Bringing the Genetics of Osteoporosis to the Clinic

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Table of Contents

Abstract/Résumé

Abstract

Osteoporosis is a common aging-related disease diagnosed by the measurement of several risk factors, the most clinically useful of which is bone mineral density (BMD). The Richards lab recently published large-scale genome-wide association study (GWAS) for BMD and showed that a large proportion of the variance in BMD was explained by genetic variants. With this information, we sought to fulfill one of the promises of the Human Genome Project - that our understanding of genetic information will impact our approach to medicine. In Chapter 2, we propose a way to implement genetic information into osteoporosis screening guidelines to reduce the number of individuals requiring expensive dual-energy X-ray absorptiometry (DXA) bone mineral density measurements. In Chapter 3, we show how the use of GWAS summary statistics for BMD and fracture can be combined with summary statistics for a circulating protein, vascular endothelial growth factor (VEGF) to better understand whether it would serve as an effective treatment target for osteoporosis. Chapter 2 involved developing a polygenic risk score model for BMD in 341,449 individuals from the UK Biobank. The utility of this model in screening for osteoporosis-related fracture risk was then tested in a cohort of 10,522 individuals who would have been eligible for screening according to the National Osteoporosis Guideline Group guidelines. We showed that by targeting assessments only to individuals with high genetic risk resulted in a 41% decrease in the number of individuals requiring DXA measurements while only decreasing the sensitivity to identify individuals requiring intervention from 99% to 93%. While Chapter 2 does not prescribe the optimal way of considering polygenic risk scores in the clinic, it demonstrates that their use can make osteoporosis screening more efficient and that future research into how they can be effectively incorporated into clinical decision-making is needed. Chapter 3 involved a Mendelian randomization study to better understand the effect of altered levels of circulating vascular endothelial growth factor on DXA-measured BMD,

BMD estimated at the calcaneus and fracture. We first obtained 10 single nucleotide polymorphisms (SNPs) that served as instrumental variables for circulating VEGF, explaining up to 52% of the variance in circulating VEGF. Then, we obtained GWAS summary statistics for these 10 SNPs on fracture and DXA-measured BMD, BMD estimated at the calcaneus to determine the effect of genetically altered levels of VEGF on osteoporosis outcomes. Because genetic variants cannot be influenced by factors that generally confound observational studies or be influenced by reverse causation, this experimental setup provided a framework for causal inference. Our results demonstrated that altering levels of circulating VEGF would not be an effective treatment or prevention for osteoporosis. Taken together, this work demonstrates that understanding the genetic determinants of bone density and fracture can help to improve clinical care by influencing screening strategies and more precisely identifying causal circulating proteins.

Résumé

L'ostéoporose est une maladie courante liée au vieillissement diagnostiquée par la mesure de plusieurs facteurs de risque, le plus utile étant la mesure de la densité minérale osseuse (DMO). Le laboratoire Richards a récemment publié une étude d'association pangénomique (GWAS) pour la DMO et a démontré qu'une grande partie de la variabilité dans la DMO serait expliquée par des variables génétiques. Avec cette information, nous avons cherché à tenir l'une des promesses du Projet Génome Humain, soit que notre compréhension du génome ait une incidence sur notre approche de la médecine. Au chapitre 2, nous proposons un moyen d'intégrer l'information génétique dans une procédure de dépistage pour l'ostéoporose afin de réduire le nombre d'individus nécessitant des mesures dispendieuses de DMO par absorptiométrie biénergétique à rayons X (DXA). Au chapitre 3, nous utilisons des statistiques récapitulatives venant d'une GWAS pour la DMO, la fracture et le niveau en sérum d'un facteur de croissance endothélial vasculaire (VEGF) afin de mieux comprendre si le VEGF peut servir d'objectif de traitement efficace pour l'ostéoporose. Le chapitre 2 porte sur le développement d'un modèle de risque polygénique pour la DMO à partir de 341,449 individus venant de la UK Biobank. L'utilité de ce modèle pour le dépistage du risque de fracture liées à l'ostéoporose a ensuite été testée dans une cohorte de 10,522 individus qui auraient été éligibles pour le dépistage conforme aux modalités définies par le National Osteoporosis Guideline Group. Nous avons établi qu'en ciblant les évaluations uniquement sur les individus présentant un risque génétique élevé, le nombre d'individus nécessitant des mesures de DXA diminuait considérablement à 41% en ne réduisant que de 99% à 93% la sensibilité de détection des individus nécessitant une intervention. Bien que le chapitre 2 n'ait pas pour objectif de recommander la façon optimale de considérer les modèles de risque polygénique dans la clinique, il montre que leur utilisation peut rendre le dépistage de l'ostéoporose plus efficace et que des recherches futures sur la manière de les intégrer rentablement à la prise de décision clinique sont nécessaires. Le chapitre 3

comprenait une étude de randomisation Mendélienne visant à mieux comprendre l'effet de la modification des niveaux en sérum de VEGF sur la DMO mesurée par DXA, sur la DMO estimée au niveau du calcanéum et sur la fracture. Nous avons d'abord obtenu 10 polymorphismes mononucléotidiques (SNP) qui ont servi d'instruments pour le niveau en sérum de VEGF, expliquant jusqu'à 52% de sa variabilité. Étant donné que ces variables génétiques ne peuvent pas être influencées par des facteurs qui confondent généralement les études d'observation ni influencées par des causalités inverses, cette configuration expérimentale a fourni un cadre pour l'inférence causale. Nos résultats ont suggéré qu'une modification des niveaux de VEGF en sérum ne constituerait pas un traitement ou une prévention efficace pour l'ostéoporose. Somme toute, ces travaux établissent que la compréhension des déterminants génétiques de la DMO et de la fracture peut aider à améliorer les soins cliniques en influençant les stratégies de dépistage pour l'ostéporose et en identifiant des protéines qui seraient de bonnes cibles pour son traitement.

List of Abbreviations

BMD: Bone mineral density

BMI: Body mass index

CIHR: Canadian Institutes of Health Research

CLSA: Canadian Longitudinal Study on Aging

DMO: Densité minérale osseuse

DXA: Dual-energy X-ray absorptiometry

EHR: Electronic health record

FA: Forearm

FN: Femoral neck

FRQS: Fonds de la recherche en santé du Québec

GWAS: Genome-wide association study

IVW: Inverse-variance weighted

LASSO: Least absolute shrinkage and selection operator

LD: Linkage disequilibrium

LS: Lumbar spine

MAF: Minor allele frequency

MR: Mendelian randomization

MrOS: The Osteoporotic Fractures in Men Study

NCATS: National Center for Advancing Translational Sciences

NIA: National Institute on Aging (NIA)

NIAMS: National Institute of Arthritis and Musculoskeletal and Skin Diseases

NIHR: National Institute for Health Research

NOGG: National Osteoporosis Guideline Group

PRS: Polygenic risk score

RCT: Randomized controlled trial

SCOOP: Screening for prevention of fractures in older women trial

SD: Standard deviation

SNP: Single nucleotide polymorphism

SOF: Study of osteoporotic fractures

SOS: Heel quantitative ultrasound speed of sound

VEGF: Vascular endothelial growth factor

eBMD: Estimated bone mineral density

gSOS: Polygenic risk score for SOS

pg/mL: Picograms per milliliter

List of Tables

Chapter 2

Table S3. VEGF-associated SNPs that were significantly associated with at least one circulating protein level using a GWAS study for 2,994 circulating protein levels in 3,301 individuals.

List of Figures

Figure S3. Sensitivity analysis on fracture excluding SNP rs6921438.

Figure S4. Sensitivity analysis on DXA-measured BMD including all VEGF-associated SNPs.

Figure S5. Sensitivity analysis on eBMD including all VEGF-decreasing alleles.

Figure S6. Sensitivity analysis on fracture including all VEGF-decreasing alleles.

Figure S7. MR-Egger analysis for DXA-measured FA, LS and FN BMD.

Figure S8. MR-Egger analysis for eBMD.

Figure S9. MR-Egger analysis for fracture.

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Preface

Contribution of Authors

The work described here was performed under the supervision of Dr. Brent Richards. It is a manuscript-based thesis format as described in the Thesis Preparation Guidelines by the Department of Graduate and Postdoctoral Studies. This thesis contains six chapters. Chapter 1 is an introduction to this thesis. Chapter 2 is in preparation to be submitted to *The BMJ*. Chapter 3 was submitted to *The Journal of Bone and Mineral Research* on June 27 2019. Chapter 4 is a discussion of Chapters 2 and 3. Chapter 5 is a conclusion with future aims for Chapters 2 and 3.

Chapter 2 is a manuscript authored by *Vincenzo Forgetta*, Julyan Keller-Baruch*, Marie Forest, Audrey Durand, Sahir Bhatnagar, John Kemp, Maria Nethander, Daniel Evans, John A Morris, Douglas P. Kiel, Fernando Rivadeneira, Helena Johansson, Nicholas C. Harvey, Dan Mellström, Magnus Karlsson, Cyrus Cooper, David M. Evans, Robert Clarke, John A. Kanis, Eric Orwoll, Eugene V McCloskey, Claes Ohlsson, Joelle Pineau, William D. Leslie, Celia M.T. Greenwood, J Brent Richards.* *Denotes equal contribution. V.F. and J.K-B. contributed to writing the manuscript and conducted the experiments and analyses presented in the paper. M.F. contributed to the initial analyses. A.D. contributed machine learning expertise and contributed to writing the manuscript. S.B., J.K, M.N., J.A.M, D.M.E., R.C. contributed to analyses. D.E. contributed data. D.P.K., F.R., N.C.H., D.M., M.K., C.C. provided comments on the manuscript. H.J. provided help with analyses. J.A.K. and E.V.M. provided comments on the manuscript and analyses. E.O. provided comments on the manuscript and provided data. C.O. provided comments on manuscript, analyses and provided data. J.P. contributed machine learning expertise. W.D.L. generated FRAX scores and provided direction on the screening program. C.M.T.G. oversaw analyses of polygenic risk score development

and provided funding. J.B.R. conceived and oversaw program, ran analyses for the screening program and obtained funding.

Chapter 3 is a manuscript authored by *Julyan Keller-Baruch, Vincenzo Forgetta, Despoina Manousaki, Sirui Zhou, J Brent Richards*. J.K-B lead the project, performed all experiments excluding those mentioned in the remainder of this paragraph, generated the figures and wrote the manuscript. V.F. contributed to writing the manuscript and worked in close collaboration with J.K-B throughout the project. D.M. contributed to writing the manuscript and enumerated the risk factors for osteoporosis and fracture that helped identify SNPs that were potentially pleiotropic (section 3.3.3). S.Z. contributed to writing the manuscript and performed the fracture GWAS meta-analysis (section 3.3.5). J.B.R conceived of the program, helped lead the project, provided funding and contributed to writing the manuscript.

Chapter 1: Introduction

1.1 The economic burden of osteoporosis

Osteoporosis is a common aging-related disease characterized by weakened bones that lead to increased risk of fracture[1]. Due to the aging population of North America, the incidence of osteoporosis is increasing, costing the Canadian healthcare system up to $3.9 billion per year[2] and the United States healthcare system over $17 billion per year[3]. To minimize costs, healthcare systems around the world have developed screening procedures that exclude individuals at low risk for the disease on the basis of measurable factors that are known to influence the risk osteoporosis-related fractures[4,5]. However, these risk factors do not generally include genetic information, which has been recently shown to explain a significant proportion of the variance in the disease[6] and is now less expensive to measure than many clinical tests. Further, genetic information can facilitate the discovery of osteoporosis determinants that have not yet been identified, leading to better or more efficient treatments for this common and costly disease. The focus of my thesis is therefore to investigate the clinical utility of the genetic determinants of bone mineral density (BMD) to improve the efficiency of osteoporosis screening programs and to identify novel drug targets for its treatment.

1.2 Bone mineral density

BMD is a heritable and complex trait that is influenced by several biological and environmental factors[7]. Low BMD is the most clinically relevant risk factor for osteoporosis, playing a fundamental role in the decision to treat individuals pharmacologically as a preventative measure against osteoporosis-related fractures[8]. BMD is generally measured using a non-invasive method, dual-energy X-ray absorptiometry (DXA)[9], at the forearm, hip or vertebral bones, each region comprising a different proportion of cortical bone and trabecular bone. Cortical bone is the dense, compact bone tissue that constitutes the majority of the human skeleton. Trabecular bone is soft, spongy bone tissue

that constitutes the remainder of the skeleton, providing structural support and flexibility to handle the stresses of movement and minor injury[10]. People with osteoporosis have an abnormally thin cortical bone and less trabecular bone. BMD can also be estimated at the heel calcaneus using quantitative ultrasound measurements, which allows for inexpensive and rapid assessment of BMD in a large number of individuals and has been shown to be predictive of osteoporosis-related fracture risk[11,12].

1.3 Risk factors for low bone mineral density and fracture

Although bone loss is expected at older ages, several risk factors will predispose individuals to abnormal amounts of bone loss and increased risk of fracture as they progress through life. These risk factors include increasing age, female sex, premature menopause, low bodyweight, glucocorticoid therapy, cigarette smoking, excessive alcohol consumption, low dietary calcium intake, vitamin D deficiency and a family history of hip fractures[13]. Because family history of hip fracture, at least in part, quantifies the inherited predisposition to low bone mineral density and fracture, a more precise measurement of this predisposition may be of clinical relevance.

1.4 Genetic determinants of bone mineral density

Genome-wide association studies (GWAS) and meta-analyses have identified single nucleotide polymorphisms (SNP) associated with BMD[14–22]. SNPs are positions in the genome that differ in their coded base pair (allele) between individuals and across populations. GWAS quantifies the association between an allele and its average effect on a trait in a population. SNPs can be measured in large cohorts using genotyping arrays which measure, at the individual-level, the alleles at hundreds of thousands of genomic positions. To increase the number of SNPs available for a GWAS, reference panels are created using whole-genome sequencing on thousands of individuals, which are

then used to impute the most likely coded alleles at genomic locations that are not covered by the genotyping array[23]. The basic form of a genome-wide association test is a linear or logistic regression of a single SNP on an outcome, which is usually a trait, or disease status, respectively. Current methods utilize linear mixed models to incorporate population stratification and cryptic relatedness into association tests[24].

1.5 Polygenic risk scores

Once a GWAS is complete, polygenic risk scores (PRS), which are predictions of a phenotype from a set of genotypes, can be calculated to estimate the overall genetic risk associated to a particular individual. It is calculated by summing the products of the count of risk alleles for a set SNPs with their corresponding effect sizes. These effect sizes can be obtained from a GWAS on the outcome, or with LASSO regression[25], which learns the effects of SNPs in a multivariable model, while simultaneously minimizing the absolute magnitude of effect sizes assigned to the genomic positions in question. It is possible that the latter can perform better in PRS generation because effect sizes can be estimated while considering all of the other SNPs in the model, whereas the former relies on effect sizes obtained from independent SNP-outcome associations.

1.6 Mendelian Randomization

In contrast to controlled experiments, observational studies are subject to a number of factors that can bias their results. One such factor is confounding, where one or more unmeasured variables associate with both the exposure of interest and the outcome, but do not lie in the causal pathway between the exposure and the outcome. Overlooking, or improperly measuring, such variables in observational studies leads to biased estimates of the effect of the exposure on the outcome. The randomized control trial (RCT) is a commonly-used approach to ensure that exposures do not

associate with confounding variables. In RCTs, patients are randomly assigned to a treatment or control group under the assumption that the groups are comparable at all but one variable of interest and its associated effects. Although the RCT is a powerful tool in clinical research, it is often not applicable to the study of potentially harmful exposures such as smoking or increased levels of a circulating biomarker. An alternative approach to assessing the causality of associations in observational studies is Mendelian Randomization (MR). Similar to RCTs, MR compares outcomes between groups of individuals that differ at a single genetic variant and its associated effects but that are comparable in all other ways. Because genetic variants are passed from parents to offspring independently of environmental factors, they can serve as instrumental variables that influence the exposure independently of factors that would generally confound observational studies. Further, because the germline genotype does not change throughout life, associations derived from MR analyses cannot be influenced by reverse causation, where levels of the exposure are influenced by the outcome itself. An MR study is performed by identifying genetic variants that are associated with an exposure of interest, then observing differences in outcomes between groups of individuals that differ at these genetic variants. If the genetic variants satisfy a set of assumptions[26] described in **Figure 1**, then they are considered valid instruments for the estimation of the causal effect of the exposure on the outcome.

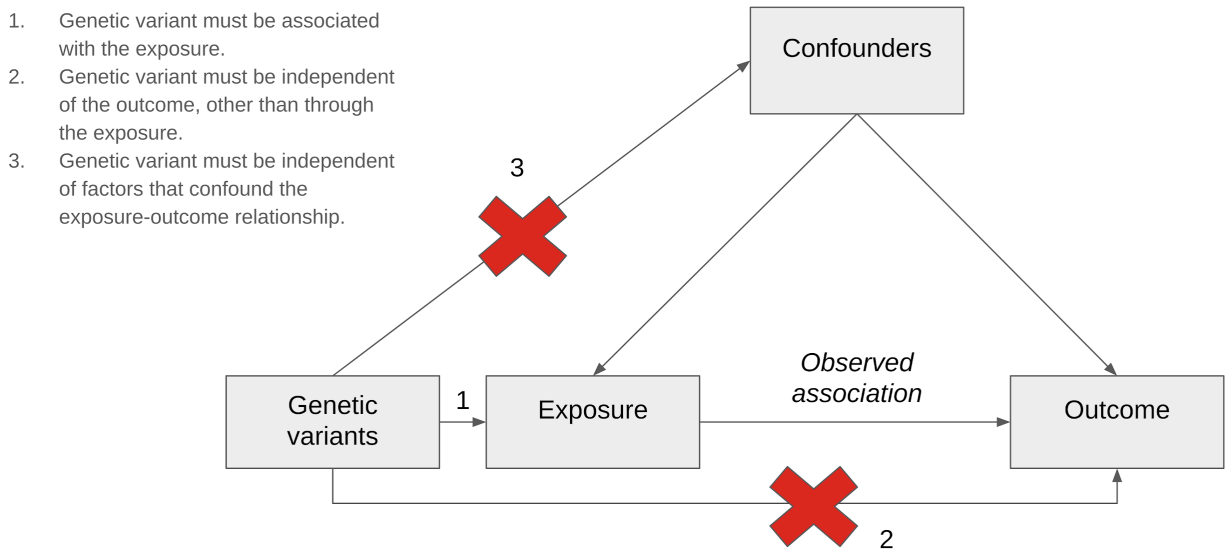

Figure 1. Mendelian randomization assumptions.

1.7 Objectives, rationales and hypotheses

The two major objectives of this thesis were to:

- Provide evidence that genomic information as a whole, rather than single genotypes, can improve the efficiency of osteoporosis screening programs, by removing from the screened population, individuals at low genetic risk.
- Identify a novel pharmacological target for the treatment or prevention of osteoporosis.

To address objective 1, we developed a real-world application of a polygenic risk score to improve osteoporosis screening. Since most screening programs identify a small proportion of the screened population to be at high risk of disease, we incorporated a polygenic risk score into a validated osteoporosis screening program in order to decrease the number of individuals that are prescribed expensive bone mineral density tests. The genetic prediction of complex traits and diseases could be clinically useful since the cost of genome-wide genotyping is decreasing rapidly and is now less expensive than many clinical tests. Further, such testing could be performed once

and provide insight into risk for many diseases. There is currently much debate in the community as to the role of polygenic risk scores, which was summarized in a *Perspective* in the *New England Journal of Medicine* in May of 2019, which concluded that, "It is time to evaluate polygenic risk scores in translational studies that assess clinical utility..."[27]. Osteoporosis might be a reasonable place to start the conversation about polygenic risk scores in screening programs, since it is less sensitive than breast cancer, or colon cancer screening.

To address objective 2, we conducted a MR study using circulating vascular endothelial growth factor (VEGF)-associated genetic variants, as identified by the largest published genome-wide association meta-analysis for circulating VEGF levels to date, providing the first evidence addressing the causal effect of altering circulating VEGF levels on bone mineral density in up to 426,824 individuals and on fracture in 76,549 cases and 470,164 controls. Several published observational and experimental studies in humans and animal models have associated lowered vascular endothelial growth factor (VEGF) with bone mineral density and adverse osteoporosis outcomes. If altered levels of VEGF is causal of adverse osteoporotic outcomes, then it could serve as a target for a novel pharmaceutical therapy. However, given that observational epidemiology studies are susceptible to confounding, it remains unclear whether this association is causal, driven by confounding or a product of the disease process itself. Further, there are currently no published randomized controlled trials investigating the skeletal effects of influencing circulating VEGF. Given that genetic variants are not vulnerable to many confounding variables or reverse causation, MR can circumvent many of the limitations that are inherent to observational studies. This provides a framework for causal inference and offers insight into the effect of a lifetime exposure to genetically decreased VEGF levels on osteoporosis outcomes, which cannot be assessed by short-term clinical trials.

Chapter 2

Preface: Bridge Between Chapter 1 and Chapter 2

A study by Lello et al.[28] showcased the utility of LASSO regression in the prediction of human height from genetic information. We decided to perform a similar study on heel bone mineral density in the UK Biobank. Given our understanding of the genetics of osteoporosis, a polygenic risk score for bone mineral density seemed likely provide clinical utility. Collaborating with bone experts from around the world, including members of FRAX® team, we hypothesized that considering a PRS for BMD alongside other commonly used clinical risk factors was a reasonable approach to implement genetic risk scores into osteoporosis screening as it avoided entirely displacing well-validated clinical practices, such as the National Osteoporosis guideline group screening (NOGG) algorithm. Implementing a PRS to the NOGG guidelines resulted in a substantial decrease in the number of individuals being needlessly prescribed an expensive bone mineral density test with a relatively small decrease to the sensitivity to correctly identify those requiring treatment. This represented the first successful implementation of genetic information into osteoporosis screening programs and set the stage for the implementation of PRS into screening programs for other common and costly diseases.

Chapter 2: A Polygenic Risk Score to Improve Screening for Fracture Risk

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2.1 Key Points

Question: Can genomics-enabled screening significantly reduce the number of individuals requiring bone mineral density measurements when screening for fragility fracture risk?

Findings: We developed "gSOS", a polygenic risk score for heel ultrasound speed of sound, in 341,449 individuals and tested its performance in 10,522 individuals from 5 different cohorts. Applying a gSOS-based filtering step in a screening program for high fracture risk reduced the proportion of the population requiring bone mineral density screening tests by 41%, while only reducing the sensitivity to identify individuals eligible for therapy from 99% to 93% and increasing specificity.

Meaning: Use of a polygenic risk score can decrease the number of people requiring bone mineral density scans in the assessment of fracture risk.

2.2 Abstract

Importance: Since most screening programs identify only a small proportion of the screened population to be eligible for an intervention, genomic-prediction of heritable risk factors could decrease the number needing to be screened by removing individuals at low genetic risk. Whether a polygenic risk score can identify people who are unlikely to benefit from bone mineral density (BMD) testing in screening for high fracture risk is unknown.

Objective: To test whether a polygenic risk score for heel quantitative ultrasound speed of sound (SOS)—a heritable risk factor for osteoporotic fracture—can identify low risk individuals who can be excluded from a fracture risk screening program.

Design, Setting and Participants: 341,449 individuals from UK Biobank with SOS measures were used to develop polygenic risk score models using LASSO regression. The optimal prediction model was determined in 5,335 separate individuals and termed "gSOS". Its utility in fracture risk screening was tested in 5 validation cohorts (N = 10,522 eligible participants) using the National Osteoporosis Guideline Group clinical screening guidelines.

Main Outcomes and Measures: The sensitivity and specificity to identify individuals requiring treatment, where the reference-standard was a BMD-based FRAX® score. The secondary outcomes were the proportions of the screened population requiring clinical risk factor-based FRAX screening or BMD-based FRAX screening.

