commission (HEALTH-F2-2008-201865-GEFOS). AGES: NIH contract N01-AG-12100 and NIA Intramural Research Program, Hjartavernd (the Icelandic Heart Association), and Althingi (the Icelandic Parliament). Icelandic Heart Association. Anglo-Australasian Osteoporosis Genetics Consortium (AOGC): National Health and Medical Research Council (Australia) (grant reference 511132). Australian Cancer Research Foundation and Rebecca Cooper Foundation (Australia). National Health and Medical Research Council (Australia). National Health and

Medical Research Council (Australia) Career Development Award (569807). Medical Research Council New Investigator Award (MRC G0800582). Health Research Council of New Zealand. Sanofi-Aventis, Eli Lilly, Novartis, Pfizer, Procter & Gamble Pharmaceuticals and Roche. National Health and Medical Research Council, Australia. Australian National Health and Medical Research Council, MBF Living Well foundation, the Ernst Heine Family Foundation and from untied educational grants from Amgen, Eli Lilly International, GE-Lunar, Merck Australia, Novartis, Sanofi-Aventis Australia and Servier. Medical Research Council UK and Arthritis Research UK. The Victorian Health Promotion Foundation and the Geelong Region Medical Research Foundation, and the National Health and Medical Research Council, Australia (project grant 628582). Action Research UK. DME is supported by an Australian Research Council Future Fellowship (FT130101709). This work was supported by a Medical Research Council programme grant (MC_UU_12013/4).. B-Vitamins for the PRevention Of Osteoporotic Fractures (BPROOF) study: supported and funded so far by The Netherlands Organisation for Health Research and Development (ZonMw, grant 6130.0031), The Hague; unrestricted grant from NZO (Dutch Dairy Association), Zoetermeer; Orthica, Almere; Netherlands Consortium Healthy Ageing (NCHA) Leiden/Rotterdam; Ministry of Economic Affairs, Agriculture and Innovation (project KB-15-004-003), The Hague; Wageningen University, Wageningen; VUmc, Amsterdam; Erasmus Medical Center, Rotterdam. Cardiovascular Health Study (CHS): National Heart Lung and Blood Institute (NHLBI) contracts HHSN268201200036C, HHSN268200800007C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086; and NHLBI grants U01HL080295, R01HL087652, R01HL105756, R01HL103612, R01HL120393, and R01HL130114 with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided through R01AG023629 from the National Institute on Ageing (NIA). Genotyping supported in part by the National Center for Advancing Translational Sciences, CTSI grant UL1TR000124, and the National Institute of Diabetes and Digestive and Kidney Disease Diabetes Research Center (DRC) grant DK063491 to the Southern California Diabetes Endocrinology Research Center. deCODE Genetics. EPIC-Norfolk: Medical Research Council G9321536 and G9800062, MAFF AN0523, EU FP5 (QLK6-CT-2002-02629), Food Standards Agency N05046, GEFOS EU FP7 Integrated Project Grant Reference: 201865, The UK's National Institute for Health Research (NIHR) Biomedical Research Centre Grant to Cambridge contributed to the costs of genotyping. Estonian Genome Center University of Tartu (EGCUT): This study was supported by EU H2020 grants 692145, 676550, 654248, Estonian Research Council Grant IUT20-60, NIASC and EIT-Health and EU through the European Regional Development Fund (project No 2014-2020.4.01.15-0012 GENTRANSMED). Erasmus Rucphen Family Study (ERF): Netherlands Organisation for Scientific Research (NWO), Erasmus University Medical Centre, the Centre for Medical Systems Biology (CMSB1 and CMSB2) of the Netherlands Genomics Initiative (NGI). Framingham Osteoporosis Study (FOS): National Institute for Arthritis, Musculoskeletal and Skin Diseases and National Institute on Ageing (R01 AR41398; DPK and R01 AR 050066; DK National Heart, Lung, and Blood Institute's Framingham Heart Study (N01-HC-25195) and its contract with Affymetrix for genotyping services (N02-HL-6-4278). The Gothenburg Osteoporosis and Obesity Determinant Study (GOOD): Swedish Research Council (K2010-54X-09894-19-3, 2006-3832 and K2010-52X-20229-05-3), Swedish Foundation for Strategic Research, ALF/LUA research grant in Gothenburg, Lundberg Foundation, Torsten and Ragnar Söderberg's Foundation, Västra Götaland Foundation, Göteborg Medical Society, Novo Nordisk Foundation, and European Commission grant HEALTH-F2-2008-201865-GEFOS. Health Aging and Body Composition Study (HealthABC): the Intramural Research Program of the National Institute of Health (NIH), National Institute on Ageing. US National Institute of Ageing (NIA) contracts N01AG62101, N01AG62103, and N01AG62106. NIA grant 1R01AG032098. The Center for Inherited Disease Research (CIDR). National Institutes of Health contract number HHSN268200782096C. Hong Kong Osteoporosis Study (HKOS): Hong Kong Research Grant Council (HKU 768610M); Bone Health Fund of HKU Foundation; KC Wong Education Foundation; Small Project Funding (201007176237); Matching Grant, Committee on research and conference (CRG) Grant and Osteoporosis and Endocrine Research Fund; and the Genomics Strategic Research Theme of the University of Hong Kong. The Osteoporotic Fractures in Men (MrOS) Study is supported by National Institutes of Health funding. The following institutes provide support: National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), National

