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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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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 (e.g., *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; $P=1.5\times10^{-68}$). 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 FRAX[4] 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] Fracture risk is a moderately heritable trait (whereby h2 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.

SUBJECTS AND 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; **Supplementary Tables 1A and 2A**), 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, **Supplementary Tables 1B and 2B**). 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 fracture[11] 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). **Supplementary Figure 1** shows the overall study design. To enable two-sample mendelian randomisation studies, we compiled summary level results from the largest 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.

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 (9,934 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 ref 26.

Fracture GWAS Meta-analysis and Replication

Genome wide genotyping was performed in each cohort by use of Illumina or Affymetrix genome wide genotyping chips (**Supplementary table 4A**) 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 previously[27] (**Supplementary table 4B**) and tested them in three more studies (**Supplementary table 4C**), 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, $P<5\times10^{-8}$) and previously known

bone mineral density fracture loci[11] were tested for replication in the 23andMe cohort (**Supplementary table 4C**).

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] smoking[34] and plasma calcium levels.[35] Body mass index[36] 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, $\alpha=4.2\times10^{-3}$). 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 two-sample mendelian randomisation framework. The mendelian randomisation approach was based on the following assumptions:

a) The genetic variants used as instrumental variables are associated with the clinical risk factors.
b) The genetic variants are not associated with any confounders of the exposure-outcome relation.
c) The genetic variants are associated with fracture only through the clinical risk factors-that is, a lack of pleiotropy (**Figure 1**).

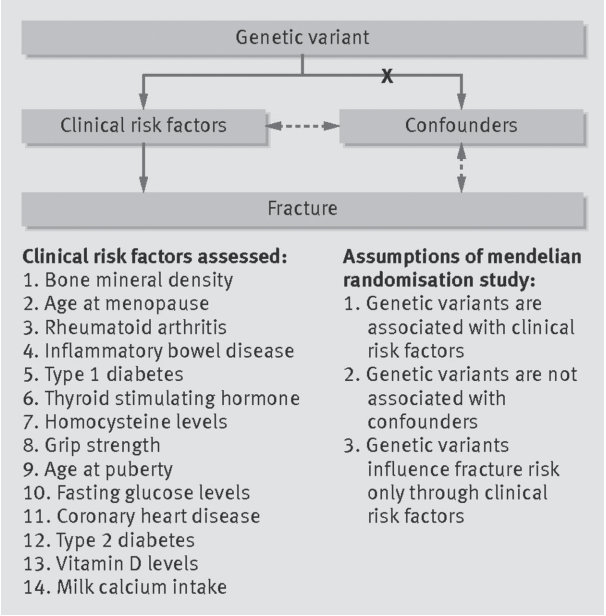

Figure 1 | Mendelian randomization study design

We used the largest previously published GWAS meta-analyses of the risk factors, at the time of analyses, to maximise statistical power (**Supplementary table 5A**) . [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 (r2>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 (**Supplementary table 5B**). 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, $\alpha=3.3\times10^{-3}$, because 15 risk factors were assessed) to account for the multiple risk factors tested. We also tested the assumptions underlying the mendelian randomisation approach (**Figure 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 density[27] 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 of susceptibility to disease.[44] Finally, we undertook mendelian randomisation power calculations[45] for all such analyses.

RESULTS

Genetic Loci Associated with Fracture

We saw was no evidence of excessive genomic inflation (λ=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 (**Figure 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 (Supplementary tables 6B, 6C, and 6D) 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 (**Supplementary Table 6E and Supplementary Figure 2**). 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]