Results: gSOS correlated with measured SOS (r^2 = 23.2%, 95% CI: 22.7-23.7%). Without genetic pre-screening, guideline recommendations were able to achieve a high sensitivity and specificity for correct treatment assignment (99.6% and 97.1%, respectively, in the validation cohorts). However, 81% of the population required clinical risk factor based-FRAX tests and 37% required BMD-based FRAX tests to achieve this accuracy. Using gSOS in screening and limiting further assessment to those with a low gSOS, resulted in small changes to the sensitivity and specificity (93.4% and 98.5%,

respectively), but substantial reductions in the proportion of individuals requiring clinical risk factor-based FRAX tests and BMD-based FRAX tests by 37% and 41% respectively.

Conclusions and Relevance: The use of a polygenic risk score in fracture risk screening can decrease the number of individuals requiring detailed assessments, including BMD, while maintaining a high sensitivity and specificity.

2.3 Introduction

Screening programs are generally designed to identify a proportion of the screened population whose risk of a clinically-relevant outcome is high enough to merit an intervention. However, usually only a small proportion of individuals who undergo screening is found to be at high risk, indicating that much of the screening expenditure is spent on individuals who will not qualify for intervention.

Osteoporosis is a common and costly disease that results in an increased predisposition to fractures.[1] Many guidelines[2–6] aimed at the prevention of osteoporosis-related fractures incorporate the fracture risk assessment tool (FRAX®),[7,8] a validated method to risk stratify individuals for treatment by assessing their 10-year probability of hip and major osteoporotic fracture. Guidelines vary widely, but often recommend a staged screening process where individuals are first screened with a clinical risk factor-based FRAX and those at increased risk of fracture are screened with a more expensive bone mineral density (BMD)-based FRAX score. Recently, a large randomized controlled trial (SCOOP) demonstrated the potential benefit of community-based fracture risk screening, by reducing rates of hip fractures in elderly women.[9] This trial used a screening strategy based on the National Osteoporosis Guideline Group (NOGG)[3] which implements fracture risk stratification through the use of FRAX scores. In this trial, the entire screened population underwent FRAX assessment using clinical risk factors and almost half (49%) had a sufficiently high probability of fracture to warrant further testing using a BMD-based FRAX. Yet, only 14% of the screened population had a resultant probability of fracture high enough to warrant intervention. This suggests that methods to improve screening efficiency could decrease the number of persons undergoing risk stratification using clinical and BMD-FRAX tests, while still correctly identifying the individuals who should be treated.

Skeletal measures that predict fracture risk are highly heritable (50-85%) and include BMD and quantitative speed of ultrasound (SOS) measurements, which are highly correlated.[10–13]

Recently, large cohort resources have enabled the genomic prediction of such heritable clinical risk factors from genotypes through polygenic risk scores,[10–15] which capture information from a large number of single nucleotide polymorphisms assayed from genome-wide genotyping. These assays assess common genetic variation at millions of single nucleotide polymorphisms and cost approximately $40 in a research context. However, the clinical utility of such polygenic risk scores is unclear, wide-spread replication of polygenic risk scores is currently lacking, and it is unknown whether they can aid in screening programs.

We therefore developed a polygenic risk score for SOS (gSOS) in a large cohort of 341,449 individuals from the UK Biobank. We then tested the generalizability and potential benefit of incorporating gSOS into NOGG screening guidelines using 5 cohorts, comprising 10,522 eligible individuals, to see if gSOS could decrease the number of people requiring more detailed assessments, such as BMD measurement, while still identifying those who require interventions to decrease their risk of fracture.

2.4 Methods

2.4.1 Overall study design and cohorts

This study included three phases (**Figure 1**). The first two phases were conducted in two distinct subsets of the UK Biobank Study cohort, and the final phase in a further subset of UK Biobank combined with 4 other cohorts. Characteristics of the cohorts are shown in **Table 1,** with the cohorts described in detail in **Table S1**.

The first phase used least absolute shrinkage and selection operator (LASSO) regression, a form of machine learning[16] to train a set of polygenic risk score models to predict SOS in the UK Biobank Training Set (N = 341,449). In phase 2, the polygenic risk score model explaining the most variance in measured SOS in the UK Biobank Model Selection Set (N = 5,335) was selected and

named “gSOS”. The ability of gSOS to explain variance in measured SOS was then tested in the UK Biobank Test Set (N = 84,768).

In phase 3, gSOS was tested for its performance in a clinical fracture risk screening program applied to a population of 10,522 individuals derived from five separate cohorts. Inclusion in the screening program required these individuals to be ≥50 years with at least one risk factor and available measurements of femoral neck BMD. This population comprised a further distinct subset of the UK Biobank Test Set (N = 2,445), as well as individuals from the Canadian Longitudinal Study of Aging [CLSA] (N = 2,931), the Study of Osteoporotic Fractures [SOF] (N = 2,094), Mr OS US (2,026) and Mr OS Sweden (N = 1,026). Together these five cohorts in phase 3 are referred to as validation cohorts.

2.4.2 SOS and BMD Measurement (Details in Supplement)

We decided to use polygenic risk scores to predict SOS, rather than BMD, because polygenic risk scores require large number of individuals with proper phenotyping and genome-wide genotyping. The largest dataset for SOS is approximately ten-fold larger than that for BMD.[17,18] SOS also predicts fracture, with similar performance characteristics compared to BMD, and the two measures are correlated (r = 0.4–0.6).[19] However, since femoral neck BMD is required for FRAX calculations used in screening programs,[20] we required that all individuals in the phase 3 analysis had femoral neck BMD measures available. Details of SOS and BMD measurement are available in the Supplement. All analyses used SOS standardized to a mean of zero and standard deviation of one.

2.4.3 Development of machine learning model to predict SOS (Figure 1)

I. Training, Model Selection and Test datasets: To develop and test gSOS, we followed best practices in clinical prediction to ensure unbiased estimates of model performance by developing models in

datasets distinct from datasets which were used to test model performance.[21] Participants in the UK Biobank with White British ancestry, measured SOS, and genotyping information (N= 426,811) were randomly assigned to either the UK Biobank Training set (80% of participants), the UK Biobank Model Selection set (1.25% of participants), or the UK Biobank Test set (18.75% of participants) (**Figure 1** & **Table 1**). Since BMD was measured in only 4,741 individuals in all of UK Biobank,[22] these individuals were assigned to the UK Biobank Test set to enable them to be used in phase 3 of the study.

II. GWAS: Using the UK Biobank Training set (N=341,449 individuals with White British ancestry), we tested the additive allelic effects of each of the 13.9 million SNPs passing QC, separately, on SOS using a series of linear mixed-models[23], adjusting for age, sex, assessment centre, and genotyping array (Supplement). The GWAS was also controlled for the top 20 principle components of ancestry to reduce effects of cryptic relatedness. Linkage disequilibrium-independent associations where obtained using PLINK by clumping SNPs in linkage equilibrium at a $r^2 > 0.05$ and selecting a single most significant SNP from within each clumped set. To reduce potential bias due to population stratification the UK Biobank Training, Model Selection and Test sets included only White British participants, whereas all other cohorts included only people of general European ancestry (defined in the Supplement).

III. Polygenic Risk Scores using LASSO: Using the UK Biobank Training set, we fitted 6 LASSO models[16] to predict SOS using only SNPs with P-values smaller than a chosen set of thresholds (**Table S2**). The UK Biobank Model Selection set was then used to identify the P-value threshold and regularization parameter (λ) that resulted in the lowest root mean square error for the prediction of SOS. This P-value threshold and regularization parameter were then taken forward for testing in the UK Biobank Test set. Hence, we ensured that the performance of only one final

polygenic risk score was evaluated in the UK Biobank Test set. We refer to this final predictor as "gSOS", in which SOS is predicted only from genotypes.

IV. Traditional Polygenic Risk Scores: Traditional polygenic risk scores[10] were derived from the GWAS for SOS performed in the UK Biobank Training set, without the use of LASSO, by including different sets of SNPs, selected by P-value threshold as described in the Supplement (**Table S2**).

2.4.4 Generation of FRAX Scores

FRAX risk scores for major osteoporotic fracture (hip, clinical vertebra, proximal humerus and wrist) can be generated with or without BMD, referred to in this paper as BMD-FRAX and clinical risk factor CRF-FRAX, respectively.[20] Therefore CRF-FRAX and BMD-FRAX were calculated for all participants in each validation cohort.[20] FRAX CRFs were assessed at the baseline visit for each cohort and included age, sex, body mass index (BMI), previous fracture, smoking, glucocorticoid use, rheumatoid arthritis and secondary causes of osteoporosis. Measures of more than two daily units of alcohol and parental history of hip fracture variables were not available in the UK Biobank and were set to "no" for this cohort, as suggested by FRAX guidelines. Not all secondary causes of osteoporosis were available for the SOF, Mr OS US and Mr OS Sweden cohorts and these variables were also set to "no" for these cohorts. Age was recorded at baseline visit. Sex was self-reported and verified by genotype. Individuals with discordant sex between self-report and genotype were excluded. CRF-FRAX and BMD-FRAX were calculated for all participants in each of the clinical cohorts, using country-specific FRAX scores.[20]

2.4.5 Genomic Screening in Fracture Risk Screening

In the absence of an international consensus on fracture risk screening,[2,4,24,25] we chose to use the assessment and management clinical algorithm developed by NOGG,[3] since it is supported by randomized controlled trial evidence.[9] NOGG uses 10-year absolute probability of fracture as calculated by FRAX and suggests treatment or reassurance based on thresholds of risk, which are age dependent and consider competing risks. NOGG guidelines (**Figure 2**) also aim to identify individuals at risk for fracture in a cost-efficient manner by reserving clinical visits and more costly BMD testing for only those at intermediate risk, i.e. where the FRAX score lies close to an intervention threshold. This intervention threshold is equivalent to the age-specific FRAX 10-year probability in women with a prior fragility fracture, since nearly all such women would be recommended an intervention.[3] Individuals without a risk factor are excluded from the CRF-FRAX assessment. By applying CRF-FRAX, individuals can be recommended for either an intervention (high risk), a BMD-FRAX (intermediate risk) or reassurance and no further participation in the screening program (low risk). Those having a BMD-FRAX can then be recommended an intervention if their resulting 10-year probability of major osteoporotic fracture exceeds the age-specific threshold, or they can be reassured. See **Figure 2**.

Despite the efficiencies gained by using this stepwise approach,[26] false-negatives can occur when interventions are not recommended to individuals who have a low CRF-FRAX-based probability and are discharged from subsequent screening, whereas if they had undergone BMD-FRAX, would qualify for intervention. Likewise, false-positives can arise when an individual is recommended for an intervention based on the CRF-FRAX score but would not have qualified for an intervention with BMD-FRAX.

Thus, using the BMD-FRAX as a reference standard, we were able to calculate the sensitivity and specificity of the NOGG screening strategy. Furthermore, resources are often expended to

measure BMD-FRAX in individuals whose final probability of fracture is too low to warrant intervention. Therefore, we also estimated the number of CRF-FRAX and BMD-FRAX tests that were performed but led to the individual being reassured without a recommended intervention.

To try to reduce the number of individuals undergoing testing, particularly BMD, who would subsequently not require intervention, we introduced a gSOS-based screening step in the NOGG algorithm, where individuals were reassured from the program if their gSOS was above a threshold (**Figure 3**). We chose the sex-specific thresholds of gSOS which reduced CRF-FRAX and BMD-FRAX testing but minimized the loss of sensitivity to identify individuals who would be recommended for treatment. This threshold was chosen using data from the UK Biobank Test set. This same gSOS thresholds were then applied in the remaining four validation cohorts (CLSA, SOF, Mr OS US and Mr OS Sweden). The sensitivity and specificity of including a gSOS screening step were calculated, where intervention according to BMD-FRAX probability was again used as the reference standard. The number of CRF-FRAX and BMD-FRAX tests performed, but not leading to an intervention, were also counted. These analyses were conducted in each validation cohort, men and women separately, and in all groups combined.

2.5 Results

2.5.1 Cohort Characteristics

Table 1 describes the FRAX risk factors for all of the cohorts. There were few clinically-relevant differences in any of the osteoporosis-related risk factors in the UK Biobank Training, Model Selection and Test sets, as expected, since these sets were generated randomly. As planned, all individuals from the UK Biobank with BMD measures were included in the UK Biobank Test set, to ensure availability of BMD-FRAX scores as the reference standard. There were few differences in demographics or CRFs between individuals with or without BMD measured. The validation cohorts

(CLSA, SOF, Mr OS US and Mr OS Sweden) provided a range of characteristics, allowing for a better assessment of generalizability of results (**Table 1**).

2.5.2 GWAS

After quality control (Supplement), 13,958,249 SNPs were included in the GWAS. The GWAS in the training set identified 1,404 independent ($r^2 \leq 0.05$) genome-wide significant loci at a P-value threshold of $< 5 \times 10^{-8}$. **Figure S1** shows the Manhattan and QQ plots for this GWAS.

2.5.3 Variance Explained in SOS in the UK Biobank Model Selection Set

The polygenic risk scores models trained with LASSO explained at most, 25.0% (95% confidence interval: 23.0-27.0%) of the variance in SOS in the UK Biobank Model Selection set (**Table S2**). **Figure S2** provides detailed information on the optimal algorithm tuning parameters. None of the traditional polygenic risk scores performed better than the polygenic risk score derived from the LASSO regression. **Figure S3** demonstrates that, as expected, the estimated effects of the activated SNPs from the LASSO algorithm were attenuated, when compared to the effects estimated from the GWAS.

2.5.4 Variance Explained in SOS in the UK Biobank Test Set

Age, sex and BMI explained 4.0% (95% CI: 3.7-4.2) of the variance in SOS. Adding all available FRAX clinical risk factors increased the variance explained to 5.3% (95% CI: 5.0-5.6%). The polygenic risk score from the UK Biobank Model Selection set explaining the most variance in measured SOS, was designated as "gSOS" and was then tested for its correlation with SOS in the UK Biobank Test set. This model (using 21,717 activated SNPs with P-value $\leq 10^{-4}$) explained 23.2% (95% CI: 22.7-23.7%) of the variance in measured SOS (**Table S2, Figure 3**).

2.5.5 Screening by NOGG Guidelines in Validation Cohorts

The validation cohorts comprised 10,522 individuals eligible for fracture risk screening (**Table 1**). Both the sensitivity and specificity of NOGG guidelines to identify individuals at high enough risk to merit an intervention, compared to the reference standard BMD-FRAX, were high (99.6% and 97.1%, respectively, **Figure 5** and **Table S3**). This high sensitivity and specificity required CRF-FRAX tests to be undertaken in 81% of the population eligible for screening with BMD-FRAX tests subsequently recommended in 37% of the population. 74% of those requiring CRF-FRAX tests were ultimately reassured without a recommendation for an intervention. As well, just over one third of all BMD-FRAX tests resulted in the individual being reassured without intervention. (**Figure 5** and **Table S3)**

2.5.6 Screening incorporating a gSOS-based screening step

Using the UK Biobank Test set we selected the threshold of gSOS that would minimize the number of BMD tests done in persons who would be ultimately reassured, but also minimize the false negative rate **(Figure S4**). Applying this threshold separately in men and women, we found that a threshold of standardized gSOS set to 0.5 and zero for men and women, respectively, balanced these goals in the UK Biobank Test set and subsequently individuals above these thresholds were excluded from further screening in the validation cohorts, prior to receiving a CRF-FRAX or BMD-FRAX Test (**Figure 4**)

Figure 5 demonstrates that applying a gSOS screening step resulted in a small decrease in sensitivity to identify eligible participants for therapy to 93.4%, but that the specificity increased slightly to 98.5%. However, the proportion of screened individuals requiring CRF-FRAX testing decreased from 81% to 51% (representing relative decrease of 37%), when compared to NOGG guidelines without a gSOS screening step. Additionally, the proportion of screened individuals

requiring BMD-FRAX testing decreased from 37% to 22% (representing a relative decrease of 41%). (**Figure 5** and **Table S3**)

The proportion of CRF-FRAX and/or BMD-FRAX tests that resulted in an individual being excluded from the screening program without a recommendation for an intervention also decreased from 74% to 46% and from 34% to 20%, respectively. (**Figure 5** and **Table S3**). Cohort-specific results are shown in **Tables S4 – S8**.

2.5.7 Women and Men Separately

The SOF cohort was comprised of only women (SOF), while Mr OS US and Mr OS Sweden, were comprised of only men providing the opportunity to explore performance characteristics by sex. Further, we divided the UK Biobank Test set and CLSA into sex-specific datasets. Amongst 4,859 women who were eligible for screening in the cohorts (SOF, UK Biobank Test set and CLSA), the sensitivity and specificity for correct treatment assignment were high (99.9% and 95%, respectively). Nevertheless 58% of the population required CRF-FRAX tests and 43% required BMD-FRAX tests. (**Table S9**)

When applying a gSOS screening step, the sensitivity decreased marginally to 94.6% and the specificity increased marginally to 98.2%. The proportion of the population requiring CRF-FRAX tests reduced from 58% to 27% (representing a relative decrease by 55%), while the proportion requiring BMD-FRAX tests reduced from 43% to 20% (representing a relative decrease by 55%). (**Table S9**)

Amongst the 5,668 men eligible for screening, the sensitivity and specificity were 96.9% and 98.2%, respectively, using CRF-FRAX alone as the screening step. In order to achieve this performance, 100% of men had a CRF-FRAX and 31% required a BMD-FRAX. The yield of high-risk individuals from these tests was low, such that 94% of men receiving a CRF-FRAX were

reassured, and 29% of those receiving a BMD-FRAX were reassured (**Table S10**). Applying a gSOS screening step to these men reduced the sensitivity to 82% while maintaining a similar specificity at 99%. However, the proportion of men requiring a CRF-FRAX reduced to 72% (representing a relative decrease of 28%), and the proportion undergoing BMD-FRAX reduced to 23% (representing a relative decrease of 25%).

2.6 Discussion

By using a polygenic risk score, gSOS, generated from 341,449 individuals, we provide evidence in 5 separate cohorts totaling 10,522 individuals, that genomics-enabled fracture risk screening can reduce the proportion of people that require BMD-based testing by 41%, while maintaining a high overall sensitivity and specificity for correct treatment assignment. While these findings are not meant to be prescriptive, they suggest a role for polygenic risk scores in screening programs that are dependent on heritable risk factors.

Screening programs for fracture risk are expensive, with estimates of approximately $50,000 USD per quality adjusted life year gained,[27] but are less expensive, or even cost-saving using NOGG screening strategies,[28,29] because NOGG decreases the number of individuals who require CRF and/or BMD-FRAX testing. Current guidelines suggest testing a large proportion of the population,[2,3,5] yet most patients are not identified to have a clinically-actionable level of risk.[9,30] This provides an opportunity for genetically-derived measures of risk to increase cost-efficiency in health care systems where investments have been made in genome-wide genotyping. Already at least seven large health care systems have invested in genome-wide genotyping of a large proportion of their population, within whom electronic health record (EHR) data are available.[31,32] Since the costs associated with genome-wide genotyping have now dropped below those of several routine clinical tests, the use of polygenic risk scores may be particularly helpful in these environments since a one-

time genotyping cost could be used to generate several polygenic risk scores. However, there is a clear need to study the translation of such polygenic risk score to clinical applications[33]—and the work presented here provides one example of how such scores could be translated to the clinic.

*Limitations***.** While nearly all FRAX risk factors were available for study, alcohol intake and parental history of fracture were not available from the UK Biobank cohorts and secondary causes of osteoporosis were not uniformly available in SOF, Mr OS US and Mr OS Sweden. Nevertheless, CLSA provided similar results to other cohorts and had all required information. Like participants in most cohort studies, the participants used in these studies are, on average, healthier than the general population.[34] Thus, external validation in a truly population-based study may provide helpful estimates of the performance of genomics-enabled fracture risk screening. This risk score has not been tested outside of individuals of European ancestry, due to lack of data, which underlines the need for large-scale GWAS datasets in individuals of non-European ancestry.[35] We recognize that different approaches could be taken to incorporate polygenic risk scores into fracture risk screening, but here we offer a simple approach that could be readily implemented in a genotyped population with required FRAX risk factors using the NOGG screening strategy, which has supportive evidence from a randomized controlled trial.[9] Further refinement could improve the efficiencies presented here, including a polygenic risk score for BMD when sample sizes are large enough to enable this.

2.7 Conclusion

In summary, we have developed and tested gSOS, a polygenic risk score for SOS, which when introduced into a fracture risk screening program can decrease the number of people requiring CRF- and BMD-FRAX assessments, while still maintaining a high sensitivity and specificity to identify individuals in whom an intervention should be recommended. These findings highlight the role that genetic prediction could play in screening programs that rely upon heritable risk factors.

2.8 Acknowledgements

This research has been conducted using the UK Biobank Resource under project number 24268. We appreciate the generosity of UK Biobank volunteers. Funding sources are listed in the Supplement, but no funders had any influence over the study design, its implementation or interpretation.

2.9 Competing Interests

JAK reports grants from Amgen, Eli Lilly and Radius Health; consulting fees from Theramex. JAK is the architect of FRAX® but has no financial interest. JBR reports investigator-initiated grants from Biogen, Eli Lilly and Glaxo-Smith-Kline, for programs unrelated to the research presented here.