Institute on Ageing (NIA), National Center for Research Resources (NCRR), and National Institute of Health (NIH) Roadmap for Medical Research under the following grant numbers: U01 AR45580, U01 AR45614, U01 AR45632, U01 AR45647, U01 AR45654, U01 AR45583, U01 AG18197, U01-AGO27810, and U11 RR024140. Prospective study of pravastatin in the elderly at risk (PROSPER): European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement No HEALTH-F2-2009-223004 PHASE. Rotterdam study I, Rotterdam study II, Rotterdam study III: Netherlands Organisation of Scientific Research (NWO) Investments (No 175.010.2005.011, 911-03-012); Research Institute for Diseases in the Elderly (014-93-015; RIDE2); Netherlands Genomics Initiative/Netherlands Consortium for Healthy Ageing (050-060-810); German Bundesministerium fuer Forschung und Technology under grants #01 AK 803 A-H and # 01 IG 07015 G, the Netherlands Organisation for Health Research and Development ZonMw VIDI 016.136.367 (funding FR, CM-G, KT). Study of Osteoporotic Fractures (SOF): supported by National Institutes of Health funding. The National Institute on Ageing (NIA) and the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) provides support under the following grant numbers: R01 AG005407, R01 AR35582, R01 AR35583, R01 AR35584, R01 AG005394, R01 AG027574, R01 AG027576, and R01 AG026720. TwinsUK1, TwinsUK2: NIHR Biomedical Research Centre (grant to Guys' and St Thomas' Hospitals and King's College London); Chronic Disease Research Foundation; Wellcome Trust; Canadian Institutes of Health Research, Canadian Funding for Innovation, Fonds de la Recherche en Santé Québec, Lady Davis Institute, Jewish General Hospital, and Ministère du Développement économique, de l'Innovation et de l'Exportation du Québec. UK Biobank: This research has been conducted using the UK Biobank Resource (application No 12703). Access to the UK Biobank study data was funded by a University of Queensland Early Career Researcher Grant (2014002959). Access to the UK Biobank study data was funded by University of Queensland Early Career Researcher Grant (2014002959) and University of Western Australia-University of Queensland Bilateral Research Collaboration Award (2014001711). NMW is supported by a National Health and Medical Research Council Early Career Fellowship (APP1104818). Women's Genome Health Study (WGHS): HL 043851 and HL69757 from the National Heart, Lung, and Blood Institute and CA 047988 from the National Cancer Institute, the Donald W Reynolds Foundation, and the Fondation Leducq Amgen. Women's Health Initiative (WHI) program is funded by the National Heart, Lung, and Blood Institute, National Institutes of Health, US. Department of Health and Human Services through contracts N01WH22110, 24152, 32100-2, 32105-6, 32108-9, 32111-13, 32115, 32118-32119, 32122, 42107-26, 42129-32, and 44221. Young Finns study (YFS): has been financially supported by the Academy of Finland: grants 286284 (TL), 134309 (Eye), 126925, 121584, 124282, 129378 (Salve), 117787 (Gendi), and 41071 (Skidi); the Social Insurance Institution of Finland; Competitive State Research Financing of the Expert Responsibility area of Tampere, Turku and Kuopio University Hospitals (grant X51001); Juho Vainio Foundation; Paavo Nurmi Foundation; Finnish Foundation for Cardiovascular Research; Finnish Cultural Foundation; Tampere Tuberculosis Foundation; Emil Aaltonen Foundation; Yrjö Jahnsson Foundation; Signe and Ane Gyllenberg Foundation; and Diabetes Research Foundation of Finnish Diabetes Association; and EU Horizon 2020 (grant 755320 for TAXINOMISIS). Barcelona cohort osteoporosis (BARCOS): Red de Envejecimiento y fragilidad RETICEF, CIBERER, Instituto Carlos III. Fondos FEDER. Fondo de Investigación Sanitaria (FIS PI13/00116). Spanish MINECO (SAF2014-56562-R), Catalan Government (2014SGR932). Austrius-A, Austrius-B: was supported by BioPersMed (COMET K project 825329), and the Competence Center CBmed (COMET K1 centre 844609), funded by the Austrian Federal Ministry of Transport, Innovation and Technology (BMVIT) and the Austrian Federal Ministry of Economics and Labour/ the Federal Ministry of Economy, Family and Youth (BMWA/BMWF) and the Styrian Business Promotion Agency (SFG). Cantabria-Camargo study (Cabrio-C), Cantabria osteoporosis case-control study (Cabrio-CC): Instituto de Salud Carlos III-Fondo de Investigaciones Sanitarias Grants PI 06/34, PI09/539, PI12/615 and PI15/521 (that could be cofunded by European Union-FEDER funds). Calcium Intake Fracture Outcome Study (CAIFOS): Healthway Health Promotion Foundation of Western Australia, Australasian Menopause Society and the Australian National Health and Medical Research Council Project Grant (254627, 303169 and 572604). Canadian Multicentre Osteoporosis Study (CaMos): was supported by a grant from the Canadian Institutes for Health Research (CIHR) (grant No MOP111103). JBR and JAM are funded by the Canadian Institutes of Health Research, Fonds du Recherche Québec Santé, and Jewish

