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Development of a MetaPGS for Accurate Prediction of Osteoporotic Fracture

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DEVELOPMENT OF A METAPGS FOR ACCURATE PREDICTION OF OSTEOPOROTIC FRACTURE

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Abstract

Introduction: Early identification of individuals at high-risk for osteoporotic fractures who may benefit from preventive intervention is essential. However, the predictive accuracy of the currently used fracture risk assessment tool remains suboptimal. The first aim of this research is to construct genome-wide polygenic scores for the femoral neck (PGS_FNBMD_{ldpred}) and total body BMD (PGS_TBBMD_{ldpred}) and to estimate their potential in identifying individuals with a high risk of osteoporotic fractures. The second aim is to validate the predictive performance of two previously established PGSs (PGS_FNBMD_{ldpred} and PGS_TBBMD_{ldpred}) in an external cohort of 9,000 postmenopausal women of European ancestry. The third aim is to develop and evaluate a novel approach called metaPGS, which combines genetic information from multiple fracture-related traits to further improve the predictive accuracy of genetic information in fracture risk assessment.

Methods: The first manuscript constructed genome-wide PGS for femoral neck and total body BMD. We externally tested the PGSs, both by themselves and in combination with available clinical risk factors, in 455,663 European ancestry individuals from the UK Biobank. The predictive accuracy of the developed genome-wide PGS was also compared with previously published restricted PGS employed in fracture risk assessment. The PGSs developed in the first study were then externally validated in the second study using the Women's Health Initiative (WHI) study data. The magnitude of the association between each PGS and Major Osteoporotic Fractures (MOF)/Hip Fractures (HF) risk was assessed by using the Cox Proportional Hazard Model. To investigate whether adding PGS would improve the predictive ability of FRAX, we formulated four models: (1) Base model: FRAX risk factors; (2) Base model +

PGS_FNBMD_{ldpred}; (3) Base model + PGS_TBBMD_{ldpred}; (4) Base model + metaPGS. The reclassification ability of models with PGS was further assessed using the Net Reclassification Improvement (NRI) and the Integrated discrimination improvement (IDI). In the third study, to develop the novel metaPGS combining PGSs of multiple fracture-related traits/diseases, we first derived individual PGS from genome-wide association studies of 16 fracture-related traits. Then, we employed an elastic-net logistic regression model to examine the association between the 16 PGSs and fractures while controlling for covariates such as age, sex, and the first four principal components. The optimal metaPGS model was chosen based on the highest area under the receiving-operating characteristic curve (AUC). The metaPGS was constructed by combining the 11 most significant individual PGSs selected using the elastic regularized regression model. We evaluated the predictive power of the metaPGS alone and in combination with clinical risk factors recommended by guidelines. The ability of the models to reclassify fracture risk was also assessed using NRI and IDI.

Results: In the first study, for each unit decrease in PGSs, the genome-wide PGSs were associated with up to 1.17-fold increased fracture risk. The genome-wide total body PGS (PGS_TBBMD_{ldpred}) (HR: 1.17; 95%CI 1.15-1.19, p<0.0001) showed a significantly higher association with fractures compared to the restricted total body BMD (PGS_TBBMD_{81}) (HR: 1.03; 95%CI 1.01-1.05, p=0.001). In the reclassification analysis, compared to the FRAX base model, the models with PGS_FNBMD_{63}, PGS_TBBMD_{81}, PGS_FNBMD_{ldpred}, and PGS_TBBMD_{ldpred} improved the reclassification of fracture by 1.2% (95% CI, 1.0% to 1.3%), 0.2% (95% CI, 0.1% to 0.3%), 1.4% (95% CI, 1.3% to 1.5%), and 2.2% (95% CI, 2.1% to 2.4%), respectively. The second study failed to validate the findings discovered in the first study. The results showed that these PGSs were not significantly correlated with MOF or HF in the

WHI cohort. Additionally, incorporating genetic information into the FRAX tool showed minimal improvement in the predicted probabilities of hip fracture risk for elderly Caucasian women. In the third study, the metaPGS had a significant association with incident fractures (HR: 1.22, 95% CI: 1.19 - 1.27), which was stronger than previously developed bone mineral density (BMD)-related individual PGSs. The metaPGS had comparable predictive power to established risk factors such as age, body weight, and early menopause. The association between the metaPGS and incident fractures remained significant after adjusting for clinical risk factors, indicating added predictive value beyond established clinical risk factors. Adding the metaPGS to the FRAX model improved the discrimination of fractures from non-fracture cases. Conclusions: The findings indicate that integrating PGS data into clinical diagnosis has the potential to enhance the efficacy of screening programs at a population level. The metaPGS approach shows promise for stratifying fracture risk in the European population, as it combines genetic data from various fracture-related traits, improving fracture risk prediction.