2.10 Tables and Figures

2.10.1 Tables

Table 1. Cohort Characteristics

	Cohorts Used for Model Development			Cohorts Used to Test gSOS-Based Screening				
Participant Characteristics	**UK Biobank Training Set**	**UK Biobank Model Selection Set**		**UK Biobank Test**	**CLSA**	**SOF**	**Mr OS US**	**Mr OS Sweden**
Sample Size	341,449	5,335		4,741	6,704	3,426	4,657	1,880
Number Individuals Eligible for Screening (%)	-	-		2,445 (51.6)	2,931 (43.7)	2,094 (61.1)	2,026 (43.5)	1,026 (54.6)
Age (SD)	56.8 (8.0)	56.6 (8.1)		55.8 (7.6)	62.6 (9.9)	71.5 (5.3)	74 (6)	75.4 (3.2)
Women (%)	186,569 (55)	2,863 (54)		2,489 (52.5)	3,396 (50.7)	3,426 (100)	0 (0)	0 (0)
Smoker (%)	27,181 (8.0)	397 (7.4)		966 (20.4)	581 (8.7)	270 (7.9)	145 (3.1)	178 (9.5)
Previous Fracture (%)	34,917 (10.2)			386 (8.1)	1,032 (15.4)	1,210 (35.3)	1,084 (23.3)	637 (33.9)
Corticosteroids use (%)	3,330 (1.0)	51 (0.8)		79 (1.7)	258 (3.9)	363 (10.6)	98 (2.1)	34 (1.8)
Alcohol User (%)	-	-		-	1189 (17.7)	98 (2.9)	182 (3.9)	52 (2.8)
Fall Within Last 12 Months (%)	69,057 (20.2)	1,052 (20.0)		1,500 (31.6)	699 (10.4)	1,021 (28.4)	984 (21.1)	298 (15.9)
Rheumatoid Arthritis (%)	3,312 (1.0)	41 (0.8)		28 (0.6)	191 (2.9)	252 (7)	226 (4.9)	27 (1.4)
Secondary Osteoporosis (%)	14,541 (4.3)	215 (4.0)		192 (4.1)	313 (4.7)	-	-	-
Parental History of Fracture (%)	-	-		-	820 (12.2)	404 (14.4)	599 (16.8)	164 (8.7)
Baseline Clinical FRAX Score for MOF	5.1 (3.1)	5.0 (3.1)		4.8 (2.7)	8.1 (6.8)	18.7 (9.5)	9.5 (4.7)	11.1 (6.3)
Baseline Clinical FRAX Score for Hip Fracture	0.75 (0.9)	0.73 (0.9)		0.7 (0.8)	2.1 (4)	6.4 (7)	3.8 (3.8)	5.3 (5.5)
Baseline BMD FRAX Score for MOF	-	-		4.9 (2.6)	7.5 (5.8)	17.1 (9.5)	8.1 (4.4)	13.1 (5.6)
Baseline BMD FRAX Score for Hip Fracture	-	-		0.7 (1)	1.5 (3)	5 (6.7)	2.5 (3.3)	7.1 (4.9)
gSOS	-	-0.002 (1.0)		0.043 (0.98)	-0.005 (1)	0 (0.99)	-0.033 (0.98)	-0.708 (0.5)

2.10.2 Figures

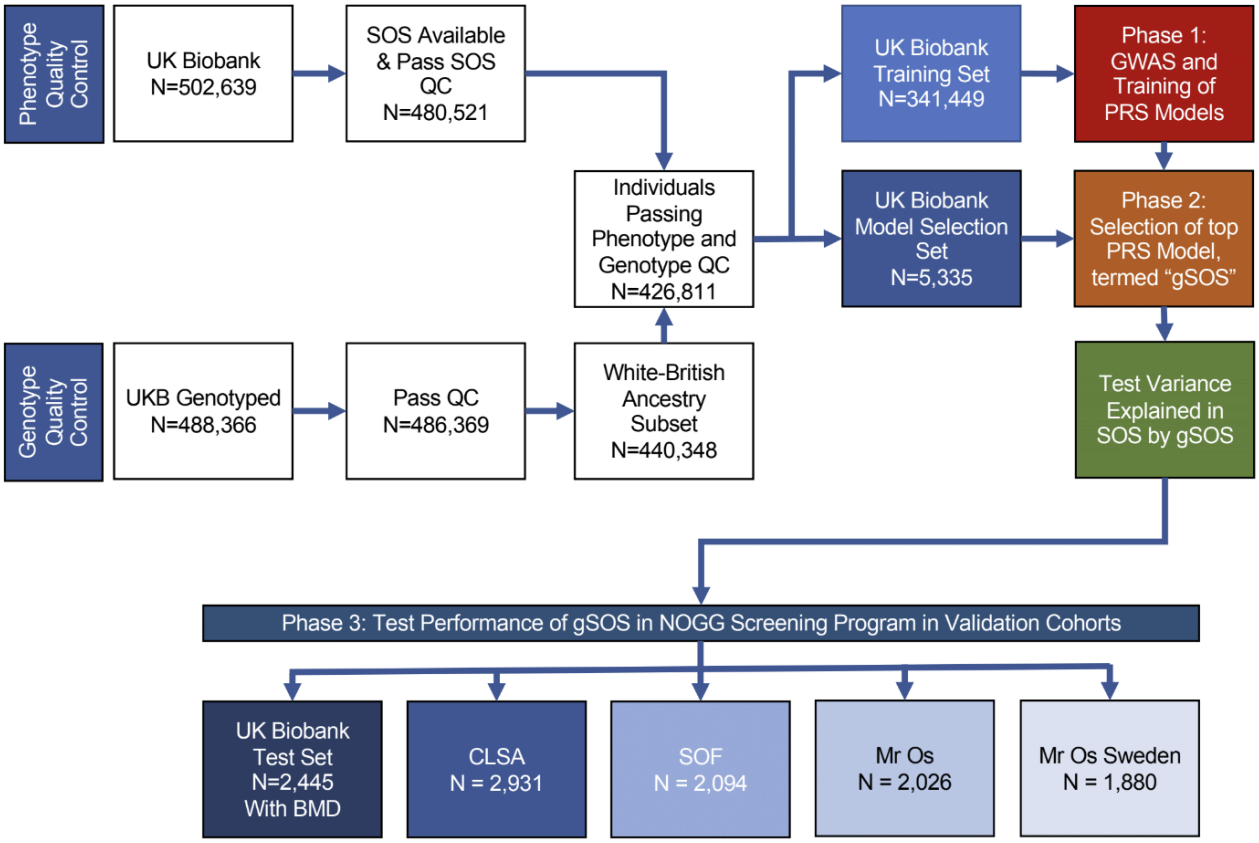

Figure 1. Overall study design.

PRS: Polygenic Risk Score. QC: Quality Control. CLSA: Canadian Longitudinal Study of Aging, SOF: Study of Osteoporosis Fracture.

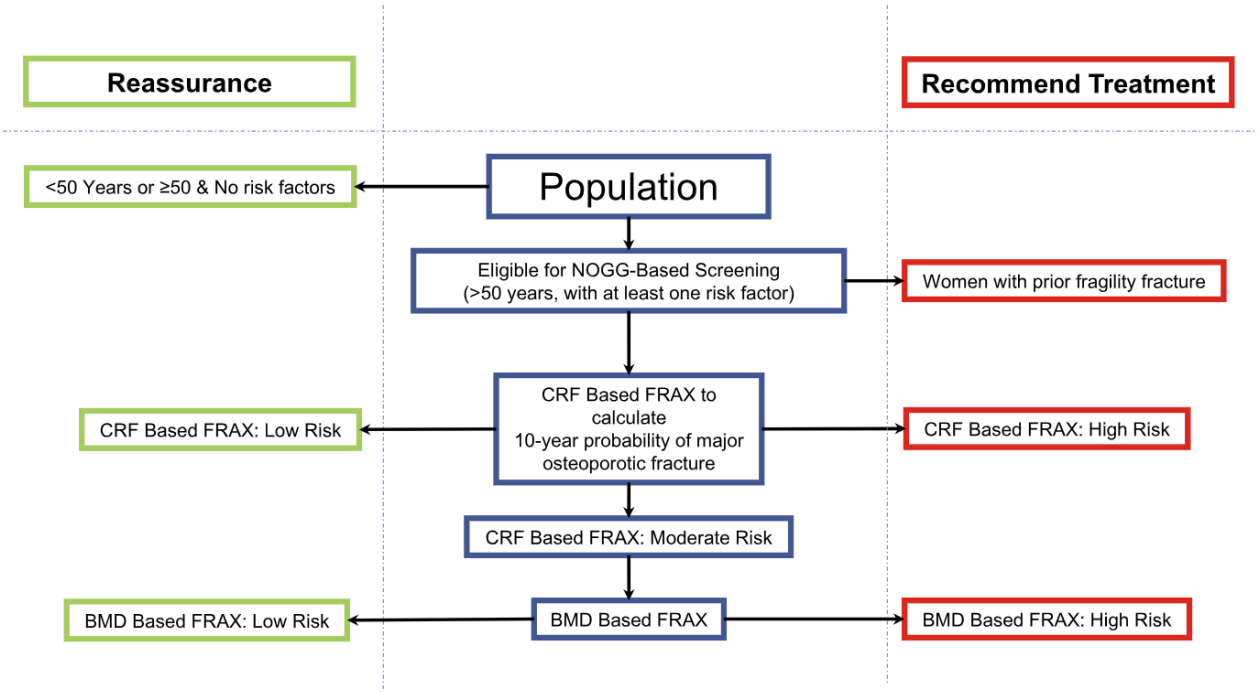

Figure 2. NOGG Screening Strategy.

Both CRF and BMD FRAX generate ten year probabilities of major osteoporotic fracture, which are used to designate risk of fracture.

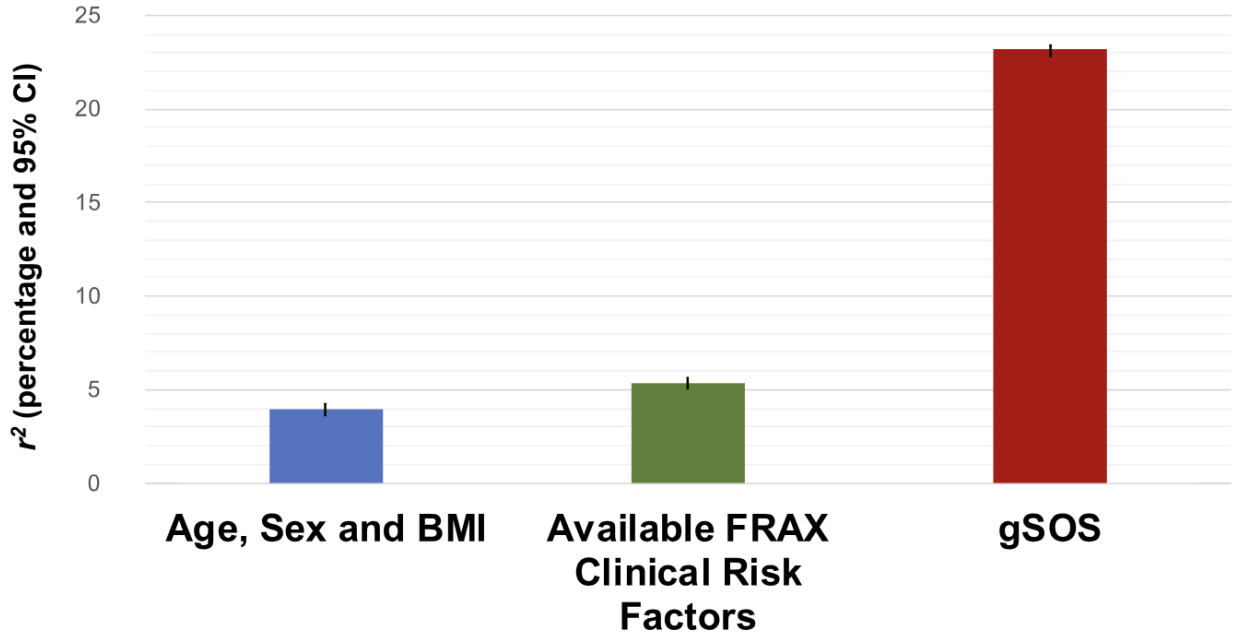

Figure 3. Variance explained in SOS by clinical risk factors and gSOS in the UK Biobank Test Set.

Available FRAX Clinical Risk Factors included: Age, Sex, BMI, Smoking, Previous Fracture, use of Glucocorticoids, Rheumatoid Arthritis and Secondary Osteoporosis. BMI = body mass index. 95% CI = 95% Confidence Intervals.

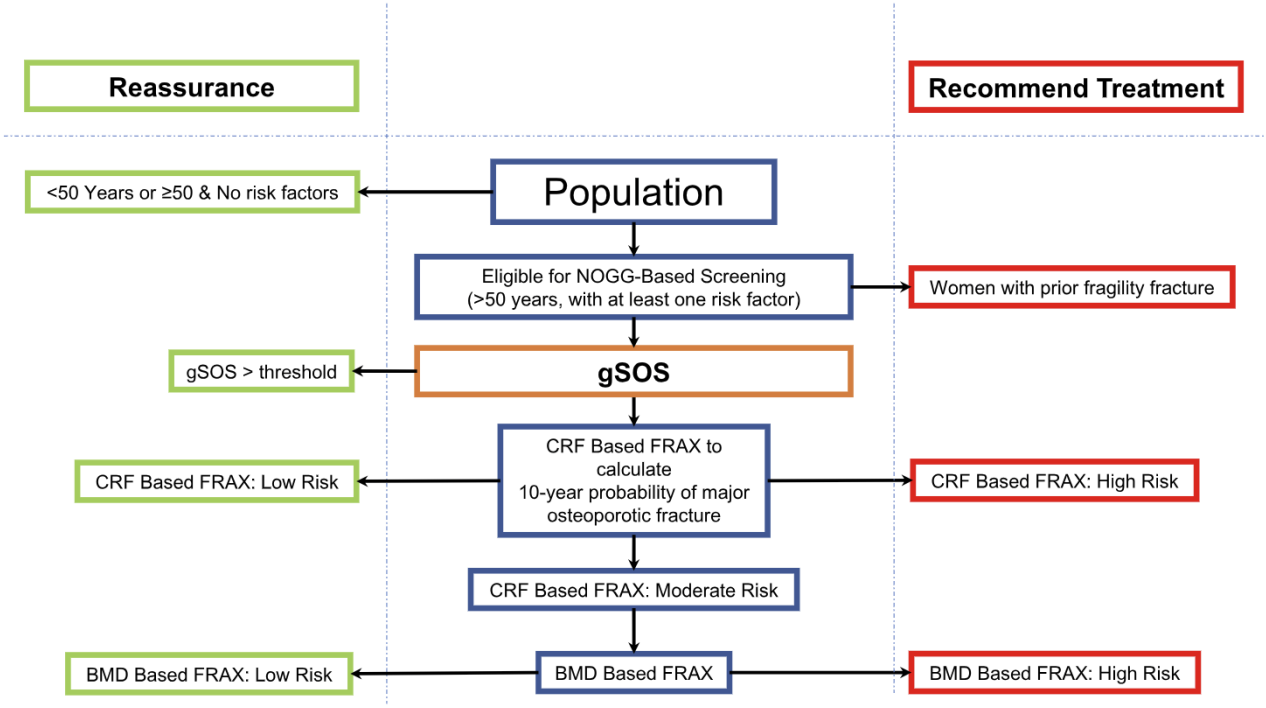

Figure 4. NOGG screening strategy with a gSOS screening step.

Both CRF and BMD FRAX generate ten year probabilities of major osteoporotic fracture, which are used to designate risk of fracture. gSOS is standardized to have a mean of zero and standard deviation of one.

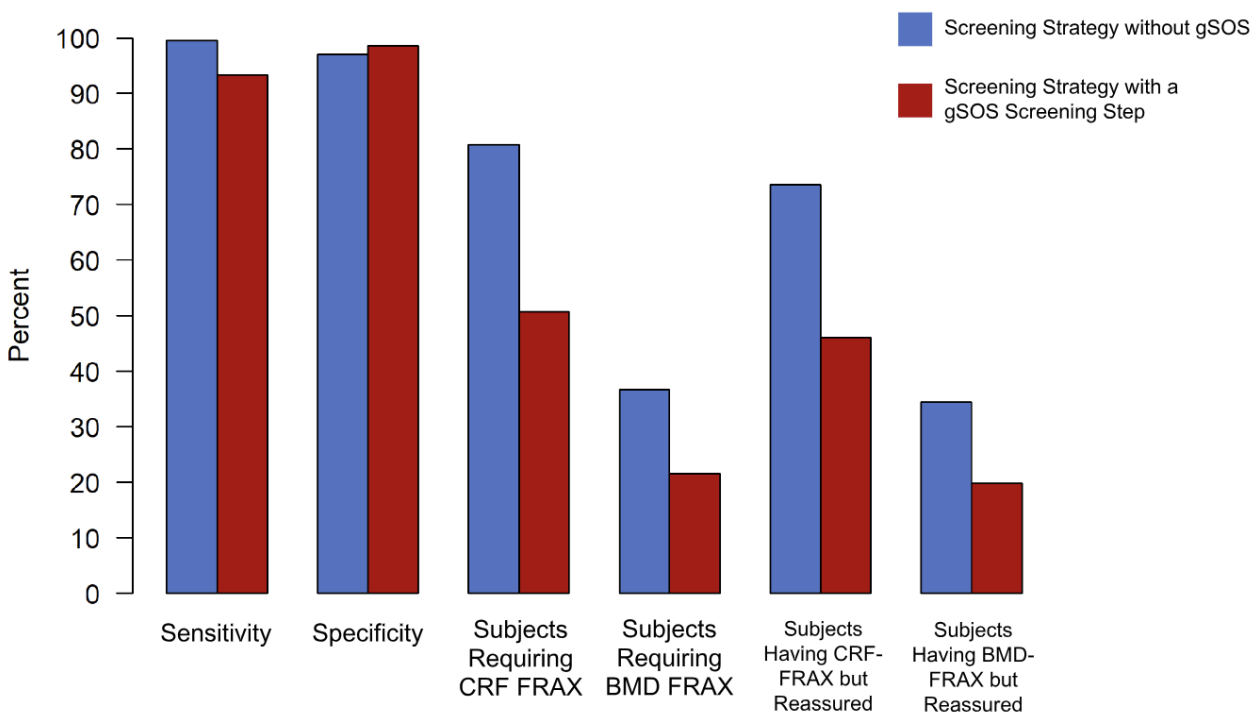

Figure 5. Performance characteristics of screening with and without a gSOS screening step.

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2.12 Supplement

2.12.1 Supplementary information

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Measurement of SOS and DXA-BMD

Full details of SOS measurement in UK Biobank are available here:

https://biobank.ctsu.ox.ac.uk/crystal/docs/Ultrasoundbonedensitometry.pdf

Full details of BMD measurement in UK Biobank are available here:

http://biobank.ctsu.ox.ac.uk/crystal/docs/DXA_explan_doc.pdf

SOS measurement in UK Biobank

Briefly, a Sahara Clinical Bone Sonometer (Hologic Corporation, Bedford, Massachusetts, USA) was used for quantitative ultrasound assessment of calcanei in UK Biobank participants. Details of the complete protocol are publicly available on the UK Biobank website (see above URLs). Participants were initially measured at baseline (N = 487,428) and had their left calcaneus (N = 317,815), right calcaneus (N = 4,102) or both calcanei (N = 165,511) measured. Prior to quality control, ultrasound data were available for 488,366 individuals at either baseline and/or follow-up assessment. To reduce the impact of outlying measurements we first identified subjects that had both heels measured and removed those with highly discrepant (i.e. left vs. right) SOS and/or BUA

measurements. To achieve this, subjects were stratified by sex and bivariate scatter plots comparing left and right heel measures of SOS and BUA were generated separately. Outliers were identified by manual inspection and removed. The same method was used to identify and remove individuals with highly discordant SOS v BUA measured for each heel. Strict quality control was thereafter applied to male and female subjects separately using the following exclusion thresholds: SOS [Male: (≤1,450 and ≥1,750 m/s), Female (≤1,455 and ≥1,700 m/s)] and BUA [Male: (≤27 and ≥138 dB/MHz), Female (≤22 and ≥138 dB/MHz)]. Individuals exceeding the threshold for SOS or BUA or both were removed from the analysis. A unique list of individuals with a valid measure for the left calcaneus (N = 477,380) and/or right (N = 181,953) were identified separately across the three time points. Individuals with a valid right calcaneus measure were included in the final data set when no left measures were available, giving a preliminary working dataset of N=481,100, (left = 475,724 and right = 5,376) unique individuals. Bivariate scatter plots of calcaneal measured were again visually inspected and 579 additional outliers were removed, leaving a total of 480,521 valid QUS measures (264,371 females and 216,150 males). Descriptive statistics of the cohort, after quality control, are detailed in Morris et al.[18]

Femoral Neck BMD Measurement in UKBiobank (see Table S1 for other cohort details)

A GE-Lunar iDXA instrument was used to measure bone mineral density at the femoral neck in UK Biobank participants. Details of the complete protocol are publicly available on the UK Biobank website ("URLs"). We use data fields Data-Field 23299 and 23208, which correspond to left and right 'Femur neck BMD (bone mineral density)', respectively. There were 5,184 individuals with either left, right or both femoral neck BMD measurements, of which 4,834 are within the White British subset. Individuals who were not in the White British subset were excluded. To reduce the impact of outlying measurements in the White British subset, we excluded individuals who had left

and right measurements that had greater than one standard deviation between left and right femoral neck BMD measurements (N=93). The remaining individuals (N=4,741) were assigned the left measurement if only left (N=1) or both (N=4739) were measured and assigned the right measurement if only right was measured (N=1). For all subsequent fracture prediction, femoral neck BMD was transformed to NHANES T-scores standardized to the mean of young women, calibrated to the GE-Lunar machine, using the formula provided by the manufacturer: NHANES T-Score = (femoral neck BMD [g/cm^2]-1.03796) / 0.139.

GWAS for SOS in UK Biobank Test Set

Selection of SNPs and participants for GWAS

SNPs were first filtered for stringent quality control metrics, retaining only SNPs with a minor allele frequency (MAF) > 0.0005 and an imputation quality score (INFO score) >0.3 (**Figure 1**), leaving 13,958,791 SNPs for analysis. Ancestry was determined only for UK Biobank participants with high-quality genotype data (N=486,369). Using flashpca,[36] genotype data comprising 38,539 LD-pruned HapMap3 SNPs (MAF > 0.01, minor allele count > 5, Hardy-Weinberg Equilibrium P-value < 1e^{-6}) were projected onto previously computed principal components using the same SNPs set from 1000 Genomes Phase 3 (N=2,504). Cluster analysis as implemented in by the EMCluster R package was used to extract the UK Biobank individuals that are within the same cluster as the GBR (British in England and Scotland) 1000 Genomes population, resulting in 486,369 participants.

Genome-wide association study (GWAS)

In the training dataset, tests of association were performed between SOS and each SNP, using an additive coding for the number of minor alleles, and with the BOLT-LMM software.[23] Age, sex, assessment centre, genotyping array and the first 20 principal components of ancestry were calculated

from the white British subset and included as covariates in each of these models. These covariates were included to increase the power of the GWAS by reducing the residual error variance.

Non-White British, Population Stratification and Cryptic Relatedness

To identify non-White British we used flashpca,[36] on UK Biobank directly genotyped SNPs comprising 38,539 LD-pruned HapMap3 SNPs (MAF > 0.01, minor allele count > 5, Hardy-Weinberg Equilibrium P-value < 1e-6) which were projected onto previously computed principal components using the same SNPs set from 1000 Genomes Phase 3 (N=2,504). Cluster analysis as implemented in by the EMCluster R package was used to extract the UK Biobank individuals that are within the same cluster as the GBR (British in England and Scotland) 1000 Genomes population.

LASSO Regression and Polygenic Risk Scores Models to Predict SOS

LASSO Regression model:

For 6 P-value thresholds (**Table S2**), we selected all SNPs with P-values smaller than the threshold and used L1-penalized least absolute shrinkage and selection operator (LASSO) regression[16] to predict SOS in the training dataset. LASSO regression controls for model overfitting by introducing a regularization term, λ, that shrinks all estimated parameters towards zero. This machine learning method achieves improved prediction when only a subset of all predictor variables independently contributes to the prediction. Importantly, this subset of predictors need not be genome-wide significant, nor need they be independent of each other. Only SNP data was used to build these LASSO models, and the number of SNPs considered ranged from 642,127 to 104,836. We used the biglasso implementation of LASSO given the size of the dataset,[37] but nevertheless we required use of Amazon cloud computing services and recoding the algorithm to use disk storage rather than memory for interim calculations. Each biglasso model was fit for a series of values of the

regularization parameter, λ. In the model selection dataset, we identified the optimal value of the regularization parameter to minimize root mean square error for each of the 6 SNP sets, and we further identified which P-value threshold gave the best results (**Table S2**). The regularization parameter controls the number of SNPs contributing to the prediction models, and this varied from 40,864 to 6,823 across the 6 SNP sets.

Traditional Polygenic risk score models

Polygenic risk scores were built by calculating weighted sums of the number of eBMD-reducing alleles (0, 1 or 2 for each SNP) carried by each person. The slope estimates from the GWAS regressions in the training dataset were used as the weights.[10] Six different p-value thresholds were used (**Table S2**), and then these SNP sets were filtered to eliminate highly correlated SNPs using GCTA-COJO.[38] This method conditions upon the lead SNP per locus (i.e. per region identified containing significant SNPS) by approximating the genotype-phenotype data with correlation matrices and summary statistics. For a p-value threshold of 5e-3, 44,091 SNPs were used to build the polygenic risk score; in contrast for a p-value threshold of 5e-8, only 1893 SNPs were used. The 6 resulting polygenic risk scores were evaluated in the Model Selection dataset to identify which one gave the smallest root mean squared prediction error.

FRAX Clinical Risk Factors in UK Biobank

To identify smokers in the cohort we use UK Biobank data fields 1239 ("Current Tobacco Smoking", recording smokers as those answering "Yes, on most or all days") and 20116 ("Smoking status", recoding smokers as those marked "Current"). Self-reported rheumatoid arthritis was not recorded, as this is often confused with osteoarthritis.[39]

Glucocorticoid use

Each individual has a list of "Treatment/medication" codes of length greater than or equal to zero. Individuals having at least one of the following codes in their list was assigned a positive flag for glucocorticoid use: '1140868364', '1140874930', '1140874950', '1140874954', '1140888628', '1140874956', '1140874976', '1140883026', '1141157402', '1140874896', '1140884704', '1140910424', '1140910484', '1141157294' and '1141173346'. These codes represent medications 'prednisone', 'prednisolone', 'prednesol 5mg tablet', 'hydrocortistab 20mg tablet', 'hydrocortistab 1% cream', 'hydrocortone 10mg tablet', 'methylprednisolone', 'methylprednisolone+neomycin', 'prednisolone product', 'hydrocortisone', 'cortisone product', 'hc - hydrocortisone', 'cortisol product', 'hydrocortisone product' and 'cortisone' respectively.