General Hospital. Edinburgh Osteoporosis Study (EDOS): was supported by a grant from Arthritis Research UK (grant number 15389). European Prospective Osteoporosis Study (EPOS): EU Biomed 1 (BMHICT920182, CIPDCT925012, ERBC1PDCT 940229, ERBC1PDCT930105), Medical Research Council G9321536 and G9800062, Wellcome Trust Collaborative Research Initiative 1995, MAFF AN0523, EU FP5 (QLK6-CT-2002-02629), Food Standards Agency N05046, GEFOS EU FP7 Integrated Project Grant Reference: 201865. The UK's National Institute for Health Research (NIHR) Biomedical Research Centre Grant to Cambridge contributed to the costs of genotyping. Geelong Osteoporosis Study (GEOS): Canadian Institutes for health research operating grant funding reference #86748. Genetic analysis of osteoporosis in Greece (GROS): University of Athens, Greece (Kapodistrias 2009). Hertfordshire Cohort Study (HCS): supported by Medical Research Council UK; Arthritis Research UK; National Institute for Health Research (NIHR) Musculoskeletal BRU Oxford; National Institute for Health Research (NIHR) Nutrition BRC Southampton. Hong Kong: The projects have been supported by The Hong Kong Jockey Club Charities Trust, VC discretionary fund of The Chinese University of Hong Kong, and Research Grants Council Earmarked Grant CUHK4101/02M. Korean osteoporosis study in Asan Medical Center (KorAMC): a grant of the Korea Health Technology R&D Project, the Ministry of Health and Welfare, Republic of Korea (project No HI14C2258); a grant of the Korea Health Technology R&D Project, the Ministry of Health and Welfare, Republic of Korea (project No HI15CO377). Longitudinal Aging Study Amsterdam (LASA): largely supported by a grant from the Netherlands Ministry of Health Welfare and Sports, Directorate of Long term Care. MINOS study was supported by a grant from the Merck-Sharp-Dohme Chibret company. Malta osteoporotic fracture study (MOFS): financial support was received from the European Union Strategic Educational Pathways Scholarship scheme (STEPS). The Osteoporotic Fractures in Men (MrOS) Sweden: financial support was received from the Swedish Research Council (K2010-54X-09894-19-3, 2006-3832), Swedish Foundation for Strategic Research, ALF/LUA research grant in Gothenburg, Lundberg Foundation, Torsten and Ragnar Söderberg's Foundation, Västra Götaland Foundation, Göteborg Medical Society, Novo Nordisk foundation, and European Commission grant HEALTH-F2-2008-201865-GEFOS. Odense androgen study (OAS): World Anti-Doping Agency, Danish Ministry of Culture, Institute of Clinical Research of the University of Southern Denmark. Prevalence of osteoporosis in Slovenia (Slo-preval): was created as part of projects financially supported by the Slovenian research agency: P3-298 Geni, Hormoni in osebnostne spremembe pri hormonskih motnjah; Z1-3238: Geniski in okoljski dejavniki tveganja za razvoj motnje pri remodelaciji kosti; J2-3314 Genetski faktorji in hormoni pri presnovnih boleznih; and J3-2330 Genetski dejavniki pri osteoporozi. TWINGENE: supported in part by the Ragnar Söderberg Foundation (E9/11); the National Science Foundation (Early Concept Grants for Exploratory