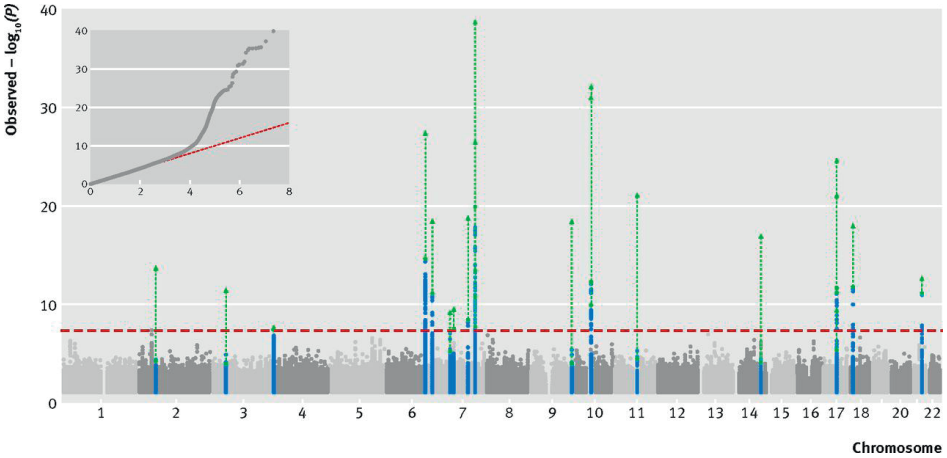

Figure 2 | Manhattan plot of -log10 association P values for discovery meta-analysis, and quantile-quantile plot (QQ plot) of the distribution of observed -log10 association P values against the expected null distribution for discovery meta-analysis. Dashed horizontal red line=genome wide significant (GWS) threshold ($P<5\times10^{-8}$); 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 −log10 association P values after replication in the 23andMe cohort

Table 2 | Fracture risk factors assessed and number of samples in each genome wide association study

Locus	Candidate gene	SNP	Distance to gene (kb)	EA	EAF	Discovery stage*		Replication stage*		Combined*			
						Odds ratio (95% CI)	P	Odds ratio (95% CI)	P	Odds ratio (95% CI)	P	No of fractures	I^2
2p16.2	*SPTBN1*	rs4233949	-23.21	G	0.61	1.03 (1.02-1.05)	6.9×10^{-5}	1.04 (1.05-1.05)	8.9×10^{-11}	1.03 (1.02-1.04)	2.8×10^{-14}	185,057	22.4
3p22.1	*CTNNB1*	rs430727	107.2	T	0.45	1.03 (1.02-1.05)	1.0×10^{-4}	1.03 (1.02-1.04)	1.1×10^{-8}	1.03 (1.02-1.04)	5.0×10^{-12}	185,057	0
6q22.33	*RSPO3*	rs10457487	0	C	0.51	1.06 (1.05-1.08)	2.3×10^{-15}	1.04 (1.03-1.05)	1.7×10^{-15}	1.05 (1.04-1.06)	4.8×10^{-28}	185,057	5
6q25.1	*ESR1*	rs2982570	0	C	0.58	1.05 (1.04-1.07)	8.1×10^{-12}	1.03 (1.02-1.04)	5.2×10^{-10}	1.04 (1.03-1.05)	4.5×10^{-19}	185,057	23
7q31.31	*WNT16, CPED1*	rs2908007	-3.25, 24.27	A	0.6	1.08 (1.06-1.10)	1.2×10^{-20}	1.05 (1.04-1.06)	5.6×10^{-22}	1.06 (1.05-1.07)	2.3×10^{-39}	185,055	0
7q21.3	*C7orf76, SHFM1*	rs6465508	0,0	G	0.34	1.05 (1.03-1.07)	4.0×10^{-9}	1.04 (1.03-1.05)	4.1×10^{-12}	1.04 (1.03-1.05)	2.0×10^{-19}	185,056	35
7p14.1	*STARD3NL*	rs6959212	-89.01	T	0.34	1.04 (1.02-1.06)	6.9×10^{-6}	1.02 (1.01-1.04)	1.1×10^{-5}	1.03 (1.02-1.04)	8.8×10^{-10}	185,057	15.6
7p12.1	*GRB10, COBL*	rs1548607	40.33, -182.4	G	0.32	1.05 (1.03-1.07)	3.2×10^{-8}	1.02 (1.01-1.04)	2.1×10^{-4}	1.03 (1.02-1.05)	4.7×10^{-10}	185,052	40
9q34.11	*FUBP3*	rs7851693	0	G	0.35	1.03 (1.01-1.06)	1.3×10^{-4}	1.05 (1.06-1.06)	4.8×10^{-16}	1.04 (1.03-1.05)	5.0×10^{-19}	185,057	23.5
10q21.1	*MBL2, DKK1*	rs11003047	-90.63	G	0.11	1.09 (1.07-1.12)	6.2×10^{-12}	1.08 (1.07-1.10)	1.4×10^{-21}	1.09 (1.07-1.10)	9.5×10^{-33}	185,057	0
11q13.2	*LRP5*	rs3736228	0	T	0.15	1.05 (1.03-1.07)	3.0×10^{-5}	1.07 (1.05-1.08)	2.8×10^{-18}	1.06 (1.05-1.08)	1.0×10^{-21}	185,056	24.6
14q32.12	*RPS6KA5*	rs1286083	0	T	0.82	1.04 (1.02-1.06)	8.8×10^{-5}	1.05 (1.04-1.07)	3.0×10^{-14}	1.05 (1.04-1.06)	1.6×10^{-17}	185,085	43.3