Definition of Rheumatoid Arthritis:

ICD10

M06 = Other rheumatoid arthritis

M06.0 = Seronegative rheumatoid arthritis

M06.00 = Seronegative rheumatoid arthritis (multiple sites)

M06.01 = M06.01 Seronegative rheumatoid arthritis (Shoulder region)

M06.02 = Seronegative rheumatoid arthritis (Upper arm)

M06.03 = Seronegative rheumatoid arthritis (Forearm)

M06.04 = Seronegative rheumatoid arthritis (Hand)

M06.05 = Seronegative rheumatoid arthritis (Pelvic region and thigh)

M06.06 = Seronegative rheumatoid arthritis (Lower leg)

M06.07 = Seronegative rheumatoid arthritis (Ankle and foot)

M06.08 = Seronegative rheumatoid arthritis (Other)

M06.09 = Seronegative rheumatoid arthritis (Site unspecified)

M06.8 = Other specified rheumatoid arthritis

M06.80 = Other specified rheumatoid arthritis (Multiple sites)

M06.81 = Other specified rheumatoid arthritis (Shoulder region)

M06.82 = Other specified rheumatoid arthritis (Upper arm)

M06.83 = Other specified rheumatoid arthritis (Forearm)

M06.84 = Other specified rheumatoid arthritis (Hand)

M06.85 = Other specified rheumatoid arthritis (Pelvic region and thigh)

M06.86 = Other specified rheumatoid arthritis (Lower leg)

M06.87 = Other specified rheumatoid arthritis (Ankle and foot)

M06.88 = Other specified rheumatoid arthritis (Other)

M06.89 = Other specified rheumatoid arthritis (Site unspecified)

M06.9 = Rheumatoid arthritis, unspecified

M06.91 = Rheumatoid arthritis, unspecified (Shoulder region)

M06.92 = Rheumatoid arthritis, unspecified (Upper arm)

M06.93 = Rheumatoid arthritis, unspecified (Forearm)

M06.94 = Rheumatoid arthritis, unspecified (Hand)

M06.95 = Rheumatoid arthritis, unspecified (Pelvic region and thigh)

M06.96 = Rheumatoid arthritis, unspecified (Lower leg)

M06.97 = Rheumatoid arthritis, unspecified (Ankle and foot)

M06.98 = Rheumatoid arthritis, unspecified (Other)

M06.99 = Rheumatoid arthritis, unspecified (Site unspecified)

ICD9

714 = Rheumatoid arthritis and other inflammatory polyarthropathies

714.0 = 714.0 Rheumatoid arthritis

714.00 = Rheumatoid arthritis (multiple sites)

714.01 = Rheumatoid arthritis (shoulder region)

714.02 = Rheumatoid arthritis (upper arm)

714.03 = Rheumatoid arthritis (forearm)

714.04 = Rheumatoid arthritis (hand)

714.05 = Rheumatoid arthritis (pelvic region and thigh)

714.06 = Rheumatoid arthritis (lower leg)

714.07 = Rheumatoid arthritis (ankle and foot)

714.08 = Rheumatoid arthritis (other specified site)

714.09 = Rheumatoid arthritis (site unspecified)

714.2 = 714.2 Other r.a. with visceral or systemic involvement

714.21 = Other r.a. with visceral or systemic involvement (shoulder region)

714.22 = Other r.a. with visceral or systemic involvement (upper arm)

714.23 = Other rheumatoid arthritis with visceral or systemic involvement (forearm)

714.24 = Other rheumatoid arthritis with visceral or systemic involvement (hand)

714.25 = Other r.a. with visceral or systemic involvement (pelvic region and thigh)

714.26 = Other r.a. with visceral or systemic involvement (lower leg)

714.27 = Other r.a. with visceral or systemic involvement (ankle and foot)

714.28 = Other r.a. with visceral or systemic involvement (other specified site)

714.29 = Other r.a. with visceral or systemic involvement (unspecified site)

Secondary Causes of Osteoporosis

Individuals reporting type 1 diabetes, menopause prior to age 45, chronic liver disease or osteogenesis imperfecta were recorded as having a secondary cause of osteoporosis.

Type 1 diabetes

Type-1 diabetes was defined as follows: Individuals having a self-reported non-cancer illness code '1222' in data-field 20002 were assigned a positive type-1 diabetes status. Others were assigned a negative status.

Menopause prior to age 45

Women who indicated that their periods had stopped through a touchscreen questionnaire (Data-Field 2724) were asked at what age this occurred (Data-Field 3581). Women who answered "Do not know" or "Prefer not to answer" were assigned a value of zero. Women who provided an age greater than or equal to 45 were assigned a value of 0. Women who provided an age less than 45 were assigned a value of 1. All other individuals in the cohort were assigned a value of zero.

Chronic liver disease

Individuals having one of the following ICD9 codes were assigned a positive chronic liver disease status: 571, 5710, 5711, 5712, 5713, 5714, 5715, 57150, 57151, 57152, 57158, 57159, 5716, 5717, 5718, 5719. These ICD9 codes correspond to 'Chronic liver disease and cirrhosis', 'Alcoholic fatty liver', 'Acute alcoholic hepatitis', 'Alcoholic cirrhosis of liver', 'Alcoholic liver damage, unspecified', 'Chronic hepatitis', 'Cirrhosis of liver without mention of alcohol', 'Cirrhosis of liver without mention of alcohol (congestive)', 'Cirrhosis of liver without mention of alcohol (postnecrotic)', 'Cirrhosis of liver without mention of alcohol (childhood function)', 'Portal fibrosis

without cirrhosis of liver without mention of alcohol', 'Cirrhosis of liver without mention of alcohol (other and unspecified)', 'Biliary cirrhosis', 'Other chronic nonalcoholic liver disease', 'Unspecified chronic liver disease without mention of alcohol', respectively.

Osteogenesis imperfecta

Individuals having one or both of the ICD9 code 75650 and ICD10 code Q780 were assigned a positive osteogenesis imperfecta status. The codes correspond to 'Osteodystrophies (osteogenesis imperfecta)' and 'Osteogenesis imperfecta', respectively. All other individuals in the cohort were assigned a negative osteogenesis imperfecta status.

2.12.2 Supplementary tables

Table S1. Study cohorts.

Short name	Full name	Study design	Study Type	Country of Origin	Ethnicity	BMD Assessment method (Lunar, Hologic)	Short Study Description	Genome-Wide Genotyping & Imputation	References
UKB Training	UK Biobank Training Set	Cohort	General population	Britain	Mixed, but predominantly White British	GE-Lunar iDXA	UK Biobank is a large-scale health resource that follows 502,628 volunteer participants in the United Kingdom. UK Biobank has ethical approval from the Northwest Multi-centre Research Ethics Committee, and informed consent was obtained from all participants.	Affymetrix UK Biobank array, followed by genotype imputation to the Haplotype Reference Consortium.	UK Biobank: An Open Access Resource for Identifying the Causes of a Wide Range of Complex Diseases of Middle and Old Age. PLoS Med. 12, e1001779 (2015).
UKB Model Selection	UK Biobank Model Selection Set	Cohort	General population	Britain	Mixed, but predominantly White British	GE-Lunar iDXA	UK Biobank is a large-scale health resource that follows 502,628 volunteer participants in the United Kingdom. Participants within the UK Biobank have been genome-wide genotyped using Affymetrix arrays, followed by genotype imputation to the Haplotype Reference Consortium. UK Biobank has ethical approval from the Northwest Multi-centre Research Ethics Committee, and informed consent was obtained from all participants.	Affymetrix UK Biobank array, followed by genotype imputation to the Haplotype Reference Consortium.	UK Biobank: An Open Access Resource for Identifying the Causes of a Wide Range of Complex Diseases of Middle and Old Age. PLoS Med. 12, e1001779 (2015).
UKB Test	UK Biobank Test Set	Cohort	General population	Britain	Mixed, but predominantly White British	GE-Lunar iDXA	UK Biobank is a large-scale health resource that follows 502,628 volunteer participants in the United Kingdom. Participants within the UK Biobank have been genome-wide genotyped using Affymetrix arrays, followed by genotype imputation to the Haplotype Reference Consortium. UK Biobank has ethical approval from the Northwest Multi-centre Research Ethics Committee, and informed consent was obtained from all participants.	Affymetrix UK Biobank array, followed by genotype imputation to the Haplotype Reference Consortium.	UK Biobank: An Open Access Resource for Identifying the Causes of a Wide Range of Complex Diseases of Middle and Old Age. PLoS Med. 12, e1001779 (2015).
CLSA	Canadian Longitudinal Study of Aging	Cohort	General population	Canada	Mixed, but predominantly European	Hologic QDR	The Canadian Longitudinal Study of Aging (CLSA) is a cohort consisting of a comprehensive cohort with DNA samples available and a tracking cohort without biological samples. The Tracking cohort of 21,241 participants who are interviewed by telephone and the Comprehensive cohort of 30,097 participants who are interviewed in person and provide blood and urine samples. For this study, only participants with available genome-wide genotyping and BMD measures were included. Genome-wide genotyping is on-going for the entire comprehensive cohort.	Affymetrix Biobank array, followed by genotype imputation to the Haplotype Reference Consortium.	The Canadian longitudinal study on aging (CLSA). Can. J. Aging 28, 221–229 (2009).
MrOS US	Osteoporotic Fractures in Men USA	Cohort	General population	United States	Non-Hispanic white	Hologic QDR	The Osteoporotic Fractures in Men (MrOS) Study enrolled 5,994 participants in 2000¬ to 2002 at six clinical centers in the U.S. Eligible participants were community-dwelling men who were at least 65 years of age, able to walk without assistance from another person, and had not had bilateral hip replacements. Written informed consent was obtained from all participants, and the Institutional Review Board at each study site approved the study.	Genome-wide genotyped on Illumina HumanOmni1-Quad, followed by genotype imputation to the Haplotype Reference Consortium.	Orwoll E, Blank JB, Barrett-Connor E, et al. Design and baseline characteristics of the osteoporotic fractures in men (MrOS) study--a large observational study of the determinants of fracture in older men. Contemporary clinical trials. Oct 2005;26(5):569-585. Blank JB, Cawthon PM, Carrion-Petersen ML, Harper L, Johnson JP, Mitson E, Delay RR 2005 Overview of recruitment for the osteoporotic fractures in men study (MrOS). Contemp Clin Trials 26:557–568
SOF	Study of Osteoporotic Fractures	Cohort	General population	United States	Non-Hispanic white	Hologic QDR	The Study of Osteoporotic Fractures (SOF) is a prospective multicenter study. The cohort originally comprised 9704 community dwelling women recruited from population-based listings in four U.S. areas. Inclusion criteria were 1) 65 years of age or older, (2) ability to walk without the assistance of	Genome-wide genotyped on Illumina HumanOmni1-Quad, followed by genotype imputation to the Haplotype Reference Consortium.	Cummings SR, Nevitt MC, Browner WS, et al. Risk factors for hip fracture in white women. Study of Osteoporotic Fractures Research Group. The New England journal of medicine. Mar 23

							another, (3) absence of bilateral hip replacements, (4) ability to provide self-reported data, (5) residence near a clinical site for the duration of the study, (6) absence of a medical condition that (in the judgment of the investigator) would result in imminent death, and (7) ability to understand and sign an informed consent. Written informed consent was provided, and the IRB at each enrollment site approved the study.		1995;332(12):767-773.Steiger P, Cummings SR, Black DM, Spencer NE, Genant HK. Age-related decrements in bone mineral density in women over 65. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. Jun 1992;7(6):625-632.Ensrud KE, Palermo L, Black DM, et al. Hip and calcaneal bone loss increase with advancing age: longitudinal results from the study of osteoporotic fractures. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. Nov 1995;10(11):1778-1787.
Mr Os Sweden	Study of Osteoporotic Fractures Sweden	Cohort	General population	Sweden	Swedish	Gothenburg subset: Hologic QDR Malmö subset: Lunar Prodigy DXA	The Osteoporotic Fractures in Men (MrOS) study is a multicenter, prospective study including older men in Sweden, Hong Kong and the United States. The MrOS Sweden study (n=3014) consists of three sub-cohorts from three different Swedish cities (n=1005 in Malmö, n=1010 in Gothenburg, and n=999 in Uppsala). Study subjects (men aged 69 to 81 years) were randomly identified using national population registers. A total of 45% of the subjects who were contacted participated in the study. To be eligible for the study, the subjects had to be able to walk without assistance, provide self-reported data, and sign an informed consent. The study was approved by the ethics committees at the Universities of Gothenburg, Lund, and Uppsala. Informed consent was obtained from all study participants.	Gothenburg part: Genotyped with Illumina HumanOmni1_Quad_v1-0 B array, followed by genotype imputation to the Haplotype Reference Consortium. Malmö part: Genotyped with HumanOmniExpress-12v1_B build 36, followed by genotype imputation to the Haplotype Reference Consortium.	PMID: 16598372 Mellström D, Johnell O, Ljunggren O, Eriksson AL, Lorentzon M, Mallmin H, Holmberg A, Redlund-Johnell I, Orwoll E, Ohlsson C 2006 Free testosterone is an independent predictor of BMD and prevalent fractures in elderly men: MrOS Sweden. J Bone Miner Res 21:529-535.

Table S2. Variance explained in SOS by clinical risk factors, polygenic risk scores and LASSO-based polygenic risk scores.

		UK Biobank Test Set			
	Risk Factors	**Sample Size**	**r2**	**Lower 95% CI**	**Upper 95% CI**
Clinical Risk Factors	Age, sex, BMI	80,027	3.97%	3.71%	4.24%
	All Available FRAX Clinical Risk Factors	80,027	4.51%	4.23%	4.79%

				UK Biobank Model Selection Set			
		GWAS P-value Cut	**# Clumped SNPs**	**Nindv**	**r2**	**Lower 95% CI**	**Upper 95% CI**
Simple Polygenic Risk Scores	SNPs Clumped	5.00E-03	44,091	5,335	15.30%	13.60%	17.16%
		5.00E-04	13,267	5,335	17.90%	16.05%	19.78%
		5.00E-05	6,045	5,335	18.00%	16.15%	19.88%
		5.00E-06	3,695	5,335	18.50%	16.62%	20.38%
		5.00E-07	2,566	5,335	17.90%	16.04%	19.76%
		5.00E-08	1,893	5,335	17.60%	15.80%	19.51%
	SNPs Not Clumped	5.00E-06	166,576	5,335	6.40%	5.18%	7.72%
		5.00E-07	130,130	5,335	6.00%	4.82%	7.29%
		5.00E-08	104,836	5,335	5.60%	4.46%	6.85%

			UK Biobank Training Set			UK Biobank Model Selection Set					UK Biobank Test Set				
	GWAS P cutoff	**Nsnp**	**Data set split**	**Nindv**	**Nλ**	**Nasnp†**	**Nindv**	**λminRMSE**	**r2***	**Lower 95% CI**	**Upper 95% CI**	**Nindv**	**λminRMSE**	**r2***	**Lower 95% CI**	**Upper 95% CI**
LASSO-Regression Approach	5.00E-03	642,127	80:1.25:18.75	341,449	45	40,864	5335	0.0032	24.53%	22.54%	26.55%					
	5.00E-04	345,111	80:1.25:18.75	341,449	45	21,717	5335	0.0028	24.99%	22.99%	27.02%	80,027	0.0028	23.18%	22.66%	23.69%
	5.00E-05	227,478	80:1.25:18.75	341,449	100	13,793	5335	0.0021	24.89%	22.89%	26.91%					
	5.00E-06	166,576	80:1.25:18.75	341,449	100	10,348	5335	0.0016	24.75%	22.75%	26.77%					
	5.00E-07	130,130	80:1.25:18.75	341,449	100	7,999	5335	0.0016	23.86%	21.88%	25.87%					
	5.00E-08	104,836	80:1.25:18.75	341,449	100	6,823	5335	0.0013	23.38%	21.41%	25.38%					

*r2 at the λ that achieved the minimum root mean-squared error (λmin).

†Nasnp: Activated SNPs, that is those with training beta not equal to 0 at λmin.

Only individuals in the White British Subset were used for this analysis.

Data set split refers to the percentage of individuals in each dataset: Model Training, Model Selection and Model Testing

Table S3. Performance of NOGG Guidelines with and without gSOS screening step in all validation cohorts.

(Validation cohorts included: UK Biobank Test set, CLSA, SOF, Mr Os, Mr Os Sweden)

Without gSOS Screening Step							
			BMD Frax				
			Treat	Not Treat			
	NOGG	Treat	2553	227			
		Not Treat	11	7736			
					Total	**10,527**	
					Sensitivity	99.6%	
					Specificity	97.1%	
					Percent Requiring CRF FRAX Tests	80.8%	
					Percent Requiring BMD FRAX Tests	36.7%	
					Percent of Subjects Receiving CRF FRAX Test But Reassured	73.6%	
					Percent of Subjects Receiving BMD FRAX Tests But Reassured	34.4%	
With gSOS Screening Step							
			BMD Frax				
			Treat	Not Treat			
	NOGG	Treat	2390	116			
		Not Treat	170	7846			
							Decrease by
					Total	**10,522**	
					Sensitivity	93.4%	
					Specificity	98.5%	
					Percent Requiring CRF FRAX Tests	50.8%	37%
					Percent Requiring BMD FRAX Tests	21.6%	41%
					Percent of Subjects Receiving CRF FRAX Test But Reassured	46.1%	37%
					Percent of Subjects Receiving BMD FRAX Tests But Reassured	19.9%	42%

Table S4. Performance of NOGG guidelines with and without gSOS screening step in men and women from the UK Biobank Test Set.

Without gSOS Screening Step						
		BMD Frax				
		Treat	Not Treat			
NOGG	Treat	217	9			
	Not Treat	3	2218			
				Total	**2,447**	
				Sensitivity	99%	
				Specificity	100%	
				Percent Requiring CRF FRAX Tests	93%	
				Percent Requiring BMD FRAX Tests	41%	
				Percent of Subjects Receiving CRF FRAX Test But Reassured	91%	
				Percent of Subjects Receiving BMD FRAX Tests But Reassured	40%	
With gSOS Screening Step						
		BMD Frax				
		Treat	Not Treat			
NOGG	Treat	208	2			
	Not Treat	11	2224			
						Decrease by
				Total	**2,445**	
				Sensitivity	95%	
				Specificity	100%	
				Percent Requiring CRF FRAX Tests	53%	43%
				Percent Requiring BMD FRAX Tests	20%	51%
				Percent of Subjects Receiving CRF FRAX Test But Reassured	51%	43%
				Percent of Subjects Receiving BMD FRAX Tests But Reassured	19%	52%

Table S5. Performance of NOGG guidelines with and without gSOS screening step in men and women from the CLSA cohort.

Without gSOS Screening Step						
		BMD Frax				
		Treat	Not Treat			
NOGG	Treat	833	55			
	Not Treat	2	2042			
				Total	**2932**	
				Sensitivity	99.8%	
				Specificity	97.4%	
				Percent Requiring CRF FRAX Tests	78.5%	
				Percent Requiring BMD FRAX Tests	35.0%	
				Percent of Subjects Receiving CRF FRAX Test But Reassured	69.7%	
				Percent of Subjects Receiving BMD FRAX Tests But Reassured	31.7%	
With gSOS Screening Step						
		BMD Frax				
		Treat	Not Treat			
NOGG	Treat	768	25			
	Not Treat	66	2072			
						Decrease by
				Total	**2931**	
				Sensitivity	92.1%	
				Specificity	98.8%	
				Percent Requiring CRF FRAX Tests	50.3%	36%
				Percent Requiring BMD FRAX Tests	20.9%	40%
				Percent of Subjects Receiving CRF FRAX Test But Reassured	44.7%	36%
				Percent of Subjects Receiving BMD FRAX Tests But Reassured	18.5%	42%

Table S6. Performance of NOGG guidelines with and without gSOS screening step in the SOF cohort.

Without gSOS Screening Step						
		BMD Frax				
		Treat	Not Treat			
NOGG	Treat	1362	79			
	Not Treat	1	653			
				Total	**2,095**	
				Sensitivity	100%	
				Specificity	89%	
				Percent Requiring CRF FRAX Tests	42%	
				Percent Requiring BMD FRAX Tests	30%	
				Percent of Subjects Receiving CRF FRAX Test But Reassured	31%	
				Percent of Subjects Receiving BMD FRAX Tests But Reassured	27%	
				*one was added to the false negative cell, because it was zero		
With gSOS Screening Step						
		BMD Frax				
		Treat	Not Treat			
NOGG	Treat	1297	30			
	Not Treat	65	702			
						Decrease by
				Total	**2,094**	
				Sensitivity	95%	
				Specificity	96%	
				Percent Requiring CRF FRAX Tests	19%	56%
				Percent Requiring BMD FRAX Tests	13%	56%
				Percent of Subjects Receiving CRF FRAX Test But Reassured	13%	58%
				Percent of Subjects Receiving BMD FRAX Tests But Reassured	11%	59%

Table S7. Performance of NOGG guidelines with and without gSOS screening step in the Mr Os US cohort.

Without gSOS Screening Step						
		BMD Frax				
		Treat	Not Treat			
NOGG	Treat	76	58			
	Not Treat	1	1892			
				Total	**2,027**	
				Sensitivity	99%	
				Specificity	97%	
				Percent Requiring CRF FRAX Tests	100%	
				Percent Requiring BMD FRAX Tests	36%	
				Percent of Subjects Receiving CRF FRAX Test But Reassured	93%	
				Percent of Subjects Receiving BMD FRAX Tests But Reassured	35%	
				*one was added to the false negative cell, because it was zero		
With gSOS Screening Step						
		BMD Frax				
		Treat	Not Treat			
NOGG	Treat	66	43			
	Not Treat	10	1907			
						Decrease by
				Total	**2,026**	
				Sensitivity	87%	
				Specificity	98%	
				Percent Requiring CRF FRAX Tests	73%	27%
				Percent Requiring BMD FRAX Tests	28%	24%
				Percent of Subjects Receiving CRF FRAX Test But Reassured	68%	28%
				Percent of Subjects Receiving BMD FRAX Tests But Reassured	26%	25%

Table S8. Performance of NOGG guidelines with and without gSOS screening step in the Mr Os Sweden cohort.

Without gSOS Screening Step						
		BMD Frax				
		Treat	Not Treat			
NOGG	Treat	65	26			
	Not Treat	4	931			
				Total	**1026**	
				Sensitivity	94%	
				Specificity	97%	
				Percent Requiring CRF FRAX Tests	100%	
				Percent Requiring BMD FRAX Tests	45%	
				Percent of Subjects Receiving CRF FRAX Test But Reassured	91%	
				Percent of Subjects Receiving BMD FRAX Tests But Reassured	43%	
With gSOS Screening Step						
		BMD Frax				
		Treat	Not Treat			
NOGG	Treat	51	16			
	Not Treat	18	941			
						Decrease by
				Total	**1026**	
				Sensitivity	74%	
				Specificity	98%	
				Percent Requiring CRF FRAX Tests	70%	30%
				Percent Requiring BMD FRAX Tests	33%	27%
				Percent of Subjects Receiving CRF FRAX Test But Reassured	63%	31%
				Percent of Subjects Receiving BMD FRAX Tests But Reassured	31%	27%

Table S9. Performance of NOGG guidelines with and without gSOS screening step in women from validation cohorts.

(Replication cohorts included: UK Biobank Test set, CLSA & SOF)							
Without gSOS Screening Step							
			BMD Frax				
			Treat	Not Treat			
	NOGG	Treat	2300	128			
		Not Treat	3	2428			
					Total	**4,859**	
					Sensitivity	99.9%	
					Specificity	95.0%	
					Percent Requiring CRF FRAX Tests	58.4%	
					Percent Requiring BMD FRAX Tests	43.4%	
					Percent of Subjects Receiving CRF FRAX Test But Reassured	49.9%	
					Percent of Subjects Receiving BMD FRAX Tests But Reassured	40.9%	
With gSOS Screening Step							
			BMD Frax				
			Treat	Not Treat			
	NOGG	Treat	2176	47			
		Not Treat	124	2509			
							Decrease by
					Total	**4,856**	
					Sensitivity	94.6%	
					Specificity	98.2%	
					Percent Requiring CRF FRAX Tests	26.5%	55%
					Percent Requiring BMD FRAX Tests	19.7%	55%
					Percent of Subjects Receiving CRF FRAX Test But Reassured	22.3%	55%
					Percent of Subjects Receiving BMD FRAX Tests But Reassured	18.1%	56%

Table S10. Performance of NOGG guidelines with and without gSOS screening step in men from all cohorts.