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Table of Contents

List of Tables

List of Figures

List of Appendices

List of Abbreviations

BMD	Bone Mineral Density
FRAX	Fracture Risk Assessment Tool
PGS	Polygenic Score
HR	Hazard Ratios
ICD-10	International Classification of Disease, Ninth Revision
MOF	Major Osteoporotic Fractures
HF	Hip Fractures
FN	Femoral Neck
TB	Total Body
GWAS	Genome-wide Association Study
SNP	Single Nucleotide Polymorphism
HGS	Hand Grip Strength
ALM	Appendicular Lean Mass
WBLM	Whole Body Lean Mass
VD	Vitamin D
SCC	Serum Calcium Concentration
HC	Homocysteine
TSH	Thyroid Stimulating Hormone Level
FG	Fasting Glucose
FI,	Fasting Insulin
T1D	Type 1 Diabetes
T2D	Type 2 Diabetes
RA	Rheumatoid Arthritis
IBD	Inflammatory Bowel Disease
HBS	Hip Bone Size
CAD	Coronary Artery Disease

Chapter 1 Introduction

1.1 Osteoporotic fracture

Osteoporosis is a major health issue, particularly among older individuals, characterized by a decrease in bone mineral density (BMD) and a decline in the structure integrity of bone tissue (Sözen, Tümay, Özışık, & Başaran, 2017a). This condition weakens bones and increases the risk of fractures, which can occur spontaneously or after minor trauma (NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy, 2001). As the most relevant sequelae of osteoporosis, the worldwide prevalence of osteoporotic fracture has surpassed 200 million (Cooper, Campion, & Melton, 1992), with an estimated 8.9 million fractures occurring annually, over 1.5 million of which take place in America (Johnell & Kanis, 2006c). The lifetime risk of osteoporotic fracture is approximately 50% in women and around 30% in men aged 50 years and older (Nguyen, Nguyen D., Ahlborg, Center, Eisman, & Nguyen, 2007). Notably, the incidence rate of fracture increases exponentially with age (Ensrud, 2013). Given the rapidly aging population, previous studies have highlighted fragility fracture as a prevalent and severe skeletal disorder that is expected to increase in prevalence in the coming decades. It is projected that the incidence of hip fracture (HF) will rise from 1.7 million in 1990 to 6.3 million in 2050 (Cooper et al., 1992; Gullberg, Johnell, & Kanis, 1997).

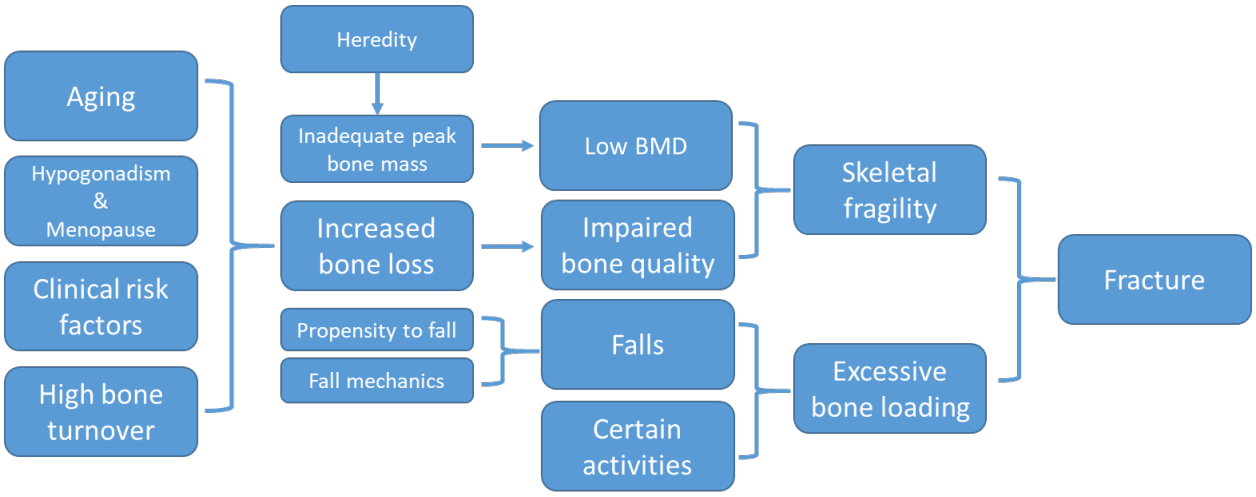
1.2 Pathogenesis of osteoporosis and risk factors of osteoporotic fracture