Table 2 | Fracture risk factors assessed and number of samples in each genome wide association study (*continued*)

Locus	Candidate gene	SNP	Distance to gene (kb)	EA	EAF	Discovery stage*		Replication stage*		Combined*			
						Odds ratio (95% CI)	P	Odds ratio (95% CI)	P	Odds ratio (95% CI)	P	No of fractures	I^2
17q21.31	*SOST, DUSP3, MEOX1*	rs2741856	-4.26, -16.65 88.02	G	0.92	1.11 (1.08-1.14)	2.4×10^{-12}	1.08 (1.06-1.11)	5.3×10^{-15}	1.10 (1.07-1.11)	3.1×10^{-25}	184,977	0
18p11.21	*FAM210A, RNMT*	rs4635400	0, -7.15	A	0.36	1.06 (1.04-1.07)	1.5×10^{-12}	1.03 (1.02-1.04)	2.7×10^{-9}	1.04 (1.03-1.05)	1.1×10^{-18}	185,057	22
21q22.2	*ETS2*	rs9980072	141.9	G	0.73	1.06 (1.04-1.08)	8.4×10^{-12}	1.03 (1.01-1.04)	1.8×10^{-5}	1.04 (1.03-1.05)	3.4×10^{-13}	185,057	36

EA=effect allele; EAF=effect allele frequency; I^2=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).

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, $P=2\times10^{-24}$ for femoral neck bone mineral density, with similar results for lumbar spine bone mineral density, -0.53, $P=1\times10^{-20}$). 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 (**Supplementary Table 7**).

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, -0.48)	2×10^{-24}
Lumbar spine bone mineral density	-0.53 (-0.64, -0.42)	1×10^{-20}
Age at menopause	-0.12 (-0.23, -0.003)	0.04
Rheumatoid arthritis	0.02 (-0.10, 0.14)	0.74
Inflammatory bowel disease	-0.01 (-0.13, 0.11)	0.90
Homocysteine levels	0.22 (0.07, 0.37)	0.004
Grip strength	-0.10 (-0.21, 0.01)	0.07
Age of puberty	0.03 (-0.05, 0.11)	0.43
Fasting glucose	-0.05 (-0.19, 0.09)	0.46
Coronary heart disease	-0.05 (-0.09, 0.19)	0.48
Type 2 diabetes	-0.07 (-0.22, 0.08)	0.35
Vitamin D levels	0.23 (-0.52, 0.98)	0.56

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 (**Figure 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, $P=1.5\times10^{-68}$). 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-hydroxyvitamin 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 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 (**Supplementary Table 8**). However, despite some indication of causality of fasting glucose levels on fracture risk (**Supplementary Table 8**) in the median weighted analyses, it did not surpass the multiple testing threshold.