(Validation cohorts included: UK Biobank Test set, CLSA, Mr OS US, Mr OS Sweden							
Without gSOS Screening Step							
			BMD Frax				
			Treat	Not Treat			
	NOGG	Treat	253	99			
		Not Treat	8	5308			
					Total	**5,668**	
					Sensitivity	96.9%	
					Specificity	98.2%	
					Percent Requiring CRF FRAX Tests	100%	
					Percent Requiring BMD FRAX Tests	31%	
					Percent of Subjects Receiving CRF FRAX Test But Reassured	94%	
					Percent of Subjects Receiving BMD FRAX Tests But Reassured	29%	
With gSOS Screening Step							
			BMD Frax				
			Treat	Not Treat			
	NOGG	Treat	214	69			
		Not Treat	46	5337			
							Decrease by
					Total	**5,666**	
					Sensitivity	82%	
					Specificity	99%	
					Percent Requiring CRF FRAX Tests	72%	28%
					Percent Requiring BMD FRAX Tests	23%	25%
					Percent of Subjects Receiving CRF FRAX Test But Reassured	67%	29%
					Percent of Subjects Receiving BMD FRAX Tests But Reassured	21%	26%

Table S11. Performance of NOGG guidelines with and without gSOS screening step in women from the UK Biobank Test Set.

Without gSOS Screening Step						
		BMD Frax				
		Treat	Not Treat			
NOGG	Treat	205	8			
	Not Treat	1	1055			
				Total	**1,269**	
				Sensitivity	100%	
				Specificity	99%	
				Percent Requiring CRF FRAX Tests	86%	
				Percent Requiring BMD FRAX Tests	69%	
				Percent of Subjects Receiving CRF FRAX Test But Reassured	83%	
				Percent of Subjects Receiving BMD FRAX Tests But Reassured	67%	
				*note that one was added to the false negative cell		
With gSOS	**Screening Step**					
		BMD Frax				
		Treat	Not Treat			
NOGG	Treat	197	2			
	Not Treat	8	1061			
						Decrease by
				Total	**1,268**	
				Sensitivity	96%	
				Specificity	100%	
				Percent Requiring CRF FRAX Tests	38%	55%
				Percent Requiring BMD FRAX Tests	31%	56%
				Percent of Subjects Receiving CRF FRAX Test But Reassured	37%	56%
				Percent of Subjects Receiving BMD FRAX Tests But Reassured	30%	56%

Table S12. Performance of NOGG guidelines with and without gSOS screening step in women from the CLSA cohort

Without gSOS Screening Step						
		BMD Frax				
		Treat	Not Treat			
NOGG	Treat	733	41			
	Not Treat	1	720			
				Total	**1,495**	
				Sensitivity	100%	
				Specificity	95%	
				Percent Requiring CRF FRAX Tests	58%	
				Percent Requiring BMD FRAX Tests	41%	
				Percent of Subjects Receiving CRF FRAX Test But Reassured	48%	
				Percent of Subjects Receiving BMD FRAX Tests But Reassured	38%	
				*note that one was added to the false negative cell		
With gSOS	**Screening Step**					
		BMD Frax				
		Treat	Not Treat			
NOGG	Treat	682	15			
	Not Treat	51	746			
						Decrease by
				Total	**1,494**	
				Sensitivity	93%	
				Specificity	98%	
				Percent Requiring CRF FRAX Tests	28%	52%
				Percent Requiring BMD FRAX Tests	20%	52%
				Percent of Subjects Receiving CRF FRAX Test But Reassured	23%	52%
				Percent of Subjects Receiving BMD FRAX Tests But Reassured	18%	52%

Table S13. Performance of NOGG guidelines with and without gSOS screening step in men from the UK Biobank Test Set

Without gSOS Screening Step						
		BMD Frax				
		Treat	Not Treat			
NOGG	Treat	12	1			
	Not Treat	2	1163			
				Total	**1,178**	
				Sensitivity	86%	
				Specificity	100%	
				Percent Requiring CRF FRAX Tests	100%	
				Percent Requiring BMD FRAX Tests	12%	
				Percent of Subjects Receiving CRF FRAX Test But Reassured	99%	
				Percent of Subjects Receiving BMD FRAX Tests But Reassured	11%	
				*note that 1 was added to the false positive cell		
With gSOS Screening Step						
		BMD Frax				
		Treat	Not Treat			
NOGG	Treat	11	0			
	Not Treat	3	1163			
						Decrease by
				Total	**1,177**	
				Sensitivity	79%	
				Specificity	100%	
				Percent Requiring CRF FRAX Tests	68%	32%
				Percent Requiring BMD FRAX Tests	9%	27%
				Percent of Subjects Receiving CRF FRAX Test But Reassured	67%	32%
				Percent of Subjects Receiving BMD FRAX Tests But Reassured	8%	29%

Table S14. Performance of NOGG guidelines with and without gSOS screening step in men from the CLSA cohort

Without gSOS Screening Step						
		BMD Frax				
		Treat	Not Treat			
NOGG	Treat	100	14			
	Not Treat	1	1322			
				Total	**1437**	
				Sensitivity	99.0%	
				Specificity	99.0%	
				Percent Requiring CRF FRAX Tests	100.0%	
				Percent Requiring BMD FRAX Tests	28.7%	
				Percent of Subjects Receiving CRF FRAX Test But Reassured	92.1%	
				Percent of Subjects Receiving BMD FRAX Tests But Reassured	24.8%	
With gSOS Screening Step						
		BMD Frax				
		Treat	Not Treat			
NOGG	Treat	86	10			
	Not Treat	15	1326			
						Decrease by
				Total	**1437**	
				Sensitivity	85.1%	
				Specificity	99.3%	
				Percent Requiring CRF FRAX Tests	73.8%	26%
				Percent Requiring BMD FRAX Tests	22.1%	23%
				Percent of Subjects Receiving CRF FRAX Test But Reassured	67%	27%
				Percent of Subjects Receiving BMD FRAX Tests But Reassured	19%	25%

2.12.3 Supplementary figures

Figure S1-A. Manhattan plot from GWAS of SOS

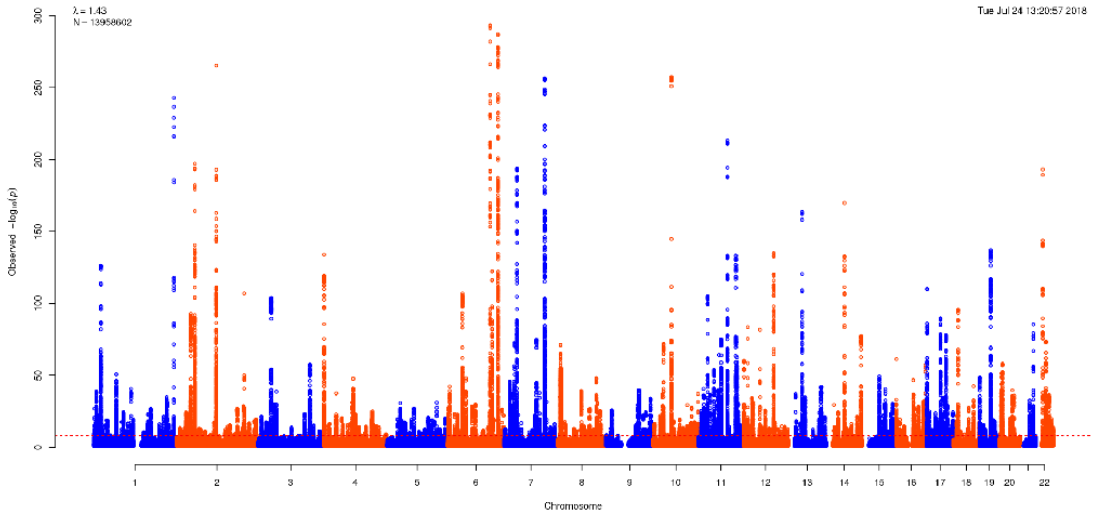


Figure S1-B. QQ plot from GWAS of SOS

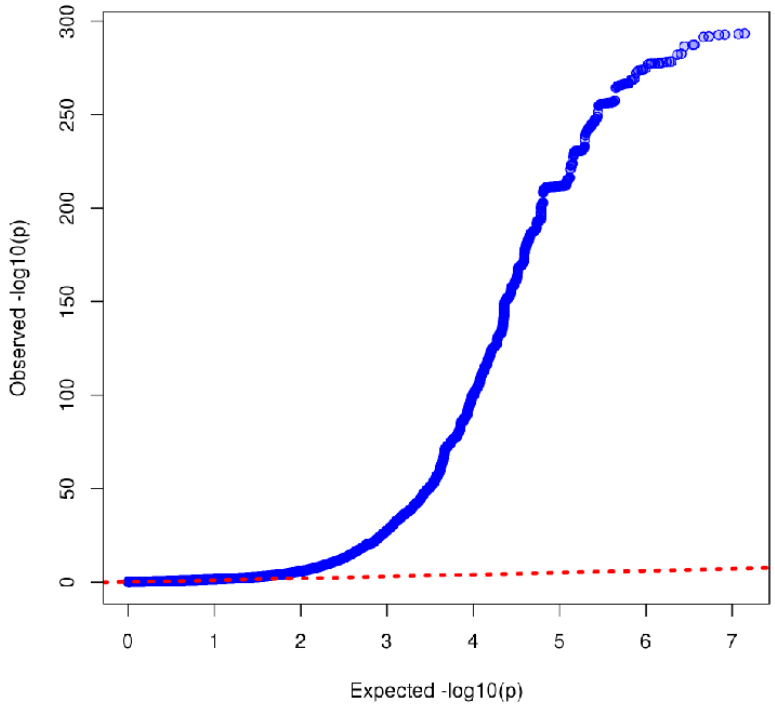


Figure S2. Performance of each SNP set using LASSO regression in the model selection set

Each feature set consists of a set of SNPs associated with SOS at a specified p-value threshold (sub-panel titles). For each feature set, we fit a regularized model to the training set over a range of regularization constants (λ) (top-left), with each λ resulting in a variable subset of activated features (bottom-left). The model with the minimal RMSE in the model selection set (top-right) was selected to compare the variance explained (r^2, bottom right) among all feature sets.

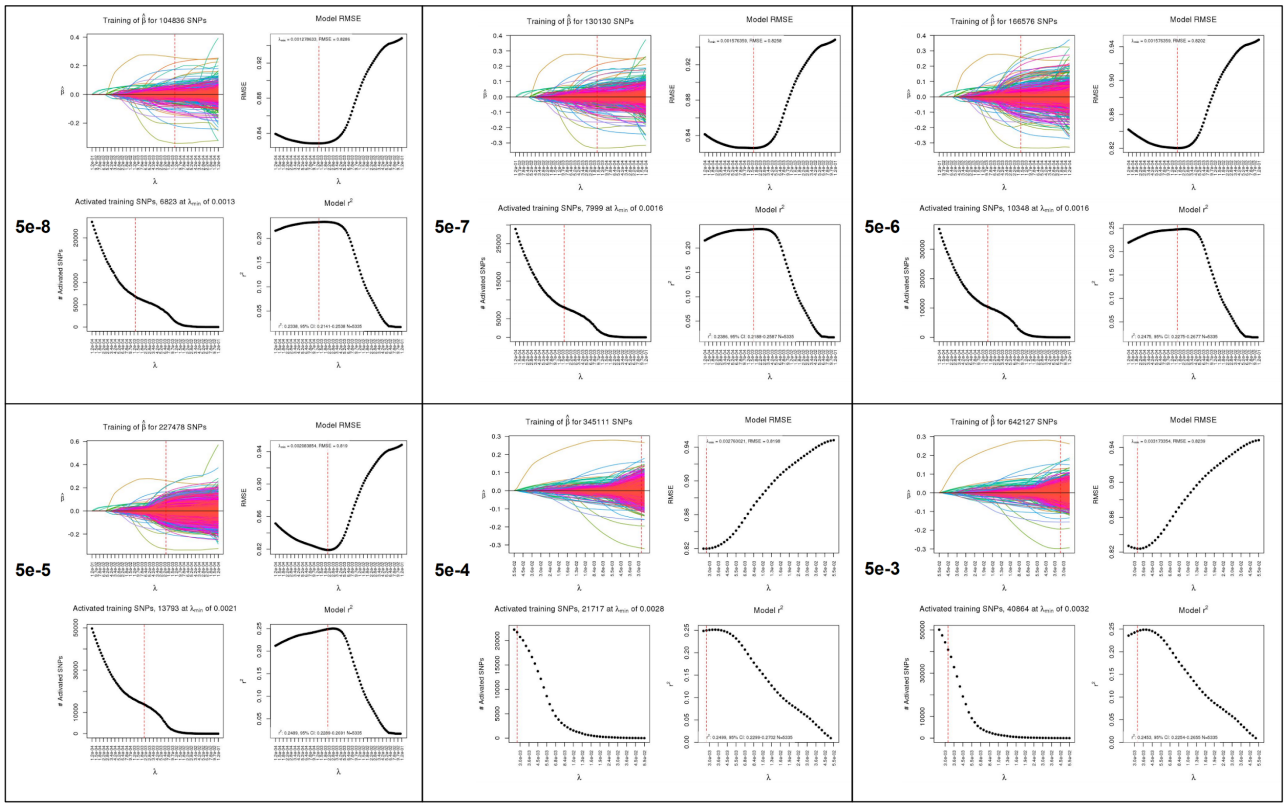
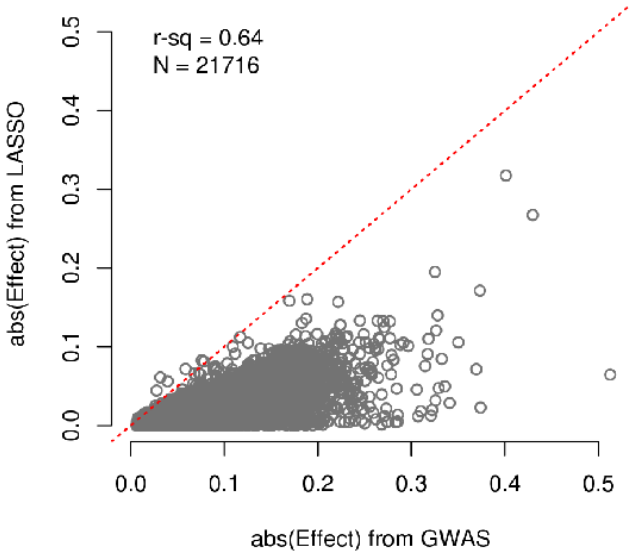


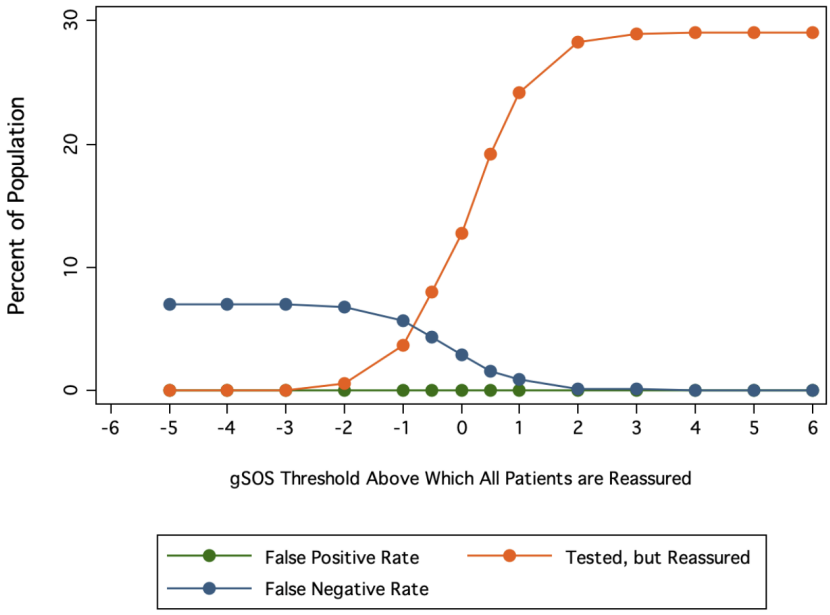
Figure S3. Correlation of betas from GWAS and betas from gSOS for activated SNPs



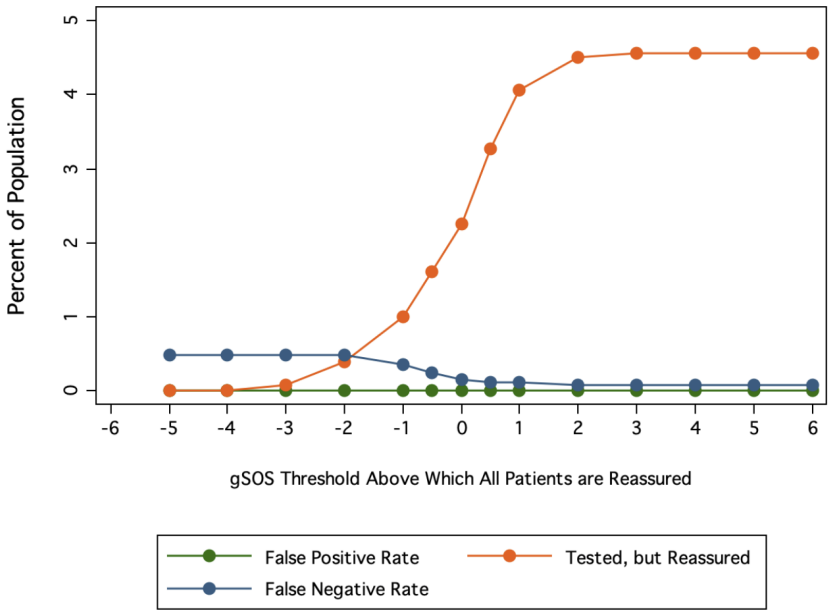
Activated SNPs are those SNPs chosen by the machine learning algorithm to be in gSOS, the final selected model

Figure S4. Effects of gSOS threshold on treatment assignment

Women



Men



Chapter 3

Preface: Bridge Between Chapter 2 and Chapter 3

Several published observational and experimental studies in humans and animal models have associated lowered VEGF with bone mineral density and adverse osteoporosis outcomes. However, given that these observational studies were susceptible to confounding, it remained unclear whether this association was causal, driven by confounding or a product of the disease process itself. Using the largest circulating VEGF genome-wide association meta-analysis to date[29], that included genetic variants explaining up to 52% of the variance in levels of circulating VEGF, we hypothesized that combining this information with our current knowledge of the genetic determinants of osteoporosis, would help us better understand whether circulating VEGF could serve an effective target for the treatment or prevention of osteoporosis-related fractures. The following chapter presents a Mendelian randomization study providing evidence that pharmacologically altering levels of circulating VEGF is unlikely to have clinically-relevant effects on osteoporosis outcomes.

Chapter 3: Genetically decreased circulating vascular endothelial growth factor and osteoporosis outcomes: A Mendelian randomization study.

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3.1 Abstract

Vascular endothelial growth factor (VEGF) is important for bone formation and has been associated with osteoporosis in humans. Therefore, we conducted a two-sample Mendelian randomization study to test whether genetically decreased circulating VEGF was associated with decreased bone mineral density (BMD) and increased risk of fracture.

Summary statistics from a genome-wide association meta-analysis of circulating VEGF level (N=16,112) were used to identify 10 genetic variants that explained up to 52% of the variance in circulating VEGF levels. Genome-wide association study meta-analyses on dual X-ray absorptiometry-derived BMD of forearm, lumbar spine, and femoral neck (N = up to 32,735), and BMD estimated from heel calcaneus ultrasound (eBMD) (N=426,824) were used to assess the effect of genetically lowered circulating VEGF levels on BMD. A genome-wide association meta-analysis consisting of 24 cohorts including a total of 76,549 cases and 470,164 controls was used to assess the effect of genetically lowered circulating VEGF levels on risk of fracture.

A natural log-transformed pg/mL decrease in circulating VEGF levels was not associated with a decrease in forearm BMD (0.02 standard deviations (SD), CI: [-0.024, 0.064], p=0.38), lumbar spine BMD (-0.005 SD, CI: [-0.03, 0.019], p=0.67), femoral neck BMD (0.004 SD, CI: [-0.017, 0.026], p=0.68), eBMD (-0.006 SD, CI: [-0.012, -0.001], p=0.031) or risk of fracture (odds ratio: 0.99, CI: [0.98, 1.0], p=0.37) in inverse-variance weighted Mendelian randomization analyses. MR-Egger analyses did not provide evidence of pleiotropic effects.

Genetically lowered circulating VEGF was not associated with a decrease in BMD or increase in risk of fracture, suggesting that efforts to influence circulating VEGF level are unlikely to have beneficial effects on osteoporosis outcomes and that previous observational associations of circulating VEGF with BMD were influenced by confounding or reverse causation.

3.2 Introduction

Osteoporosis is an aging-related disease characterized by weakened bone microarchitecture leading to increased risk of fracture[1]. While osteoporosis is a common disease with important clinical outcomes, such as hip fracture, many patients who are at high risk are not treated — in part due to side-effects of current medical therapies[2]. This has led to efforts to identify additional targets for new therapies[3]. A source of potential drug targets are circulating proteins since they are amenable to manipulation with small molecules and monoclonal antibodies. One such circulating protein is vascular endothelial growth factor (VEGF), the factor responsible for angiogenesis.

Although VEGF plays a role in bone formation[4–6], its role as a circulating protein influencing osteoporosis outcomes has not yet been clearly established[7]. Several studies in animal models and humans support a role for VEGF in bone formation and osteoporosis. For instance, VEGF has been shown to be highly expressed in osteoblastic precursor cells and stimulate bone formation[8,9]. Furthermore, VEGF influences bone remodeling in glucocorticoid-induced osteoporosis in the mini-pig animal model[10]. In mouse models, VEGF-mediated bone angiogenesis is required for proper bone gain induced by exercise[11]. These findings suggest that VEGF may influence osteoporosis outcomes.

In humans, observational epidemiology supports a role for circulating VEGF in osteoporosis[12], however, the findings were suggestive and may be subject to confounding by unmeasured, or improperly measured factors. Observational studies may also be influenced by reverse causation, where the outcome itself influences the risk factor. This may be the case in osteoporosis outcomes as the precise timing of osteoporosis onset is often not known. Moreover, osteoporosis treatment may influence levels of circulating VEGF[13–15]. Therefore, further study is needed to determine whether VEGF has a causal role in osteoporosis.

The principles of Mendelian randomization (MR) can be used to assess the causal role of

circulating biomarkers in risk of disease. Similar to a randomized control trial, MR compares outcomes between two groups of individuals that differ at one variable of interest and its associated effects. In MR, this variable is a genetic variant, yielding two groups that differ by any factor that the genetic variant relates to. Because genetic variants are passed from parents to offspring independently of environment, they can serve as instrumental variables that are largely independent of factors that would confound observational studies. Further, because the germline genotype does not change throughout life, associations derived from MR analyses cannot be influenced by reverse causation. MR has been previously used to investigate the causal role of high-density lipoprotein[16] and C-reactive protein[17] in predisposition to cardiovascular disease, and has provided evidence that PCSK9 inhibition prevents cardiovascular disease[18]. MR has also provided evidence that higher vitamin D status leads to reduced multiple sclerosis risk[19], subsequently informing the vitamin D supplementation guidelines for the Multiple Sclerosis Canada[20]. A recent MR study supported a causal role for bone mineral density (BMD) on fracture and showed that several other exposures, such as lowered levels of vitamin D levels in the general population, did not influence fracture risk[21]. MR methods may be of particular relevance for understanding the etiology of osteoporosis since the disease onset occurs long after birth and MR studies assess the effect of lifetime exposures. Understanding the risk associated with a lifetime exposure to lowered circulating VEGF could therefore elucidate the role of this circulating protein in osteoporosis.