Osteoporosis is a systemic skeletal disorder characterized by an imbalance in bone remodeling (Feng & McDonald, 2011), where osteoclasts continuously break down bone tissue while

osteoblasts rebuild it (Florencio-Silva, Sasso, Sasso-Cerri, Simões, & Cerri, 2015). If bone resorption outpaces bone formation, bone loss occurs (Manolagas, 2000), which weakens bone tissue microarchitecture and increases bone fragility.

The loss of BMD, typically associated with the aging-related decline in physiological function, is a primary contributor to increased susceptibility to fractures (Pouresmaeili, Kamalidehghan, Kamarehei, & Goh, 2018). Estrogen deficiency accelerates bone turn-over, leading to a decline in BMD and the deterioration of the bone structure, which raises the risk of fractures (Cheng, Chen, & Chen, 2022) Fragility fractures often arise as a result of compromised bone strength and are commonly caused by falls from a standing position or routine activities of daily living (Li et al., 2017). The pathogenesis of osteoporosis-related fractures is multifactorial (Clifford J. Rosen,) and Figure 1 depicts this process. Other hormones, such as growth hormone, testosterone, and insulin, also play a role in regulating bone mass (Giustina, Mazziotti, & Canalis, 2008). Alongside aging and sex steroid deficiency, several clinical risk factors, including low body weight, physical inactivity, cigarette smoking, alcohol consumption, and limited sun exposure, increase the likelihood of fragility fractures (Pisani et al., 2016). Inadequate intake of calcium, vitamin D, and protein can impede the attainment of peak bone mass and its preservation later in life (Mitchell, P. J., Cooper, Dawson-Hughes, Gordon, & Rizzoli, 2015). The utilization of certain medications, particularly glucocorticoids, can also impede bone quality and micro-architectural integrity by reducing the bone formation and hastening bone loss, resulting in secondary osteoporosis (Cosman et al., 2014a). Notably, a positive family history of fracture is a significant risk factor for fracture, implying a critical relationship between an individual's genetic constitution and susceptibility to disease (Pisani et al., 2016).

Figure 1-1 Pathogenesis of osteoporosis-related fractures



1.3 Current fracture risk assessment tool

Previous investigations have indicated that targeting individuals at high risk of fracture or those with a prior fracture can reduce the likelihood of subsequent fractures by an estimated 30-60% (Black & Rosen, 2016; Delmas, Rizzoli, Cooper, & Reginster, 2005). As a result, the assessment of fracture risk plays a crucial role in the management of osteoporosis and the prevention of fractures. By precisely identifying individuals at increased risk, appropriate interventions can be implemented to mitigate the likelihood of sustaining a fracture.

Several fracture risk assessment models have been developed in recent years, utilizing well-established risk factors. In the United States, the Fracture Risk Assessment Tool (FRAX) (Kanis, Johnell, Oden, Johansson, & McCloskey, 2008a) is the most employed assessment tool for determining fracture risk. FRAX incorporates 12 risk factors, such as anthropometric factors,

lifestyle factors, and comorbidities, which can be used either with or without femoral neck BMD (as outlined in Table 1) to estimate 10-year probabilities of major osteoporotic fracture (MOF: including hip, clinical spine, forearm, and humerus fracture) and hip fractures (HF) (Kanis et al., 2008a).

Table 1-1 Risk factors included in the Fracture Risk Assessment Tool (FRAX)

	FRAX
Risk factors (inputs)	Age Gender Femoral neck BMD Bodyweight Height History of prior fracture Parental history of hip fracture Current smoking Chronic glucocorticoid use Rheumatoid arthritis Secondary osteoporosis Alcohol (3 or more units/d)
Output	10-year risk of hip fracture 10-year risk of major osteoporotic fracture
Websites	http://www.shef.ac.uk/FRAX

The prognostic efficacy of FRAX has been extensively evaluated in previous validation studies conducted independently (Azagra et al., 2012; Bolland et al., 2011; Ensrud et al., 2009; Ettinger et al., 2013; Leslie, W. D. et al., 2011a; Leslie, William D. et al., 2010; Sandhu et al., 2010a; Tamaki et al., 2011). However, FRAX's predictive accuracy is suboptimal in terms of discrimination and calibration. A prior meta-analysis reported that the median area under the curve (AUC) value of FRAX with BMD for predicting HF and MOF was 0.78 and 0.69,

respectively (Nguyen, Tuan V. & Eisman, 2017). In general, discrimination in HF was better than MOF, but AUC values for infrequent outcomes such as HF tend to be overoptimistic.