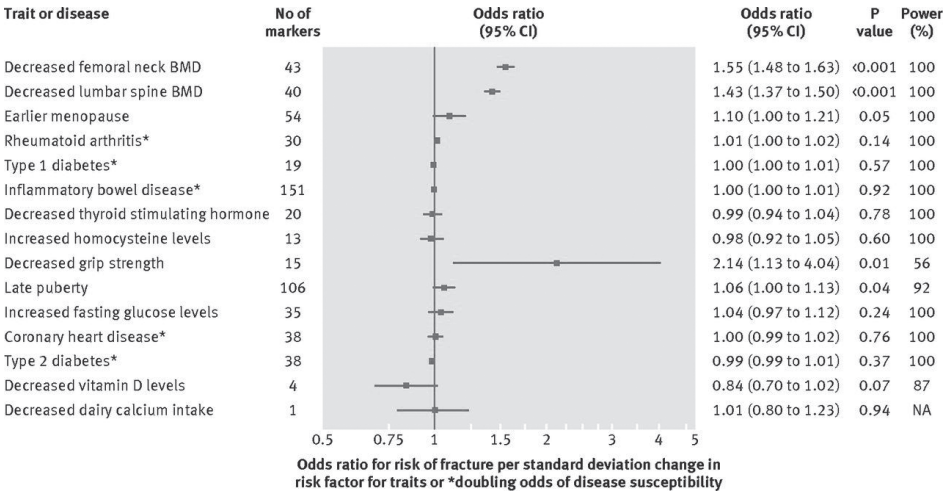

Figure 3 | Forest plot showing effect of 15 genetically determined risk factors on fracture risk. Power=statistical power to detect an odds ratio of 1.15 at $\alpha \leq 3.3 \times 10^{-3}$; NA=not applicable; BMD=bone mineral density

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 (**Supplementary table 9**), 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 ($P<5 \times 10^{-8}$). 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**).

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

		Inverse variance weighted meta-analysis			Mendelian randomisation-Egger regression§	
Trait or disease	**No of markers**	**Odds ratio (95% CI)***	**P**	**Power (%)‡**	**Intercept (95% CI)**	**P**
Decreased femoral neck BMD	43	1.55 (1.48-1.63)	1.5×10^{-68}	100	-0.001 (-0.011, 0.008)	0.83
Decreased lumbar spine BMD	40	1.43 (1.37-1.50)	2.3×10^{-55}	100	0.005 (-0.006, 0.014)	0.93
Earlier menopause	54	1.10 (1.00-1.21)	0.05	100	0.001 (-0.006, 0.007)	0.83
Rheumatoid arthritis†	30	1.01 (1.00-1.02)	0.14	100	0.010 (0.003, 0.017)	0.005
Type 1 diabetes†	19	1.00 (1.00-1.01)	0.57	100	0.003 (-0.004, 0.010)	0.39
Inflammatory bowel disease†	151	1.00 (1.00-1.01)	0.92	100	0.000 (-0.003, 0.004)	0.86
Decreased thyroid stimulating hormone	20	0.99 (0.94-1.04)	0.78	100	0.005 (-0.019, 0.009)	0.47
Increased homocysteine levels	13	0.98 (0.92-1.05)	0.60	100	0.013 (0.001, 0.026)	0.03
Decreased grip strength	15	2.14 (1.13-4.04)	0.01	56	0.107 (0.011, 0.203)	0.03
Late puberty	106	1.06 (1.00-1.13)	0.04	92	0.004 (-0.002, 0.009)	0.21
Increased fasting glucose levels	35	1.04 (0.97-1.12)	0.24	100	-0.008 (-0.014, -0.002)	0.01
Coronary heart disease†	38	1.00 (0.99-1.02)	0.76	100	0.003 (-0.007, 0.013)	0.57
Type 2 diabetes†	38	0.99 (0.99-1.01)	0.37	100	-0.009 (-0.016, -0.002)	0.02
Decreased vitamin D levels	4	0.84 (0.70-1.02)	0.07	87	-0.014 (-0.103, 0.074)	0.56
Decreased dairy calcium intake	1	1.01 (0.80-1.23)	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 $\alpha \leq 3.3\times10-3$.

§ 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, $\alpha<3.3\times10-3$) after correction for multiple testing.

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 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 ($P<5\times10^{-4}$), of which six surpassed genome-wide significance ($P<5\times10^{-8}$). 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 *CTNNB1*. 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 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 pressure[26] 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 calcium[67] 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 trials[68] (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/ cm^2 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/cm^2 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 relation[39] 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 index[72-74] 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 been 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 vs 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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https://www.bmj.com/content/362/bmj.k3225

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