In this study, we performed a MR study to provide evidence for, or against, a causal role of circulating VEGF levels on BMD, the single best predictor of osteoporosis risk and fracture susceptibility[22]. We used a two-sample MR approach[23] which entailed first obtaining genetic variants that were significantly associated with circulating VEGF levels from a meta-analysis of genome-wide association studies (GWAS) on 10 cohorts of European ancestry[24], comprising a total of 16,112 individuals. Next, we tested their validity as instrumental variables for MR analyses[25].

Finally, using all circulating VEGF-associated genetic variants, we conducted MR analyses and meta-analyzed their combined effect of circulating VEGF levels on dual-energy X-ray absorptiometry (DXA)-measured BMD at the forearm (FA) (N=8,143), lumbar spine (LS) (N=28,498) and femoral neck (FN) (N=32,735)[26], on BMD estimated from heel calcaneus ultrasound (eBMD) (N=426,811)[27] and on risk of fracture (76,549 cases and 470,164 controls)[21].

3.3 Methods

3.3.1 Instrumental variable selection and data source

The first assumption of MR studies requires that instrumental variables (which are most often single nucleotide polymorphisms (SNP) in MR studies), are associated with the exposure of interest[25], in this case circulating levels of VEGF. We identified 10 genome-wide significant SNPs for circulating VEGF ($p<5x10^{-8}$) and collected their effect size estimates from the largest published GWAS meta-analysis for circulating VEGF levels to date consisting of 16,112 individuals of European ancestry. Together, these 10 SNPs explained up to 52% of the variance in circulating VEGF levels. The mean variance explained across the cohorts in the meta-analysis was 33%[24]. The meta-analyzed cohorts were the Age Gene/Environment Susceptibility Reykjavik Study (n=1,548), the Cilento study (n=1,115), the Framingham Heart Study (n=7,048), the Ogliastra Genetic Park (n=897), the Prospective Investigation of the Vasculature in Uppsala Seniors Study (n=945), the Val Borbera study (n=1,759), the Gioi (n=470) population, the Sorbs population (n=659), the STANISLAS Family Study (n=676) and a sample of hypertensive adults (n=995). Units of circulating VEGF levels were in pg/mL and were natural log-transformed[24].

3.3.2 Independence from confounding factors.

The second assumption of MR studies requires that there are no unmeasured confounders of the

association between the genetic variants and the outcome[25]. This assumption can be violated if the genetic variants are subject to population stratification, that is if they are associated with ancestry, which in turn is associated with the outcome. Prior to the MR analysis, SNPs from the VEGF, FA, LS and FN DXA-measured BMD, eBMD and fracture GWAS meta-analyses were confirmed to have been adjusted for population stratification using methods described in their respective studies[21,24,28] Further, all cohorts analyzed in the GWAS meta-analyses consisted uniquely of individuals of European descent.

3.3.3 Horizontal pleiotropy assessment

The third assumption of MR studies requires that instrumental variables influence the outcome only through their effect on the exposure[25]. To explore potential violations of this assumption, each of the 10 VEGF-associated SNP was queried against the Phenoscanner database[29] of GWAS and was excluded from the analysis if it was significantly associated to any known risk factor for osteoporosis or fracture, after Bonferroni correction for the number of SNPs queried ($p<0.05/10=0.005$). Well-validated risk factors for osteoporosis included advancing age, previous fracture, glucocorticoid therapy, parental history of hip fracture, low body weight, current cigarette smoking, excessive alcohol consumption, rheumatoid arthritis and secondary osteoporosis (e.g. hypogonadism or premature menopause, malabsorption, chronic liver disease, inflammatory bowel disease[30]). Other potential pleiotropic pathways were also considered, such as history of a fall, lower-extremity weakness, cognitive impairment, balance problems, psychotropic drug use, arthritis, history of stroke, orthostatic hypotension, dizziness and anemia[31–43].

To further exclude the possibility that any of the 10 VEGF-associated SNPs influenced the outcome independently of circulating VEGF levels, they were queried against a GWAS of 2,994 circulating protein levels conducted on 3,301 individuals[44]. SNPs that were significantly associated

to a circulating protein after Bonferroni correction for the number of circulating proteins in the study and the number of SNPs queried ($p<0.05/(10x2994)=1.67x10^{-6}$) were considered potentially pleiotropic and were excluded from the main MR analysis. We note that the above sensitivity analyses do not necessarily differentiate between horizontal and vertical pleiotropy, where only the former would bias MR studies.

3.3.4 Independence of instrumental variables

MR studies require that instrumental variables are independent of one another.[45] To verify that the VEGF-associated SNPs met this assumption, linkage disequilibrium (LD) was measured between those that shared a chromosome using LDLink[46], considering the following 1000 Genomes reference populations: Utah Residents from North and West Europe, Toscani in Italia, Finnish in Finland, British in England and Scotland, and Iberian population in Spain.

3.3.5 Association of instrumental variables with BMD and fracture

Summary statistics, including effect sizes for the association of instrumental SNPs with BMD, were obtained using three separate GWAS of FA, LS and FN DXA-measured BMD from Zheng et al.[26], the largest GWAS on DXA-measured BMD to date. eBMD summary statistics were obtained from the largest GWAS on this phenotype to date[27]. Summary statistics for the association of instrumental SNPs with fracture were obtained from a fracture fixed effect meta-analysis comprising a total of 24 cohorts from two recently published fracture GWAS, which included 23 cohorts from Genetic Factors for Osteoporosis consortium[21], the EPIC-Norfolk study[21] and the UK Biobank 's full release[27]. Proxies for SNPs that were not present in an outcome GWAS were identified using LDLink[46] ($r^2>0.8$, considering 1000 Genomes populations: Utah Residents from North and West Europe, Toscani in Italia, Finnish in Finland, British in England and Scotland, and Iberian population

in Spain) and corresponding summary statistics were assigned to instrumental SNPs assuring that proxy effect directions were aligned with the correlating VEGF-decreasing allele of the instrumental SNP.

3.3.6 MR estimates

MR estimates for instrumental SNPs were computed using the Wald method[47] and were meta-analyzed by computing an inverse-variance weighted (IVW) average. The Python code written to compute MR estimates is presented at the following link: https://github.com/richardslab/MR-VEGF-Osteoporosis

3.3.7 Sensitivity analyses

To investigate the extent to which the IVW estimate was influenced by the SNP with the largest effect on circulating VEGF levels, the IVW meta-analysis was repeated after its exclusion.

SNPs that were associated with non-VEGF circulating protein levels in Sun et al.[44] were excluded from the main analysis to avoid potential violations of the MR assumptions. However, it is possible that the circulating proteins associated to these SNPs are members of pathways that influence circulating VEGF level and have no effect on BMD or fracture risk other than through circulating VEGF. Or, these same proteins may be influenced directly by VEGF. In such cases, the use of these SNPs as instrumental variables for VEGF would be valid. As a second sensitivity analysis, MR analysis was repeated using all 10 VEGF-associated SNPs[24], acknowledging that nonzero MR estimates could be driven by pathways that are independent of VEGF and that null MR estimates could be the additive outcome of two or more opposing nonzero effects.

To estimate the extent to which MR estimates were influenced by pathways that are independent of circulating VEGF, MR-Egger regression analyses were performed using the

Mendelian Randomization R package[48], using the intercept as a test for horizontal pleiotropy.

3.4 Results

3.4.1. Selection of Instrumental Variables

Choi et al. 2016[24] identified 10 genome-wide significant SNPs for circulating VEGF level that, together, explained up to 52% of the variance in circulating VEGF in individuals of European ancestry[24] (**Table 1**). LD was measured between SNPs on the same chromosome, and high LD was not found between the tested SNP pairs (**Table 2**).

By querying the 10 VEGF-associated SNPs in the Phenoscanner database, we found that rs10761741 [*JMJD1C*], rs6993770 [*ZFPM2*], rs2639990 [*ZADH2*], rs4782371 [*ZFPM1*], were significantly associated with at least one secondary cause of osteoporosis after correcting for multiple hypothesis testing ($p<0.05/10=0.005$) and removed them the main MR analysis (**Table S1**). Additionally, rs10761741 [*JMJD1C*] was associated with cognitive function ($p=1.52x10^{-3}$) and rs6993770 [*ZFPM2*] was associated with myocardial infarction ($p=8.65x10^{-4}$) and Parkinson's disease ($p=2.28x10^{-3}$), which can all increase fall susceptibility and, by consequence, risk of fracture (**Table S2**). This provided yet another rationale for their exclusion from the main MR analyses.

To exclude the possibility that any of the 10 VEGF-associated SNPs associated with biological pathways that act upon the studied outcomes independently of circulating VEGF, each of the 10 VEGF-associated SNPs were queried against the Sun et al. circulating protein GWAS. We found that rs6921438 [*LOC100132354*], the SNP of largest effect in **Table 1**, rs34528081 [*VEGFA*] and rs2375981 [*KCNV2*] were uniquely associated with circulating levels of VEGF (**Table S3**). While rs10761741 [*JMJD1C*] and rs6993770 [*ZFPM2*] were associated with circulating VEGF level after Bonferroni correction for the number of proteins in the study and the number of SNPs queried ($p<0.05/(10x2994)=1.67x10^{-6}$), they were also associated with 14 and 86 circulating protein levels

respectively, prompting their removal from the main MR analyses (**Table S3**). The Sun et al. study[44] was possibly not powered to detect the association of the remaining SNPs with VEGF level. rs114694170 [*MEF2C*] was significantly associated with Noggin ($p=1.78x10^{-10}$), a protein known to bind and inactivate proteins that stimulate angiogenesis through the production of VEGF-A by osteoblasts[49]. However, this protein has also been shown to be implicated with bone formation via pathways that are independent of VEGF[50]. Further, rs114694170 is located in *MEF2C* in which deletions have been shown to be responsible for severe mental retardation with stereotypic movements, epilepsy and cerebral malformations[51], all of which are risk factors for falls susceptibility and fracture. Due to the potentially pleiotropic nature of this genomic region, rs114694170 was excluded from the main MR analysis.

3.4.2 Association of VEGF-associated SNPs with BMD and fracture

Summary statistics for the association of the VEGF-associated SNPs with DXA-measured BMD on FA, LS, and FN are shown in **Table 3**. Summary statistics for the association of the VEGF-associated SNPs with eBMD and fracture are shown in **Tables 4 and 5**, respectively. For the DXA-measured BMD and eBMD analyses, rs11965885 ($R^2=0.83$) was used as a proxy for rs34528081 as summary statistics for the latter were not available.

While the p-value for the association of rs6921438 with eBMD was less than 0.05, none of the 10 VEGF-associated SNPs were significantly associated with DXA-measured BMD or eBMD after Bonferroni correction for the number of independent SNPs tested ($p\geq0.05/10=0.005$) (**Tables 3 and 4**). Likewise, none of the VEGF-associated SNPs were significantly associated with fracture after Bonferroni correction for the number of SNPs tested ($p\geq0.05/10=0.005$) (**Table 5**).

3.4.3 MR for association of VEGF with FA, LS, FN BMD, eBMD and fracture risk

MR estimates for the valid instruments (rs34528081, rs6921438, rs1740073, rs7043199 and rs2375981) were computed individually for all three DXA-measured BMD outcomes and then meta-analysed using an IVW average of the individual MR estimates. For all three DXA-BMD outcomes, we observed that a natural log-transformed pg/mL decrease in circulating VEGF was not associated with a change in DXA-measured BMD for any of the instrumental SNPs. Of note, the variant rs6921438, that decreases circulating VEGF by 0.64 natural log-transformed pg/mL per VEGF-decreasing allele, showed null effects on FA, LS and FN DXA-measured BMD (**Table 3**). The IVW estimates of VEGF on DXA-measured BMD were also null for all 3 outcomes (0.02 standard deviations (SD), CI: [-0.024, 0.064], p=0.38), (-0.005 SD, CI: [-0.03, 0.019], p=0.67), (0.004 SD, CI: [-0.017, 0.026], p=0.68) for FA, LS, FN, DXA-measured BMD, respectively. (**Figure 1**).

MR estimates for the valid instruments were also computed for eBMD along with an IVW meta-analysis of the individual MR estimates. All of the SNPs had a null effect on eBMD except for rs6921438 which showed a small negative effect of circulating VEGF on eBMD (-0.008 SD, CI: [-0.014, -0.002], p=0.009) that was different from zero after Bonferroni correction for the number of SNPs tested ($p<0.05/5=0.01$) (**Figure 2**). The IVW estimate showed a small suggestive negative effect of VEGF on eBMD (-0.006 SD, CI: [-0.012, -0.001], p=0.031) in response to a natural log-transformed pg/mL decrease in circulating VEGF.

For the fracture study, MR estimates for the valid instruments and their resulting IVW estimate did not demonstrate a change in odds of fracture in response to a natural log-transformed pg/mL decrease in circulating VEGF (odds ratio: 0.99, CI: [0.98, 1.01], p=0.37) (**Figure 3**).

3.4.4 Sensitivity analysis

Of the 10 VEGF-associated SNPs, one SNP, rs6921438, located ~171 kb downstream of the VEGF

gene[52] had the largest effect on VEGF with a 0.64 natural log-transformed pg/mL decrease in circulating VEGF level per VEGF-decreasing allele. This effect size is three times larger than that of the SNP with the second largest effect on VEGF (rs2375981) that results in a 0.21 natural log-transformed pg/mL decrease in circulating VEGF levels per VEGF-decreasing allele. To investigate the extent to which the IVW estimate was driven by rs6921438, we excluded it from the FA, LS, FN DXA-measured BMD and eBMD analyses. Consistent with the findings from **Figure 1**, IVW estimates did not demonstrate a change in FA, LS, FN DXA-measured BMD or eBMD, albeit with larger confidence intervals, (0.03 SD, CI: [-0.089, 0.149], p=0.62), (-0.033 SD, CI: [-0.1, 0.035], p=0.34), (0.018 SD, CI: [-0.04, 0.076], p=0.54), (0.006 SD, CI: [-0.009, 0.02], p=0.46), respectively (**Figures S1 and S2**). This sensitivity analysis was repeated for the fracture study and, consistent with the findings from **Figure 3**, the resulting IVW estimate did not demonstrate a change in odds of fracture in response to a natural log-transformed pg/mL decrease in circulating VEGF (odds ratio: 1.04, CI: [0.99, 1.09], p=0.17) (**Figure S3**).

We then repeated the DXA-measured BMD and eBMD analyses using all 10 VEGF-associated SNPs acknowledging that some MR estimates may be influenced by pleiotropic effects, in violation of the third assumption of MR studies requiring that instrumental variables influence the outcome only through their effect on the exposure (**Figures S4 and S5**). Consistent with the findings from **Figure 1**, all newly introduced SNPs (rs114694170, rs6993770, rs10761741, rs4782371 and rs2639990), including the resulting IVW averages, showed MR estimates that spanned the null with the exception of rs114694170 that showed a 0.26 SD decrease in DXA-measured FN BMD in response to a natural log-transformed pg/mL decrease in circulating VEGF (CI: [-0.48, -0.038], p=0.022). (**Figure S4.C**). This sensitivity analysis was repeated for the fracture study and none of the estimates, including the IVW average, demonstrated a change in odds of fracture in response to a natural log-transformed pg/mL decrease in circulating VEGF (**Figure S6**).

To estimate the extent to which the MR estimates from the second sensitivity analysis were influenced by pathways that are independent of VEGF, we performed MR-Egger regression analyses using the 10 VEGF-associated SNPs on the studied outcomes. For the FA, LS and FN DXA-measured BMD analyses, we observed intercepts of -0.009 SD (CI:[-0.025, 0.007], p=0.25), -0.006 SD (CI: [-0.015, 0.003], p=0.18) and -0.007 SD (CI: [-0.016, 0.003], p=0.18), respectively (**Figure S7**). For the eBMD analysis, we observed an intercept of 0.002 SD (CI: [0.000, 0.005], p=0.04) (**Figure S8**). For the fracture analysis we observed an intercept of 0.002 SD (CI: [-0.004, 0.009], p=0.48) (**Figure S9**). None of the regressions had an intercept that was different from zero after Bonferroni correction for the number of MR-Egger analyses performed (p>0.05/5=0.01), and thus provided no evidence of pleiotropic effects.

3.5 Discussion

Using large sample sizes for circulating VEGF, BMD and fracture, we showed that a decrease in circulating VEGF was not associated with clinically-relevant changes in FA, LS or FN DXA-measured BMD, eBMD or odds of fracture in individuals of European descent. Despite large changes in circulating VEGF level captured by genetic variants, no effects were observed on BMD or fracture and the confidence intervals of these null effects excluded clinically-relevant changes for these outcomes. These findings suggest that circulating VEGF does not have an important causal effect on osteoporosis outcomes in humans.

These findings are inconsistent with an observational study which suggested a role for circulating VEGF on BMD[12] suggesting that this study may have been influenced by confounding and/or reverse causation. Our results suggest that pharmacological efforts to influence circulating VEGF are unlikely to have beneficial skeletal effects. Further, MR may be the only feasible study design to assess the role of a lifetime exposure to lowered VEGF on BMD, as randomized control trials would generally only assess the effect of targeting circulating VEGF over a relatively short period of time.

While MR can overcome some of the limitations of observational studies, the possibility that one or more of our instrumental SNPs exhibit undetected pleiotropy is difficult to eliminate. Therefore, it is possible that the resulting IVW estimates of circulating VEGF on BMD and fracture risk are affected by factors that alter BMD and fracture risk independently of VEGF. However, this becomes increasingly unlikely as the number instruments suggesting a null effect of VEGF on BMD or fracture increases. Furthermore, the SNP with the largest effect on VEGF sits near the *VEGF* gene and likely influences its transcription. Such *cis*-associated SNPs are less likely to be influenced by horizontal pleiotropy. Our results remained consistent in both sensitivity analyses, with the exception of rs114694170 that showed a decrease in DXA-measured FN BMD associated with a decrease in

circulating VEGF. Although this SNP was not associated with measured clinical risk factors for osteoporosis or fracture, it lies in the *MEF2C* gene which has been associated with severe mental retardation, stereotypic movements, epilepsy and cerebral malformations[53]. Its validity as an instrument is therefore questionable, as individuals with one or more of these phenotypes can be subject to lifestyle habits that would result in lowered BMD[54]. Finally, it is important to note that our study is limited to the effect of circulating VEGF level on BMD and fracture. Our results cannot be generalized to the role of intracellular VEGF levels on BMD or fracture. Several studies have shown that intracellular VEGF influences bone-marrow stem cell differentiation independently of the role of VEGF in circulating[55] and it remains unclear whether the targeting of intracellular VEGF levels can be used as a preventive treatment for fracture. It is also possible that a life-long exposure to lowered circulating VEGF results in compensatory developmental processes that bias the apparent effect circulating VEGF on BMD and fracture toward the null. In such a case, influencing circulating VEGF levels in adults could in fact have a non-null effect on BMD or fracture. This phenomenon is called canalization[25] and has not been directly assessed, since MR studies can only assess the relationship between a biomarker and a disease at the time point in the life-course where the genetic variant has been associated with circulating VEGF. We have also not examined effects of VEGF level in individuals with extreme levels of circulating VEGF, and our results do not permit comment on such individuals. Lastly, because the GWAS was conducted on individuals of European descent, the results may not generalize to other populations.

In conclusion, this study provides evidence against a causal role for circulating VEGF on BMD and fracture in humans. These findings suggest that efforts to influence circulating VEGF are unlikely to have beneficial skeletal effects and that previous observational associations of circulating VEGF and lowered BMD in humans may be due to confounding or reverse causation.

3.6 Acknowledgements

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3.7 Tables and Figures

3.7.1 Tables

Table 1. Summary statistics for 10 genome-wide significant variants on circulating VEGF level from Choi et al., 2016.

Effect alleles represent a decrease in circulating VEGF level.

SNP	Chromosome	Position (hg19)	Effect Allele	Effect Allele Frequency	Effect	Standard Error	*p-value*	Gene	Location
rs114694170	5	88180196	T	0.96	-0.15	0.023	1.09×10^{-11}	*MEF2C*	intron
rs34528081	6	43704417	T	0.6	-0.09	0.01	1.83×10^{-17}	*VEGFA*	intergenic
rs6921438	6	43925607	A	0.46	-0.64	0.008	1.66×10^{-1449}	*LOC100132354*	intergenic
rs1740073	6	43947398	C	0.64	-0.09	0.01	4.40×10^{-17}	*C6orf223*	intergenic
rs6993770	8	106581528	T	0.3	-0.16	0.01	3.83×10^{-55}	*ZFPM2*	intron
rs7043199	9	2621145	A	0.21	-0.1	0.013	4.16×10^{-14}	*VLDLR-AS1*	intron
rs2375981	9	2692583	G	0.46	-0.21	0.01	9.49×10^{-99}	*KCNV2*	intergenic
rs10761741	10	65066186	G	0.57	-0.08	0.009	2.99×10^{-19}	*JMJD1C*	intron
rs4782371	16	88568831	T	0.67	-0.07	0.011	1.26×10^{-9}	*ZFPM1*	intron
rs2639990	18	72915551	C	0.09	-0.11	0.018	5.85×10^{-10}	*ZADH2*	intron

Table 2. Linkage disequilibrium between SNPs on the same chromosome.

Coefficients of determination (R^2) were calculated using 1000 Genomes populations: Utah Residents from North and West Europe, Toscani in Italia, Finnish in Finland, British in England and Scotland, and Iberian population in Spain.

Chromosome	SNP_1	SNP_2	R^2
6	rs6921438	rs1740073	0.001
6	rs6921438	rs34528081	< 0.001
6	rs1740073	rs34528081	0.0007
9	rs7043199	rs2375981	< 0.001

Table 3. Summary statistics for the VEGF-associated SNPs from the DXA-measured FA, LS and FN BMD GWAS

rs11965885 was used as a proxy (R^2=0.83) for rs34528081 as summary statistics for the latter were not available in the FA, LS, or FN GWAS.

				Forearm			Lumbar spine			Femoral neck		
SNP	**Chromosome**	**Effect allele**	**Effect allele frequency**	**Effect**	**Standard error**	***p*-value**	**Effect**	**Standard error**	***p*-value**	**Effect**	**Standard error**	***p*-value**
rs114694170	5	T	0.057	-0.001	0.035	0.981	0.025	0.02	0.221	0.039	0.017	0.025
rs11965885	6	T	0.462	0.015	0.016	0.363	0.014	0.009	0.118	1.5×10^{-4}	0.008	0.985
rs6921438	6	A	0.483	-0.012	0.016	0.467	0.001	0.009	0.931	-0.001	0.007	0.846
rs1740073	6	C	0.437	0.012	0.016	0.461	0.011	0.009	0.247	0.014	0.008	0.086
rs6993770	8	T	0.274	0.002	0.017	0.903	-0.008	0.01	0.401	-0.002	0.008	0.788
rs7043199	9	A	0.234	-0.025	0.019	0.192	-1.9×10^{-4}	0.011	0.986	-0.006	0.009	0.522
rs2375981	9	G	0.446	-0.012	0.016	0.442	5.9×10^{-5}	0.009	0.995	-0.009	0.008	0.224
rs10761741	10	G	0.416	0.024	0.017	0.157	0.009	0.009	0.313	0.012	0.008	0.133
rs4782371	16	T	0.297	0.017	0.017	0.328	0.006	0.01	0.558	0.007	0.008	0.391
rs2639990	18	C	0.07	-0.023	0.03	0.445	-0.022	0.017	0.207	0.019	0.015	0.209

Table 4. Summary statistics for the VEGF-associated SNPs from the eBMD GWAS.

rs11965885 was used as a proxy (R^2=0.83) for rs34528081 as summary statistics for the latter were not available in the FA, LS, or FN GWAS. Effect alleles, effect sizes and effect allele frequencies were aligned to the VEGF-decreasing alleles from Table 1.