Most validation studies have suggested that the FRAX model underestimates the fracture risk. For instance, a validation study conducted on 1,422 postmenopausal women reported that FRAX consistently underestimated the risk of fracture (Bolland et al., 2011). In a Spanish cohort, FRAX accurately predicted only 46% of MOF and 41% of actual HF cases (Azagra et al., 2012). FRAX also underestimated fracture risk in White men. Results of the Canadian Multicenter Osteoporosis Study cohort showed that FRAX underestimated the risk of fracture in men (predicted 5.4% vs. observed 6.4%) (Leslie, W. D. et al., 2011b). Similarly, in another validation study on 5,891 men from the Osteoporotic Fractures in Men (MrOS) cohort, the FRAX's predicted 10-year probability of HF was 1.4% compared to the observed risk of 3%, with moderate discrimination ability (AUC: 0.76 for HF and 0.69 for MOF) (Ettinger et al., 2013). The study also revealed remarkably poor sensitivity of FRAX in the prediction of MOF. Taking the predicted 10-year risk of 20% as a cut-off value to define high versus low risk, only 15 (4%) out of 373 men who sustained a fracture during the follow-up had FRAX predicted 10-year risk of at least 20% (Ettinger et al., 2013). Although FRAX was calibrated for specific countries, its performance on US data was also concerning, with an AUC value for fracture discrimination no better than chance, especially for men (AUC 0.54) (Sandhu et al., 2010b).

1.4 Genetic advances in osteoporotic fracture

A twin study has demonstrated that the heritability of the liability to fracture may account for up to 50% of the variance (Michaëlsson, Karl, Melhus, Ferm, Ahlbom, & Pedersen, 2005). Unlike the Mendelian traits, which arise from variation in a single gene or a small set of genes with

substantial effects, the genetic etiology of fragility fracture has been established as polygenic in nature, with numerous small-effect genes contributing to the outcome. The systematic screening of the genome, made possible through Genome-wide association studies (GWAS), has facilitated the identification of genetic variants that are linked to fracture risk. To date, several GWASs have been conducted to investigate vertebral fracture, with the initial GWAS meta-analysis (GWAMA) revealing one locus on chromosome 16q24 (rs11645938) that was associated with the risk of radiographic vertebral fractures (Oei et al., 2014). A more recent GWAMA also identified a locus located on chromosome 2q13 to be significantly associated with clinical vertebral fractures (Alonso et al., 2018). In 2018, Trajanoska et al. conducted the largest GWAS on osteoporotic fractures and identified 15 fracture loci (Trajanoska, Katerina et al., 2018). Due to the limited number of loci discovered through GWAS for dichotomous disease as a direct outcome (Visscher, Brown, McCarthy, & Yang, 2012), various fracture-related endophenotypes such as BMD (Estrada et al., 2012), heel BMD (Kemp et al., 2017a), lean mass (Zillikens et al., 2017) and handgrip strength (Matteini et al., 2016) have been widely used as alternatives to explore the genetic basis of fracture. BMD has been found to be highly heritable, with heritability estimates ranging from 50% to 80%, and has been the most extensively studied trait in GWASs related to osteoporosis (Arden, Baker, Hogg, Baan, & Spector, 1996a; Arden & Spector, 1997a). The initial GWAS conducted on 8,557 individuals identified two variants that were associated with both lumbar spine and femoral neck BMD (Richards et al., 2008). Collaborative studies subsequently identified a growing number of genetic variants. The first GWAS meta-analysis (GWAMA) of the GEnetic Factors for OSteoporosis consortium identified 13 novel loci associated with BMD (Rivadeneira et al., 2009), followed by a second GEFOS GWAMA, which replicated the majority of the known BMD-related loci and identified an