Research: "Workshop for the Formation of a Social Science Genetic Association Consortium," SES-1064089) as supplemented by the National Institutes of Health's (NIH) Office of Behavioural and Social Sciences Research; and the National Institute on Ageing/NIH through Grants P01-AG005842, P01-AG005842-20S2, P30-AGO12810, and T32-AG000186-23 to the National Bureau of Economic Research. The Swedish Twin Registry is supported by the Swedish Department of Higher Education, European Commission European Network for Genetic and Genomic Epidemiology (ENGAGE: 7th Framework Program (FP7/2007-2013)/Grant agreement HEALTH-F4-2007-201413; and GenomEUtwin: 5th Framework program "Quality of Life and Management of the Living Resources" Grant QLG2-CT-2002-01254); NIH (DK U01-066134); Swedish Research Council (M-2005-1112 and 2009-2298); Swedish Foundation for Strategic Research (ICA08-0047); Jan Wallander and Tom Hedelius Foundation; and Swedish Council for Working Life and Social Research. The Umeå Fracture and Osteoporosis Study (UFO) is supported by the Swedish Research Council (K20006-72X-20155013), Swedish Sports Research Council (87/06), Swedish Society of Medicine, Kempe-Foundation (JCK-1021), and by grants from the Medical Faculty of Umeå University (ALFVLL:968:22-2005, ALFVLL:937-2006, ALFVLL:223:11-2007, ALFVLL:78151-2009) and county council of Västerbotten (SpjutspetsanslagVLL:159:33-2007). GRW and JHDB were funded by the Wellcome Trust (Strategic Award grant No 101123; Joint Investigator Award No 110141; project grant No 094134). DPK was funded by a grant from the National Institute on Arthritis Musculoskeletal and Skin Diseases R01 AR041398. The funding agencies had no role in the study design, analysis, or interpretation of data; the writing of the manuscript; or in the decision to submit the article for publication.

Competing interests: All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: support from the organisations listed in the funding statement for the submitted work; BMP serves on the data safety monitoring board of a clinical trial funded by the manufacturer (Zoll LifeCor) and on the Steering Committee of the Yale Open Data Access Project funded by Johnson and Johnson; CEE is currently employed by AstraZeneca; SR has received grants from Eli Lilly, Amgen, UCB, and Ultragenyx, and has provided consultation for Novartis; KE is currently employed by Biogen; US, UT, GT, and KSt are employed by deCODE genetics/Amgen; and AK, JYT, and DAH are employed by 23andMe, and hold stock or stock options in 23andMe.

Ethical approval: The different cohorts had approvals from respective national ethical committees for medical research.

Data sharing: All data are available for unrestricted use through the GEFOS consortium and can be downloaded here: <http://www.gefos.org/>. We request that all use of such data be properly attributed through citation of this article.

Transparency: The corresponding authors (JBR and FRi) affirm that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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Web appendix: Supplementary note and full list of authorship details