SNP	Chromosome	Effect allele	Effect allele frequency	Effect	Standard error	*p*-value
rs114694170	5	T	0.94	-0.0047	0.0041	0.40
rs11965885	6	T	0.58	0.0003	0.0020	0.74
rs6921438	6	A	0.49	0.0050	0.0019	0.02
rs1740073	6	C	0.61	-0.0010	0.0021	0.73
rs6993770	8	T	0.29	-0.0034	0.0021	0.10
rs7043199	9	A	0.23	-0.0024	0.0023	0.42
rs2375981	9	G	0.45	-0.0007	0.0020	0.60
rs10761741	10	G	0.58	0.0028	0.0020	0.08
rs4782371	16	T	0.70	-0.0028	0.0021	0.30
rs2639990	18	C	0.07	-0.0061	0.0039	0.08

Table 5. Summary statistics of the VEGF-associated SNPs on fracture.

Effect alleles, effect sizes and effect allele frequencies were aligned to the VEGF-decreasing alleles from Table 1.

SNP	Chromosome	Effect allele	Effect allele frequency	Effect	Standard error	*p*-value
rs114694170	5	T	0.94	-0.013	0.015	0.37
rs34528081	6	T	0.58	0.003	0.007	0.70
rs6921438	6	A	0.49	0.002	0.006	0.79
rs1740073	6	C	0.61	0.004	0.007	0.55
rs6993770	8	T	0.28	0.003	0.007	0.67
rs7043199	9	A	0.23	0.003	0.008	0.68
rs2375981	9	G	0.45	0.012	0.007	0.08
rs10761741	10	G	0.58	-0.001	0.006	0.90
rs4782371	16	T	0.70	-0.002	0.007	0.79
rs2639990	18	C	0.07	-0.002	0.014	0.86

3.7.2 Figures

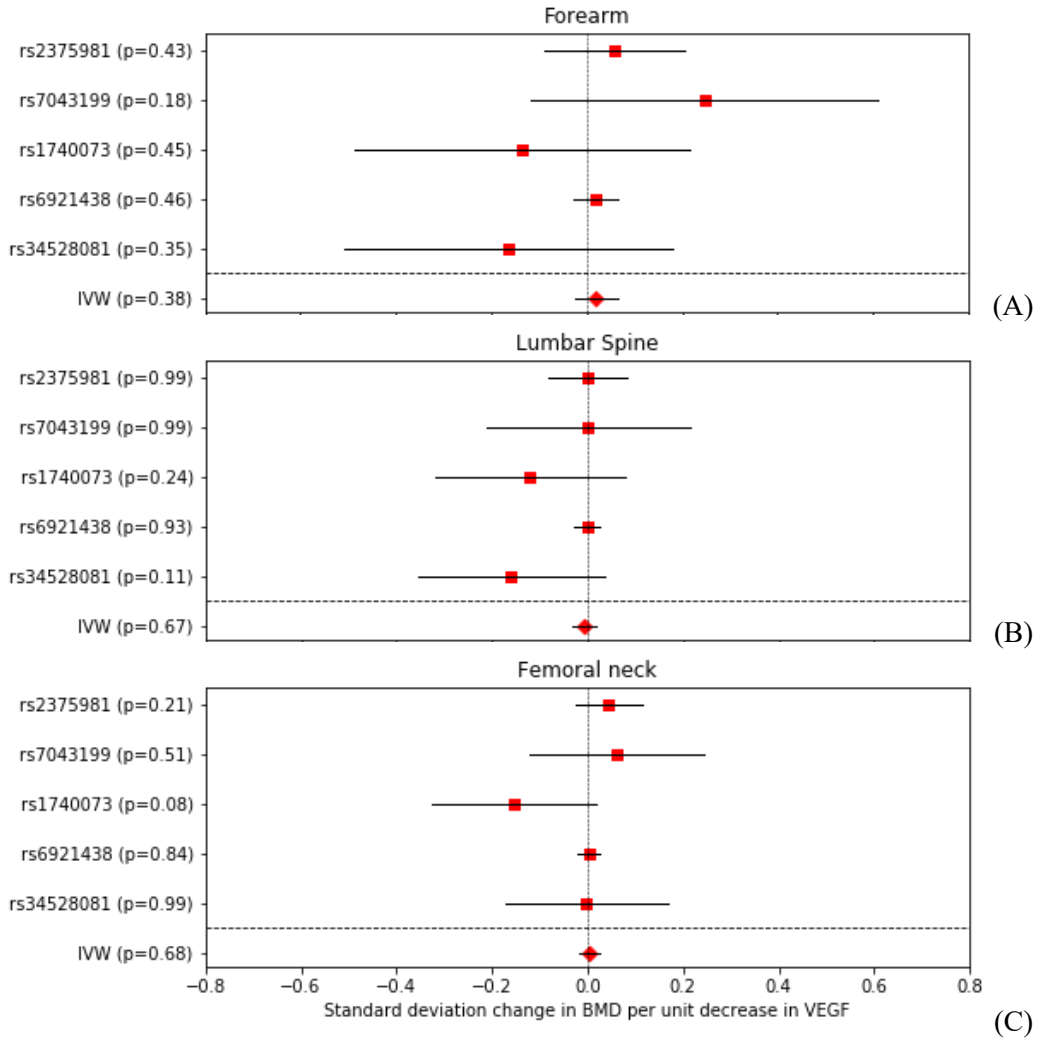

Figure 1. Circulating VEGF effect on DXA-measured BMD.

(A) Forearm, (B) Lumbar spine and (C) Femoral neck. Two-sample Mendelian Randomization (MR): individual effects and inverse-variance weighted (IVW) average. Effects are expressed as standard deviation changes in DXA-measured BMD per natural log-transformed pg/mL decrease in circulating VEGF.

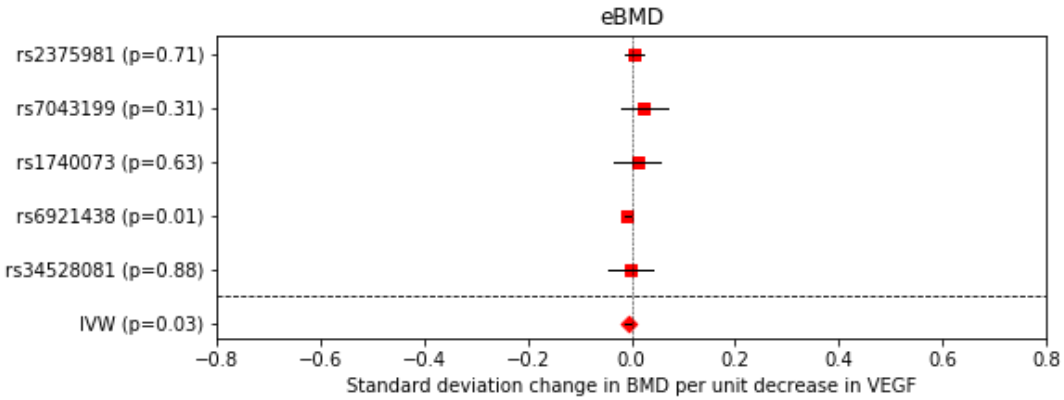

Figure 2. Circulating VEGF effects on eBMD.

Two-sample Mendelian Randomization (MR): individual effects and inverse-variance weighted (IVW) average. Effects are expressed as standard deviation changes in eBMD per natural log-transformed unit (pg/mL) decrease in circulating VEGF.

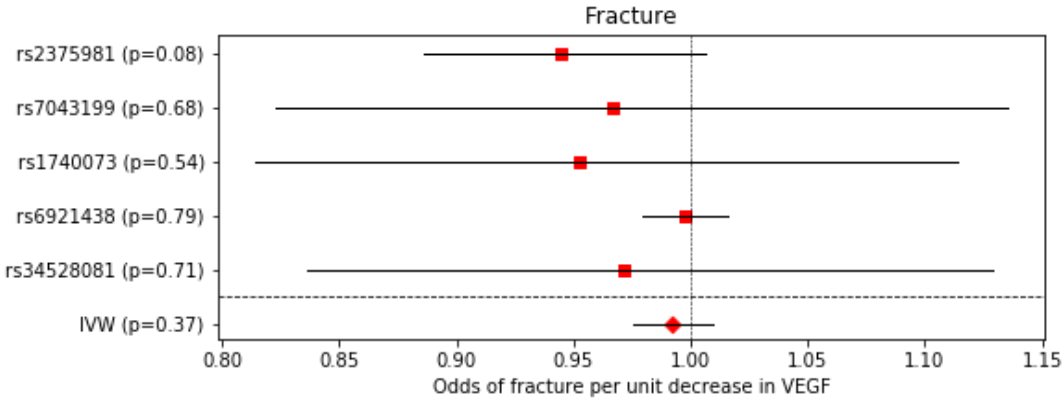

Figure 3. Circulating VEGF effects on fracture.

Two-sample Mendelian Randomization: individual effects and inverse-variance weighted (IVW) average. Effects are expressed as odds of fracture per natural log-transformed unit (pg/mL) decrease in circulating VEGF

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3.9 Supplement

3.9.1 Supplementary Tables

Table S1. VEGF-associated SNPs that were significantly associated with at least one secondary cause of osteoporosis in the Phenoscanner database.

SNP	Proxy rsID	r^2	Trait	PMID	P
rs10761741	rs7923609	0.809	Alkaline Phosphatase	22001757	6.00E-23
rs10761741	rs10761741	1	Alkaline phosphatase ALP	18940312	4.70E-08
rs10761741	rs7923609	0.809	Alkaline phosphatase ALP in plasma	22001757	5.90E-23
rs10761741	rs10761741	1	BMI	23754948	0.0002024
rs10761741	rs10761741	1	BMI in females	25673413	0.0002493
rs10761741	rs10761741	1	BMI in females less than or equal to 50 years of age	26426971	0.00027
rs10761741	rs10761741	1	Body mass index BMI	20935630	0.000923
rs10761741	rs12355784	0.805	Fat mass	17463246	0.0002381
rs10761741	rs10761741	1	Former smoker	20418890	0.004586
rs10761741	rs10761741	1	Height	25282103	0.0012
rs10761741	rs7082470	0.805	Height in males	23754948	0.0045
rs10761741	rs7923609	0.809	Liver enzyme levels alkaline phosphatase	22001757	6.00E-23
rs10761741	rs10761741	1	Obesity class 1	23563607	0.0048
rs10761741	rs10761741	1	Serum dihydrotestosterone DHT level	22936694	0.004591
rs10761741	rs10761741	1	Serum testosterone T level	22936694	9.03E-05
rs10761741	rs7923609	0.809	Serum testosterone T level in dutasteride or placebo treatment group	22936694	3.66E-08
rs10761741	rs7923609	0.809	Serum testosterone T level in dutasteride treatment group	22936694	0.000124
rs10761741	rs7923609	0.809	Serum testosterone T level in placebo group	22936694	7.90E-05
rs10761741	rs7910927	0.809	Sex hormone binding globulin levels	22829776	6.00E-35
rs10761741	rs7910927	0.809	Sex hormone binding globulin SHBG concentrations	22829776	6.10E-35
rs2639990	rs609303	0.926	Sarcoidosis	22952805	1.61E-06
rs4782371	rs868874	0.969	Alcohol dependence symptom count	23089632	1.28E-05
rs6993770	rs6993770	1	Body fat percentage	26833246	4.63E-05
rs6993770	rs6993770	1	Crohn's disease	26192919	0.004238
rs6993770	rs6993770	1	Height	23754948	0.003329
rs6993770	rs6993770	1	Height in females	23754948	0.0008
rs6993770	rs4602861	0.93	Inflammatory bowel disease	26192919	0.002103

Table S2. VEGF-associated SNPs that were significantly associated with at least one risk factor for falls in the Phenoscanner database.

SNP	Proxy rsID	r^2	Trait	PMID	P
rs10761741	rs10761731	0.984	Cognitive function	25201988	0.00152
rs10761741	rs10761739	0.992	Cognitive function	25201988	0.001692
rs10761741	rs7896518	0.996	Cognitive function	25201988	0.001736
rs10761741	rs10740118	1	Cognitive function	25201988	0.001755
rs10761741	rs10761741	1	Cognitive function	25201988	0.001795
rs10761741	rs7075195	0.988	Cognitive function	25201988	0.00186
rs10761741	rs4454603	0.809	Cognitive function	25201988	0.002614
rs10761741	rs10761742	0.809	Cognitive function	25201988	0.002837
rs10761741	rs3999089	0.801	Cognitive function	25201988	0.003086
rs10761741	rs10509186	0.805	Cognitive function	25201988	0.003452
rs10761741	rs7092784	0.805	Cognitive function	25201988	0.003483
rs10761741	rs3740331	0.805	Cognitive function	25201988	0.003508
rs10761741	rs10740125	0.805	Cognitive function	25201988	0.003526
rs10761741	rs7085621	0.801	Cognitive function	25201988	0.003526
rs10761741	rs10740126	0.805	Cognitive function	25201988	0.003527
rs10761741	rs2393977	0.805	Cognitive function	25201988	0.003546
rs10761741	rs10761762	0.805	Cognitive function	25201988	0.003557
rs10761741	rs10761756	0.805	Cognitive function	25201988	0.003607
rs10761741	rs7896783	0.805	Cognitive function	25201988	0.003608
rs10761741	rs7910927	0.809	Cognitive function	25201988	0.003747
rs10761741	rs2393966	0.805	Cognitive function	25201988	0.003936
rs10761741	rs2393969	0.809	Cognitive function	25201988	0.003941
rs10761741	rs7923609	0.809	Cognitive function	25201988	0.003985
rs10761741	rs10509189	0.805	Cognitive function	25201988	0.004149
rs10761741	rs4486511	0.805	Cognitive function	25201988	0.004151
rs10761741	rs12355784	0.805	Cognitive function	25201988	0.004239
rs10761741	rs10761779	0.805	Cognitive function	25201988	0.004768
rs6993770	rs66489920	0.811	Myocardial infarction	26343387	0.0008651
rs6993770	rs16873418	0.811	Myocardial infarction	26343387	0.0008689
rs6993770	rs2343592	0.925	Myocardial infarction	26343387	0.002136
rs6993770	rs34826779	0.921	Myocardial infarction	26343387	0.002273
rs6993770	rs4734879	0.976	Myocardial infarction	26343387	0.002279
rs6993770	rs6993770	1	Myocardial infarction	26343387	0.003836
rs6993770	rs7832219	0.972	Myocardial infarction	26343387	0.003983
rs6993770	rs16873418	0.811	Parkinson's disease	19915575	0.002278

Table S3. VEGF-associated SNPs that were significantly associated with at least one circulating protein level using a GWAS study for 2,994 circulating protein levels in 3,301 individuals.

SNP	chromosome	Effect	StdErr	P	Protein
rs114694170	5	-0.3383	0.053	1.78E-10	NOG.5846.24.3
rs6921438	6	-0.7023	0.0215	1.66E-234	VEGFA.14032.2.3
rs6921438	6	-0.4199	0.0236	7.76E-71	VEGFA.2597.8.3
rs34528081	6	-0.1296	0.0266	1.12E-06	VEGFA.14032.2.3
rs6993770	8	0.3042	0.027	2.09E-29	VEGFA.14032.2.3
rs6993770	8	0.3027	0.027	3.89E-29	COCH.7227.75.3
rs6993770	8	0.2868	0.0271	3.09E-26	PDGFD.9341.1.3
rs6993770	8	0.2607	0.0272	7.76E-22	DKK1.3535.84.1
rs6993770	8	0.2596	0.0271	1.12E-21	CTSA.3179.51.2
rs6993770	8	0.2553	0.0272	5.75E-21	DKK4.3365.7.2
rs6993770	8	0.246	0.0272	1.48E-19	ERP44.6064.4.3
rs6993770	8	0.2387	0.0272	1.78E-18	APLP2.10627.87.3
rs6993770	8	0.2375	0.0272	2.63E-18	LGALS7.9196.8.3
rs6993770	8	0.2331	0.0272	1.10E-17	ANGPT1.2811.27.1
rs6993770	8	0.2161	0.0273	2.29E-15	PDGFA.4499.21.1
rs6993770	8	0.2125	0.0273	6.76E-15	SIRT5.12461.8.3
rs6993770	8	0.212	0.0273	7.76E-15	CCL17.3519.3.2
rs6993770	8	0.2089	0.0273	1.95E-14	CPXM1.6255.74.3
rs6993770	8	0.2085	0.0273	2.14E-14	UGT2A1.8907.11.3
rs6993770	8	0.2029	0.0273	1.07E-13	PPBP.2790.54.2
rs6993770	8	0.202	0.0273	1.38E-13	SERPINE1.2925.9.1
rs6993770	8	0.1979	0.0273	4.37E-13	PDGFB.4149.8.2
rs6993770	8	-0.193	0.0273	1.58E-12	EDAR.2977.7.2
rs6993770	8	0.1901	0.0273	3.55E-12	NSG2.13409.9.3
rs6993770	8	0.1897	0.0273	3.89E-12	SYT11.7089.42.3
rs6993770	8	0.1862	0.0273	9.77E-12	SPARC.3043.49.2
rs6993770	8	0.1852	0.0273	1.26E-11	BDNF.2421.7.3
rs6993770	8	0.1849	0.0273	1.35E-11	DNAJB11.7110.2.3
rs6993770	8	0.1805	0.0274	4.07E-11	INPP5E.11370.20.3
rs6993770	8	0.1788	0.0274	6.31E-11	SELP.4154.57.2
rs6993770	8	0.1756	0.0274	1.38E-10	FUT8.8244.16.3
rs6993770	8	0.1746	0.0274	1.78E-10	MPP7.12732.13.3
rs6993770	8	0.1735	0.0274	2.29E-10	ARL1.12392.30.3
rs6993770	8	0.1722	0.0274	3.09E-10	CENPW.8864.59.3
rs6993770	8	0.1694	0.0274	6.17E-10	CST7.3302.58.1
rs6993770	8	0.1692	0.0274	6.31E-10	RHOG.12540.25.3
rs6993770	8	0.1686	0.0274	7.41E-10	CGB2.6213.10.3
rs6993770	8	0.1646	0.0274	1.82E-09	CHST11.7779.86.3
rs6993770	8	0.1625	0.0274	2.95E-09	COTL1.4905.63.1
rs6993770	8	0.1624	0.0274	3.02E-09	PCDHGA8.11259.71.3
rs6993770	8	0.1599	0.0274	5.25E-09	SPTLC1.7886.26.3
rs6993770	8	0.1595	0.0274	5.75E-09	B4GALT7.7806.33.3
rs6993770	8	0.1577	0.0274	8.51E-09	APP.3171.57.2
rs6993770	8	0.1578	0.0274	8.51E-09	SPOCK3.9906.21.3
rs6993770	8	0.1576	0.0274	8.71E-09	SATB1.13511.29.3
rs6993770	8	0.157	0.0274	1.00E-08	IL7.14049.17.3
rs6993770	8	0.1568	0.0274	1.05E-08	WFDC13.9345.436.3
rs6993770	8	0.1557	0.0274	1.32E-08	OBP2A.6526.77.3
rs6993770	8	0.1556	0.0274	1.35E-08	SERPINE2.3217.74.2
rs6993770	8	-0.1549	0.0274	1.55E-08	APBB1.14206.28.3
rs6993770	8	0.1544	0.0274	1.74E-08	SCGB2A1.5001.6.2
rs6993770	8	-0.1541	0.0274	1.86E-08	AGER.4125.52.2
rs6993770	8	0.1523	0.0274	2.75E-08	LILRB4.6453.70.3
rs6993770	8	0.1512	0.0274	3.39E-08	PAIP1.12430.78.3

rs6993770	8	0.151	0.0274	3.55E-08	C10orf54.14123.34.3
rs6993770	8	0.1502	0.0274	4.17E-08	DEFB119.8315.5.3
rs6993770	8	0.1481	0.0274	6.46E-08	ARSA.3583.54.4
rs6993770	8	0.1481	0.0274	6.46E-08	KIRREL2.7958.15.3
rs6993770	8	-0.1467	0.0274	8.71E-08	EDA.2826.53.2
rs6993770	8	-0.1467	0.0274	8.71E-08	RBM28.11927.3.3
rs6993770	8	0.1456	0.0274	1.07E-07	PROL1.6530.63.3
rs6993770	8	0.1456	0.0274	1.10E-07	SMPDL3A.4771.10.3
rs6993770	8	0.1448	0.0274	1.29E-07	CRIM1.8699.43.3
rs6993770	8	-0.1428	0.0274	1.91E-07	GSTM1.7239.9.3
rs6993770	8	0.1427	0.0274	1.95E-07	GRP.5897.58.3
rs6993770	8	0.1419	0.0274	2.29E-07	SYT17.9110.2.3
rs6993770	8	0.1418	0.0274	2.34E-07	KITLG.9377.25.3
rs6993770	8	0.1413	0.0274	2.57E-07	CCL5.5480.49.3
rs6993770	8	-0.1405	0.0274	3.02E-07	SPARCL1.4467.49.2
rs6993770	8	-0.1397	0.0274	3.47E-07	RFK.13059.33.3
rs6993770	8	0.1391	0.0274	3.89E-07	EIF4EBP2.4184.43.3
rs6993770	8	0.1386	0.0274	4.27E-07	P2RX6.7233.73.3
rs6993770	8	-0.1381	0.0274	4.79E-07	MFGE8.4455.89.2
rs6993770	8	-0.1377	0.0274	5.13E-07	PTPRD.9296.15.3
rs6993770	8	0.1373	0.0274	5.50E-07	THRA.12527.50.3
rs6993770	8	0.1373	0.0274	5.62E-07	MYSM1.11536.9.3
rs6993770	8	0.1369	0.0274	5.89E-07	RGS3.12827.37.3
rs6993770	8	0.1361	0.0274	6.92E-07	HIF1A.13089.6.3
rs6993770	8	-0.135	0.0274	8.51E-07	REG3A.9277.16.3
rs6993770	8	-0.1347	0.0274	9.12E-07	EPHB2.5077.28.3
rs6993770	8	0.1341	0.0274	1.00E-06	EDC4.13066.42.3
rs6993770	8	0.1342	0.0274	1.00E-06	MADCAM1.11258.41.3
rs6993770	8	0.134	0.0274	1.05E-06	GPX7.8345.27.3
rs6993770	8	0.1332	0.0274	1.20E-06	PDIA5.5593.11.3
rs6993770	8	0.133	0.0274	1.23E-06	N6AMT1.11096.57.3
rs6993770	8	0.1325	0.0274	1.35E-06	PLOD3.10612.18.3
rs6993770	8	0.1324	0.0274	1.38E-06	CASP2.4904.7.1
rs6993770	8	0.1318	0.0274	1.55E-06	LRRN3.10471.25.3
rs6993770	8	-0.1316	0.0274	1.58E-06	CD300E.10798.4.3
rs6993770	8	0.1315	0.0274	1.62E-06	PXDNL.11324.3.3
rs2375981	9	0.1261	0.0247	3.31E-07	VEGFA.14032.2.3
rs10761741	10	0.1783	0.0252	1.58E-12	HBEGF.14094.29.3
rs10761741	10	0.158	0.0253	4.07E-10	DKK1.3535.84.1
rs10761741	10	0.1577	0.0253	4.27E-10	DKK4.3365.7.2
rs10761741	10	0.1542	0.0253	1.05E-09	CTSA.3179.51.2
rs10761741	10	0.1443	0.0253	1.15E-08	SIRT5.12461.8.3
rs10761741	10	0.142	0.0253	2.00E-08	CCL5.5480.49.3
rs10761741	10	0.1342	0.0253	1.12E-07	CXCL11.3038.9.2
rs10761741	10	0.1338	0.0253	1.26E-07	BCL2A1.3413.50.2
rs10761741	10	-0.1304	0.0253	2.63E-07	CRISPLD2.5691.2.3
rs10761741	10	0.1304	0.0253	2.63E-07	SCGB2A1.5001.6.2
rs10761741	10	0.129	0.0253	3.47E-07	LGALS7.9196.8.3
rs10761741	10	0.129	0.0253	3.47E-07	NID2.3633.70.5
rs10761741	10	0.1252	0.0253	7.76E-07	ARL1.12392.30.3
rs10761741	10	0.1249	0.0253	8.13E-07	ARSA.3583.54.4

3.9.2 Supplementary Figures

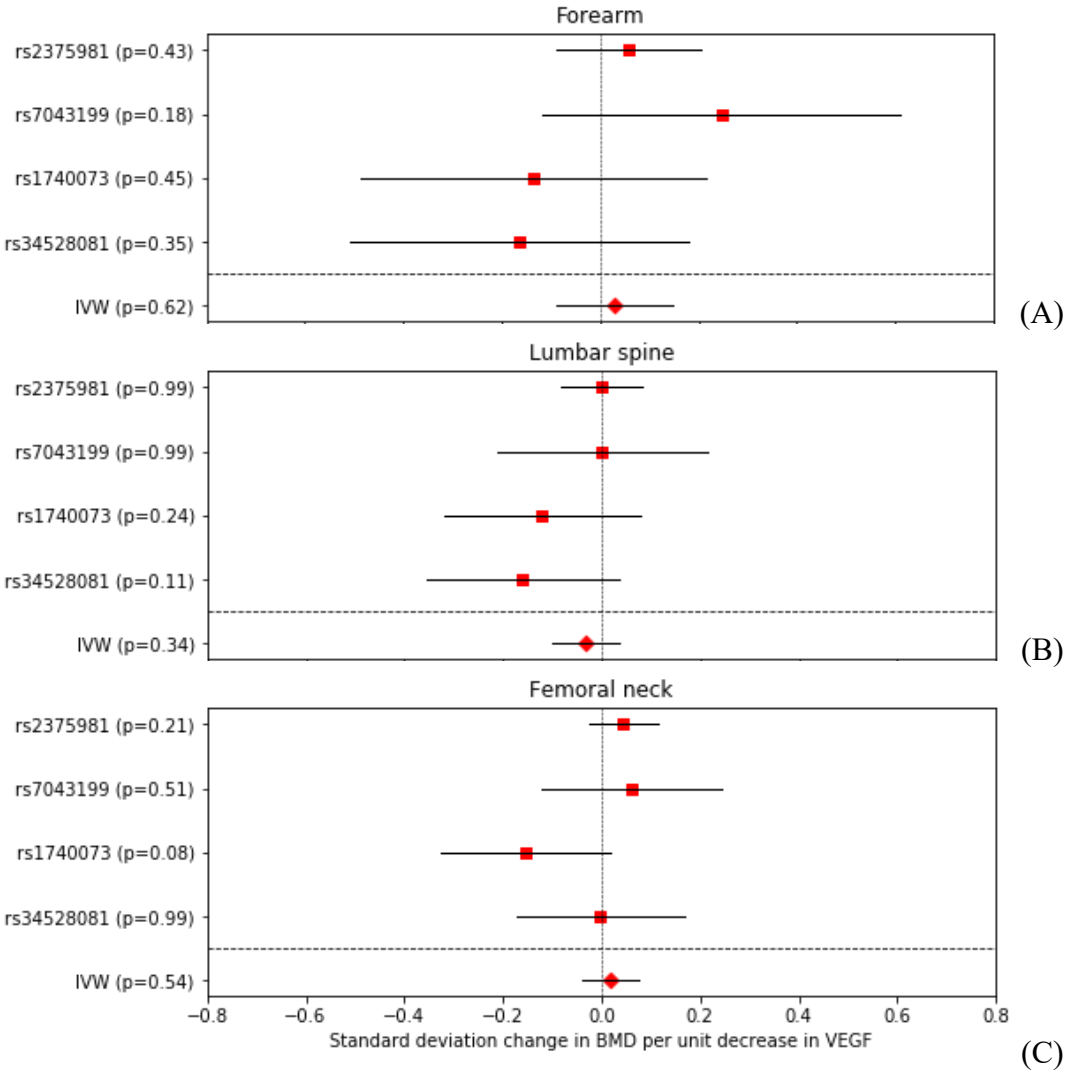


Figure S1. Sensitivity analysis on DXA-measured BMD excluding SNP rs6921438.