additional 32 novel loci (Estrada, Karol et al., 2012). Among the BMD-associated loci, 14 were also associated with osteoporotic fractures. Recent GWASs have also used BMD estimated from heel ultrasound (eBMD) as the trait of interest. The first study on eBMD identified 203 loci associated with eBMD (Kemp et al., 2017b), while the most recent one identified 518 loci, 301 of which were novel (Morris et al., 2019b). Besides the great genetic influence on BMD, twin, and family studies have also shown that other bone parameters like geometry ($\boldsymbol{h^2}$=30-70%) (Demissie et al., 2007), bone ultrasound measure ($\boldsymbol{h^2}$=40-50%) (Arden, Baker, Hogg, Baan, & Spector, 1996b), and high-resolution peripheral quantitative computed tomography (HR-pGCT) measures of bone microarchitecture ($\boldsymbol{h^2}$=20-80%) are also highly heritable (Karasik et al., 2017). To date, over 20 GWAS have been published for different bone parameters.

1.5 Polygenic score

GWASs have indicated that common single-nucleotide polymorphisms (SNPs) play a crucial role in determining susceptibility to common diseases. It is hoped that for each disease, there will be a considerable number of susceptibility SNPs that individually show only a modest association with the disease. However, when combined, they could explain a considerable proportion of the variance in disease incidence within the general population. At present, the only available method for assessing the genetic predisposition to a disease or trait at an individual level is through the use of a polygenic score (PGS).

PGS are typically constructed using the summary statistics of a GWAS, with various approaches and algorithms available to generate them. The traditional approach (Dudbridge, 2013) involves calculating the sum of genome-wide significant risk alleles (typically with $p<5*10^{-8}$) associated with a phenotype of interest in each individual. These risk alleles are weighted by the effect size estimated from the GWAS on the phenotype. However, this approach is not optimal because it

fails to account for linkage disequilibrium (LD) between genetic markers or simply discords potential informative SNPs that could be useful for constructing more accurate PGS.

Previous research has demonstrated that utilizing whole genome-wide PGS rather than a limited number of genome-wide significant SNPs can result in significantly higher predictive power (Khera, Amit V. et al., 2018). To capture additional predictive capacity, researchers have developed several novel algorithms to aggregate SNP effects across the entire genome (Choi, Mak, & O'Reilly, 2020). These advanced PGS can extend to loci with only small associations that do not meet genome-wide significance threshold and account for correlations in effect size resulting from LD. For instance, the Pruning and Thresholding (P+T) method is an approach used to construct PGS while accounting for LD. This method involves selecting an independent subset of variants from the GWAS to be used as a standard PGS. The pruning process begins by selecting the most significant variant and then excluding all markers in LD with this index variant that have an r^2 value above a predetermined cutoff. The most significant remaining marker is then selected as a second index variant, and the process continues until all index variants have been identified at a specific significance level. More advanced methods like Bayesian and variable reduction models can model the joint effect of all markers (Vilhjálmsson et al., 2015). These models use approaches commonly employed in regression analysis with correlated data and to predictor selection. Bayesian models integrate a prior probability distribution for the parameters of interest with the observed data to derive a posterior distribution. These models incorporate LD information from a reference panel and apply shrinkage to marker effects. To accurately capture the genetic architecture of the trait, prior distributions are chosen that consider the LD structure and overall heritability of the genome.

In addition to the techniques used in constructing a PGS based on a single set of GWAS summary statistics for one trait or disease, recent analytical advancements have led to the development of a more powerful genomic risk score called "metaPGS" (Inouye et al., 2018). This approach utilizes multiple sets of GWAS summary statistics, potentially overcoming limitations of power and heterogeneity that exist in single PGS construction. In other fields metaPGS has been shown to outperform single PGS. For instance, in the case of coronary artery disease (CAD), the meta-score combining multiple CAD-related PGSs has been demonstrated to enhance risk prediction beyond that of any individual PGS (Inouye et al., 2018). By using the joint predictive power of multiple PGS in a single regression model, the metaPGS approach can exploit genetic correlations between the outcome trait and a multitude of traits.

1.6 The current utility of polygenic risk scores in fracture prediction

Genomic risk prediction has an advantage over established risk factors since it can be used to estimate disease risk from birth, enabling preventive strategies to be initiated before the emergence of conventional risk factors and their discriminative capacity. However, there has been limited translational success in using genetic information for fracture prediction in clinical practice.

Previous attempts to improve fracture assessment models by incorporating BMD-related PGSs showed consistent ability to identify individuals at high risk but provided limited improvement in risk reclassification. Simulation studies suggested that a genetic profile consisting of up to 50 genetic variants could improve the accuracy of fracture prediction by 10% points of AUC (Tran et al., 2011), while recent studies in postmenopausal women of Korean background found that a genetic profile of 39 SNPs could enhance the precision of non-vertebral fracture prediction and define the risk threshold (Lee, Seung Hun et al., 2013). However, the predictive power of

previous BMD-related PGSs for fracture has been limited due to the heterogeneity of the fracture phenotype and the assumption of independence between contributing loci in the traditional PGS method, which is biased in the presence of LD and can significantly reduce the predictive performance of models. As a result, prior PGSs explained only a small fraction of the interindividual differences in genetic risk and had weak predictive power. Moreover, since fragility fracture is a multifactorial disease, PGS derived from only one trait, such as BMD, may not sufficiently capture the genetic components of fracture. Hence, the current strategy of constructing fracture using a simple method and a single trait has limitations.

Two directions have been proposed to improve the predictive power of genetic profiles for fracture assessment but not yet explored in the field. The first direction involves using more advanced PGS algorithms such as P+T, Bayesian, and Variable Reduction models to increase the predictive value of a single PGS. The second direction involves integrating genetic information from multiple fracture-related traits and risk factors of fragility fracture to generate a metaPGS. This multi-trait extension approach has been used in other fields and may further enhance the predictive accuracy of genetic profiles for fracture assessment.

1.7 Study aims and hypotheses

While fracture prediction models have advanced osteoporosis research, there is still a need for improvement. Genetics plays a significant role in the risk of many common diseases and can provide advantages in predicting disease risk from birth. However, genetic profiling is not currently included in fracture assessment models. Previous studies have suggested the potential of PGS for fracture risk prediction, but their predictive power has been limited. Recent advancements in PGS construction have overcome power and heterogeneity limitations, and the objective of this dissertation is to develop a well-powered genetic predictor that utilizes GWAS

summary statistics for multiple fracture-related traits. Additionally, the goal is to create a genetic-enhanced, individualized risk assessment tool that can accurately identify the at-risk population early in life. Therefore, the dissertation will be structured around three separate research aims and corresponding hypotheses, which are outlined below:

- Aim 1: To construct a BMD-related genome-wide polygenic score and to estimate the potential in identifying individuals at high-risk for osteoporotic fractures.
- Aim 2: To validate the clinical usefulness of genome-wide PGS in improving the accuracy of the FRAX in an external cohort of postmenopausal women.
- Aim 3: To develop and evaluate a novel metaPGS to improve the predictive accuracy of genetic information in fracture risk assessment.

Chapter 2 Manuscript 1

"The Clinical Utility of the BMD-related comprehensive Genome-wide polygenic score in identifying individuals with a high risk of osteoporotic fractures"

2.1 Abstract

2.1.1 Purpose

This study sought to construct genome-wide polygenic scores (PGS) for femoral neck and total body bone mineral density (BMD) and to estimate their potential in identifying individuals with a high risk of osteoporotic fractures.

2.1.2 Methods

Genome-wide polygenic scores were developed and validated for femoral neck and total body BMD. We externally tested the PGSs, both by themselves and in combination with available clinical risk factors, in 455,663 European ancestry individuals from the UK Biobank. The predictive accuracy of the developed genome-wide PGS was also compared with previously published restricted PGS employed in fracture risk assessment.

2.1.3 Results

For each unit decrease in PGSs, the genome-wide PGSs were associated with up to 1.17-fold increased fracture risk. Out of four studied PGSs, $\boldsymbol{PGS\_TBBMD_{81}}$ (HR: 1.03; 95%CI 1.01-1.05, p=0.001) had the weakest and the $\boldsymbol{PGS\_TBBMD_{ldpred}}$ (HR: 1.17; 95%CI 1.15-1.19, p<0.0001) had the strongest association with an incident fracture. In the reclassification analysis, compared

to the FRAX base model, the models with $\boldsymbol{PGS\_FNBMD_{63}}$, $\boldsymbol{PGS\_TBBMD_{81}}$, $\boldsymbol{PGS\_FNBMD_{ldpred}}$, and $\boldsymbol{PGS\_TBBMD_{ldpred}}$ improved the reclassification of fracture by 1.2% (95% CI, 1.0% to 1.3%), 0.2% (95% CI, 0.1% to 0.3%), 1.4% (95% CI, 1.3% to 1.5%), and 2.2% (95% CI, 2.1% to 2.4%), respectively.