(A) Forearm, (B) Lumbar spine and (C) Femoral neck. Two-sample Mendelian Randomization (MR): individual effects and inverse-variance weighted (IVW) average. Effects are expressed as standard deviation changes in DXA-measured BMD per natural log-transformed pg/mL decrease in circulating VEGF.

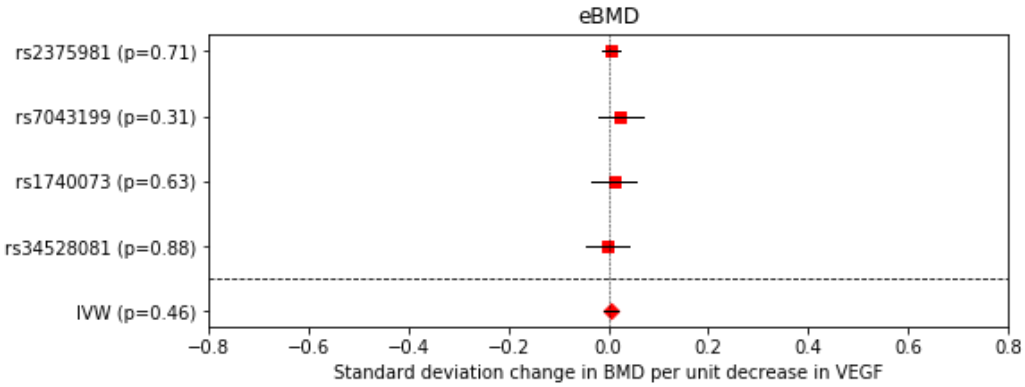


Figure S2. Sensitivity analysis on eBMD excluding SNP rs6921438.

Two-sample Mendelian Randomization (MR): individual effects and inverse-variance weighted (IVW) average. Effects are expressed as standard deviation changes in eBMD per natural log-transformed pg/mL decrease in circulating VEGF.

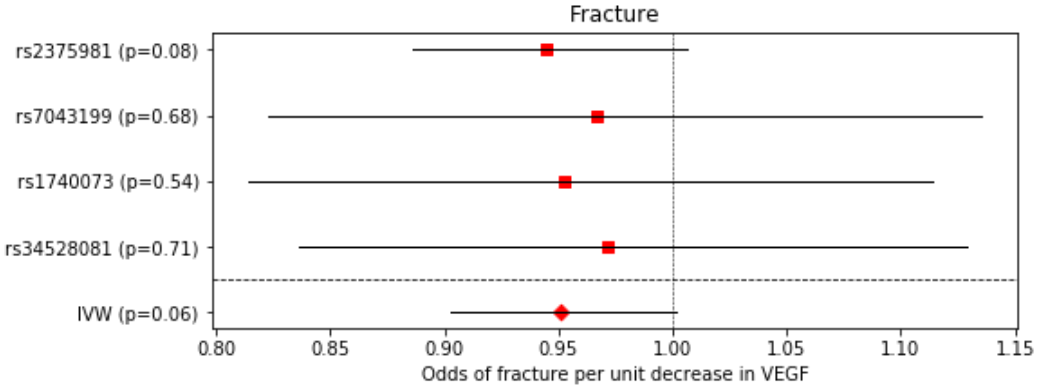


Figure S3. Sensitivity analysis on fracture excluding SNP rs6921438.

Two-sample Mendelian Randomization (MR): individual effects and inverse-variance weighted (IVW) average. Effects are expressed as odds of fracture per natural log-transformed pg/mL decrease in circulating VEGF.

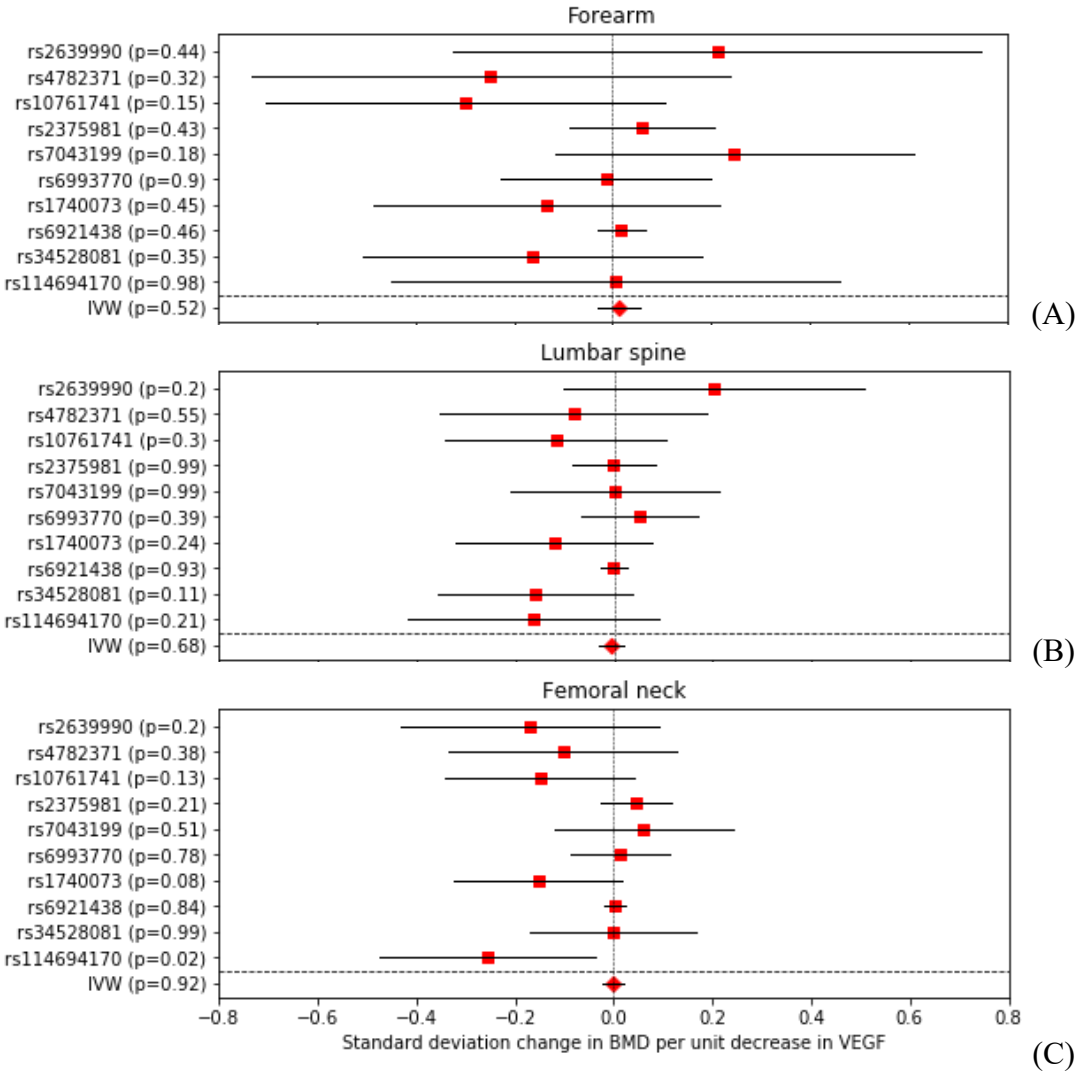


Figure S4. Sensitivity analysis on DXA-measured BMD including all VEGF-associated SNPs.

(A) Forearm, (B) Lumbar spine and (C) Femoral neck. Two-sample Mendelian Randomization (MR): individual effects and inverse-variance weighted (IVW) average. Effects are expressed as standard deviation changes in DXA-measured BMD per natural log-transformed pg/mL decrease in circulating VEGF.

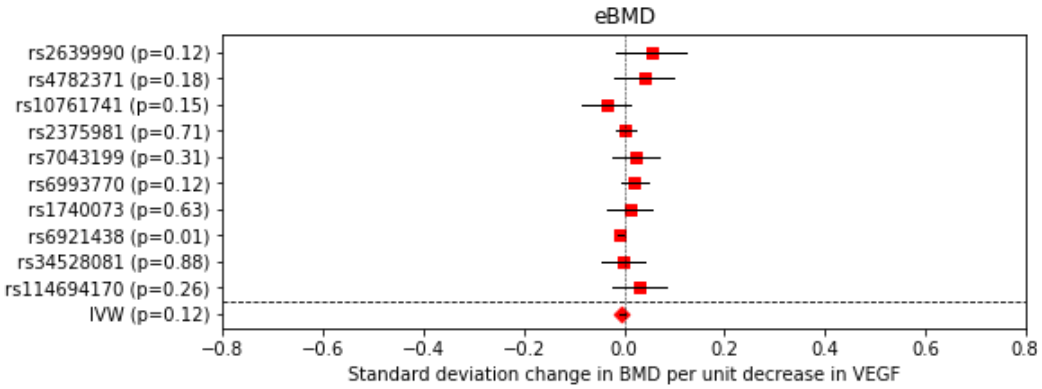


Figure S5. Sensitivity analysis on eBMD including all VEGF-decreasing alleles.

Two-sample Mendelian Randomization (MR): individual effects and inverse-variance weighted (IVW) average. Effects are expressed as standard deviation changes in eBMD per natural log-transformed pg/mL decrease in circulating VEGF.

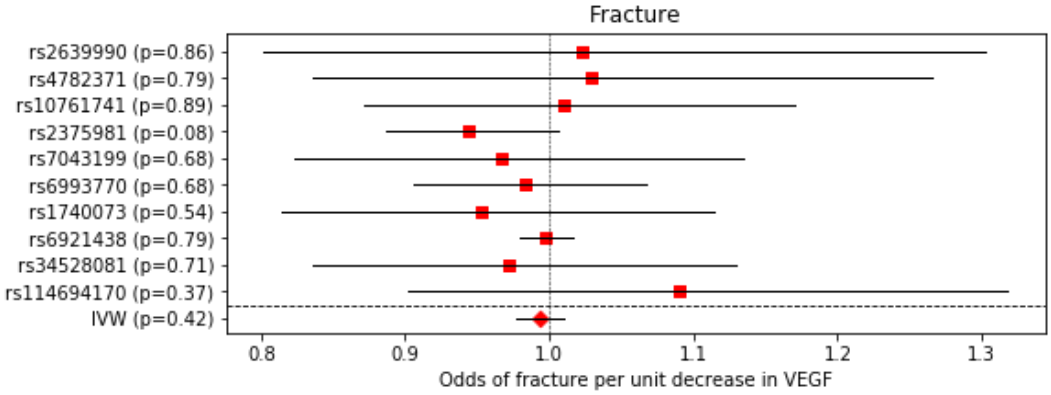
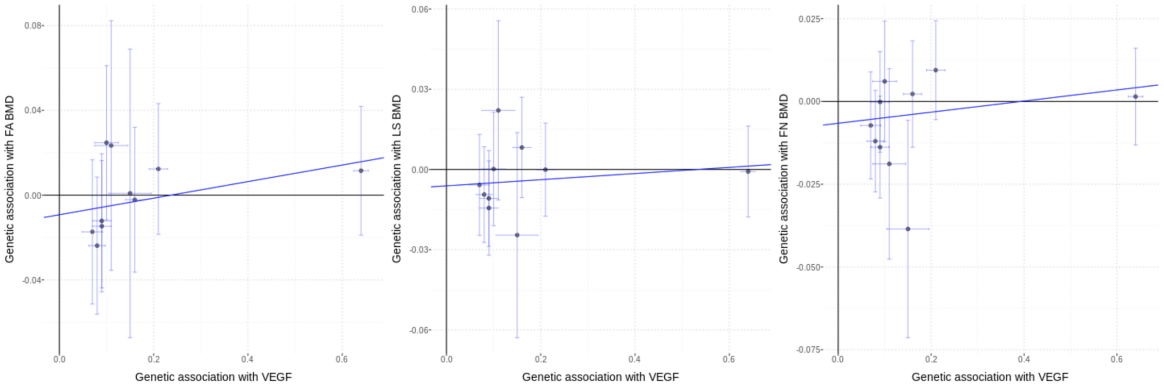


Figure S6. Sensitivity analysis on fracture including all VEGF-decreasing alleles.

Two-sample Mendelian Randomization (MR): individual effects and inverse-variance weighted (IVW) average. Effects are expressed as odds of fracture per natural log-transformed pg/mL decrease in circulating VEGF.



Method	Estimate	Std Error	95% CI	p-value
MR-Egger	0.039	0.031	-0.021, 0.100	0.206
(intercept)	-0.009	0.008	-0.025, 0.007	0.252

Residual Standard Error : 0.891
Heterogeneity test statistic = 6.3452 on 8 degrees of freedom, (p-value = 0.6086)
I^2_GX statistic: 99.8%

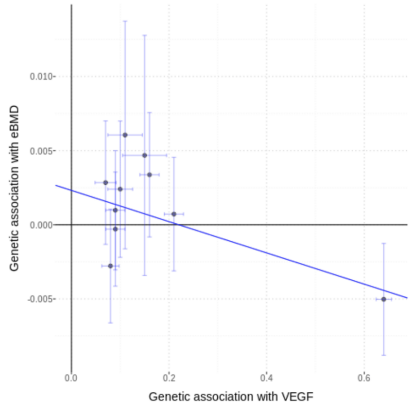
Method	Estimate	Std Error	95% CI	p-value
MR-Egger	0.012	0.017	-0.022, 0.045	0.505
(intercept)	-0.006	0.005	-0.015, 0.003	0.178

Residual Standard Error : 0.961
Heterogeneity test statistic = 7.3838 on 8 degrees of freedom, (p-value = 0.4958)
I^2_GX statistic: 99.8%

Method	Estimate	Std Error	95% CI	p-value
MR-Egger	0.017	0.018	-0.019, 0.053	0.364
(intercept)	-0.007	0.005	-0.016, 0.003	0.175

Residual Standard Error : 1.243
Heterogeneity test statistic = 12.3663 on 8 degrees of freedom, (p-value = 0.1356)
I^2_GX statistic: 99.8%

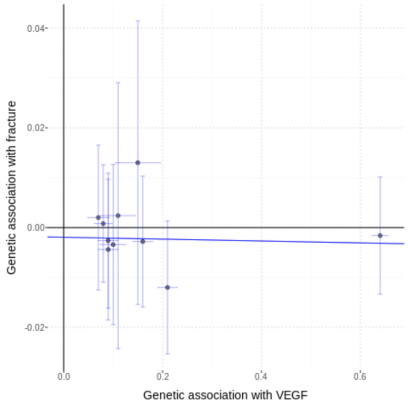
Figure S7. MR-Egger analysis for DXA-measured FA, LS and FN BMD.



Method	Estimate	Std Error	95% CI	p-value
MR-Egger	-0.011	0.004	-0.019, -0.002	0.016
(intercept)	0.002	0.001	0.000, 0.005	0.042

Residual Standard Error : 1.139
Heterogeneity test statistic = 10.3797 on 8 degrees of freedom, (p-value = 0.2394)
I^2_GX statistic: 99.8%

Figure S8. MR-Egger analysis for eBMD.



Method	Estimate	Std Error	95% CI	p-value
MR-Egger	-0.002	0.012	-0.026, 0.022	0.877
(intercept)	-0.002	0.003	-0.008, 0.005	0.559

Residual Standard Error : 0.705
Heterogeneity test statistic = 3.9724 on 8 degrees of freedom, (p-value = 0.8596)
I^2_GX statistic: 99.8%

Figure S9. MR-Egger analysis for fracture.

Chapter 4: Discussion

Osteoporosis is a common and costly disease. BMD is the single best predictor of future osteoporotic fractures. Genetic variation explains a large proportion of variance in BMD and can be used to improve osteoporosis screening guidelines. Genetic determinants of BMD can also be used, in conjunction with GWAS summary statistics for circulating biomarkers to assess the causality of circulating biomarkers in osteoporosis, leading to a better understanding of whether the manipulation of these biomarkers could serve as effective treatment or prevention strategies for osteoporosis. The purpose of this thesis was to leverage our understanding of the genetics of BMD to, first, improve the efficiency of osteoporosis screening programs and, second, to investigate the utility of targeting circulating VEGF for the treatment or prevention of osteoporosis. Below, we discuss the strengths and limitations of each chapter.

Chapter 2 of this thesis represents the first proof of concept in the use of PRS to improve upon clinical guidelines for osteoporosis screening. Building a PRS in 341,449 individuals, we showed that genetic variants explain an important proportion of the variance in BMD and demonstrated, in 5 separate validation cohorts comprising 10,522 individuals, that a PRS threshold that excluded individuals at a low genetic risk from an osteoporosis screening program substantially reduced the proportion of individuals requiring expensive BMD testing with a relatively small reduction in the sensitivity to identify individuals eligible for therapy. While our results were not intended to be prescriptive of how to use genetic information in the clinic, they did show that polygenic risk scores are now mature enough to require careful consideration as to how they can be incorporated meaningfully into clinical care.

This study was not without limitations. Our polygenic risk score was trained uniquely on individuals of White British ancestry from the UK Biobank. It is important to note that our results may not generalize to individuals of other ancestries. Ancestry-specific polygenic risk score models

and modified NOGG screening simulations are to be repeated as genomic datasets grow in size and diversity.

Chapter 3 represented an attempt to identify a novel pharmaceutical target, circulating VEGF, for the treatment of prevention of osteoporosis. However, using large sample sizes for circulating VEGF, BMD and fracture, we found that a decrease in circulating VEGF was not associated with clinically-relevant changes in BMD or odds of fracture, despite large changes in circulating VEGF captured by our instrumental variables. Although this was a null result, the findings suggested that circulating VEGF does not have an important causal effect on osteoporosis in humans, which is inconsistent with several observational studies that suggest a role for circulating VEGF on BMD. This result provides evidence that these studies may have been influenced by confounding or reverse causation and that VEGF is not an effective drug target for the treatment or prevention of osteoporosis.

Given that GWAS summary statistics for VEGF, BMD and fracture were computed on individuals of European ancestry, the lack of causality can only be discussed for such individuals. Therefore, it is possible that lowered levels of VEGF are sufficient to influence bone mineral density in groups of different ancestries and our results do not permit comment on such individuals. Further, since our study was performed using summary statistics which represent the average effect of variants on circulating VEGF in a population, our results only provide evidence that supplementing VEGF would have a null effect on osteoporosis outcomes in the general population. We cannot comment on individuals that have extreme levels of circulating VEGF. Lastly, while our results provide evidence for the role of circulating VEGF, they do not provide information about the role of intracellular VEGF on osteoporosis outcomes.

Chapter 5: Conclusion and Future Aims

This thesis was an investigation of the clinical utility of incorporating genetic information into osteoporosis screening programs and of targeting circulating VEGF for the treatment or prevention of osteoporosis. In Chapter 2, we demonstrated that polygenic risk scores can be useful to effectively exclude individuals at low genetic risk from osteoporosis screening programs. In Chapter 3, we used MR to show that previous observational studies that associated circulating VEGF to osteoporosis outcomes were likely influenced by confounding or reverse causation, providing the first evidence suggesting that manipulating levels of circulating VEGF is unlikely to be an effective treatment for osteoporosis. Several future aims can be suggested to continue these projects.

As our polygenic risk score model was only trained on individuals of White British ancestry, it may not accurately reflect true genetic risk when applied to individuals of other ancestries. Chapter 2 can be repeated on large datasets of non-European ancestry. Polygenic risk scores and modified screening guidelines should therefore be trained and validated on non-European cohorts such as the Kadoorie Biobank (www.ckbiobank.org), Biobank Japan[30] and other future trans-ethnic cohorts as they will likely result in polygenic risk score models that differ in the set of SNPs considered as well as in the effect sizes associated with SNPs that they have in common. Further, the threshold at which individuals are excluded from the modified NOGG screening guidelines should be investigated in cohorts of non-European ancestry. It is possible that the balance of environmental and genetic contributions to osteoporosis is different across ancestries, requiring a different threshold at which individuals should be excluded from the screening procedure.

Chapter 3 should also be replicated in datasets of non-European ancestry to confirm that the causal effect of VEGF on osteoporosis outcomes is null, independent of ancestry. Our results provided evidence for a lack of causality only in individuals of European ancestry and do not permit comment on individuals of other ancestries. Lastly, this analysis can be repeated on other circulating

biomarkers for which GWAS summary statistics are available to better understand their effectiveness as pharmacological targets for the treatment or prevention of osteoporosis.

In conclusion, the findings presented in this thesis represent the first successful implementation of genetic information into osteoporosis screening programs and the first evidence suggesting that VEGF supplementation is unlikely to have clinically-relevant effects on osteoporosis outcomes.

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Appendix

Ethics Approval

For Chapters 2 and 3, written and informed consent was obtained for each participant and was approved by each participating site's regional ethical review board.