2.1.4 Conclusions

Our findings suggested that an efficient PGS estimate enables the identification of strata with up to a 1.7-fold difference in fracture incidence. Incorporating PGS information into clinical diagnosis is anticipated to increase the benefits of screening programs at the population level.

2.1.5 Keywords

Disease and Disorders of/related to Bone; Fracture Risk Assessment; Genetic Research; Human Association Studies; Osteoporosis.

2.2 Introduction

Osteoporosis is an age-related, devastating bone disease characterized by low bone mineral density (BMD) and structural deterioration of bone tissue (Sözen, T., Özışık, & Başaran, 2017a), resulting in an increased risk of fracture. As the world population ages rapidly, bone fracture is becoming a major public health issue. Each year, osteoporosis is responsible for more than 8.9 million fractures globally, of which more than 1.5 million occur in the United States (Johnell & Kanis, 2006a). In 2025, osteoporotic fractures are projected to increase to over 3 million in the US (Burge et al., 2007). The increasing fracture incidence renders early identification and preventive intervention a vital goal.

Several fracture predictive tools have been developed in recent years. In the United States, the Fracture Risk Assessment Tool (FRAX) is the most widely used fracture prediction tool, which

is well-established and validated to predict 10-year probabilities of major osteoporotic fracture (MOF) and hip fracture (HF) on the basis of 12 clinical risk factors (Kanis, Johnell, Oden, Johansson, & McCloskey, 2008b). However, the performance of FRAX in discriminating fracture and non-fracture cases is too often unsatisfactory, which certainly indicates that there is still room for improvement (Briot et al., 2014a; Crandall, Schousboe, Morin, Lix, & Leslie, 2019a; Sornay-Rendu, Munoz, Delmas, & Chapurlat, 2010a).

The predisposition to osteoporotic fracture is attributable to the complex interaction between genetic and non-genetic factors (Trajanoska, K. & Rivadeneira, 2019a). As a major determinant of fracture risk, BMD measured by dual-energy X-ray absorptiometry (DXA) has been proven to be highly heritable (Andrew, Antioniades, Scurrah, Macgregor, & Spector, 2005a; Arden & Spector, 1997b; Michaëlsson, K., Melhus, Ferm, Ahlbom, & Pedersen, 2005a; Ralston, S. H. & Uitterlinden, 2010a; Wagner, Melhus, Pedersen, & Michaëlsson, 2012) and has thus been widely investigated in Genome-wide association studies (GWAS) (Estrada, Karol et al., 2012; Kim, 2018; Medina-Gomez et al., 2018a). Numerous BMD-associated genetic variants, mainly single nucleotide polymorphism (SNPs), have been discovered in the past decade (Estrada, Karol et al., 2012; Kim, 2018; Morris et al., 2019a). As a result, the polygenic score (PGS), calculated according to GWAS summary statistics and an individual's genotype profile, is often used to quantify the genetic propensity of individuals to a disease/trait (Lewis & Vassos, 2020).

Prior studies have demonstrated the potential use of BMD-decreasing PGS in predicting fracture risk; however, they provided only limited predictive power (Eriksson, J. et al., 2015; Nethander et al., 2020a; Warrington, Kemp, Tilling, Tobias, & Evans, 2015; Xiao, X. & Wu, 2021). A PGS based on 62 femur neck-related SNPs revealed a hazard ratio (HR) of 1.20 for incident fracture per one standard deviation (SD) increase (Ho-Le, Center, Eisman, Nguyen, & Nguyen, 2017a).

Another study derived a similar PGS from 63 BMD-related SNPs was also reported to have a significant association with fracture risk in adults (Mitchell, J. A., Chesi, Elci, McCormack, Roy, Kalkwarf, Lappe, Gilsanz, Oberfield, Shepherd, Kelly, Grant, & Zemel, 2016). However, previously published genetic risk scores included genetic variants restricted to those that reached genome-wide significant levels ($p < 5*\mathbf{10^{-8}}$). Due to the polygenic nature of BMD, previously established "restricted PGSs" were not able to sufficiently capture the underlying genetic predisposition, thus failing to provide a comprehensive assessment of genomic information in fracture risk prediction. PGSs calculated from millions of variants across the genome and accounting for linkage disequilibrium (LD) between variants was proven to outperform traditional PGS in the risk prediction of several diseases, such as cardiovascular disease, type II diabetes, and breast cancer (Agerbo et al., 2015; Inouye et al., 2018; Mavaddat et al., 2019). However, whether a novel BMD-related genome-wide PGS derived from an improved PGS algorithm would significantly increase the predictive power of the genetic components in fracture prediction remains unclear. Therefore, we aimed to build more robust and generalizable genome-wide PGSs for BMD to provide a more comprehensive fracture risk evaluation. We compared the accuracy of the genome-wide PGS with previously published PGS in fracture risk assessment. We also aimed to assess the added value of PGS beyond FRAX in fracture prediction. We hypothesized that genome-wide PGSs would outperform previously published "restricted PGS" in assessing fracture risk and that combining genome-wide PGS with FRAX could better identify individuals at high risk of osteoporotic fracture.

2.3 Methods

2.3.1 Study cohort

UK Biobank (UKB) is a large-scale, population-based observational study consisting of 502,617 individuals aged 40-69 recruited from across the United Kingdom between 2006 and 2010 (Sudlow, Cathie et al., 2015). A total of 488,251 participants were genotyped using Affymetrix arrays (Bycroft, Clare et al., 2018). The genotype data were quality controlled and imputed using the Haplotype Reference Consortium (McCarthy et al., 2016a). At recruitment, a standardized socio-demographic questionnaire, medical history, and other lifestyle factors were collected. Individual records were linked to the Hospital Episode Statistics (HES) records and the National Death and Cancer Registries. Compared to the general population, the UKB participants were healthier, less obese, and less likely to smoke and drink alcohol (Fry et al., 2017). Since the PGSs were derived based on predominately White GWAS participants and the people of non-European ancestry comprised only a small proportion of the UKB, we restricted the analysis to 452,936 white British individuals so as to analyze individuals with a relatively homogeneous ancestry.

2.3.2 Fracture event ascertainment

Fracture cases were identified through the Hospital Episodes Statistics linked through NHS Digital, with a hospital-based fracture diagnosis irrespective of mechanism within the primary (data field #41202; n= 435,968) or secondary (n= 435,972) diagnosis field (Appendix A). Fractures of the skull, face, hands, and feet, as well as pathological fractures due to malignancy, atypical femoral fractures, periprosthetic, and healed fractures were excluded from the analysis. The incident fracture cases were defined as having the date of ICD-10–identified fractures after the initial assessment visit. The follow-up time was calculated from the enrollment date to the

first fracture observed or the subjects' death. People who did not experience a fracture or death were followed until 12 years after enrollment.

2.3.3 Ascertaining conventional risk factors

Age, sex, height, weight, body mass index (BMI), previous fracture, current smoking status, glucocorticoid use, rheumatoid arthritis, and secondary cause of osteoporosis (Type 1 diabetes and menopause before age 45 years) were ascertained from the initial assessment visit (Appendix B). Previous fractures were defined as those reported by a questionnaire at enrollment or from ICD-10 codes that occurred before the baseline visit. Gender was self-reported and verified by genotype, and Individuals with discordant sex between self-report and genotype were excluded.

2.3.4 Data processing and quality control

Genotyping of the UKB samples was performed using Affymetrix, UK BiLEVE Axiom, and the Affymetrix UKB Axiom array. The Wellcome Trust Centre for Human Genetics performed the genotype imputation using the Haplotype Reference Consortium (HRC) and the UK10K haplotype resources, which yielded a total of 96 million imputed variants. Quality control was performed for the UKB genotype data: SNPs with minor allele frequency less than 0.1% were missing in a high fraction of subjects (>0.01) and have Hardy-Weinberg equilibrium p-value < $1*\mathbf{10^{-6}}$ were removed. Individuals who have a high rate of genotype missingness (> 0.01) were also excluded from PGS construction. After quality control, a total of 11.5 million variants were retained for analysis.

2.3.5 Polygenic score tuning

The summary statistics of two comprehensive GWA studies conducted among European predominantly cohorts for femoral neck BMD (Estrada, Karol et al., 2012) and total body BMD

(Medina-Gomez et al., 2018a) were used to derive PGSs. UKB samples were not included in any of the two discovery GWASs. The UKB dataset was split into a tuning set (n=3,000) and a testing set (n=452,936). For the tuning set, we randomly selected 1000 prevalent fracture cases and 2000 non-fracture cases of European ancestry. A set of candidate PGSs was derived for each trait by using the Pruning and Thresholding (P+T) method and the LDPred2 computational algorithm in the tuning set.

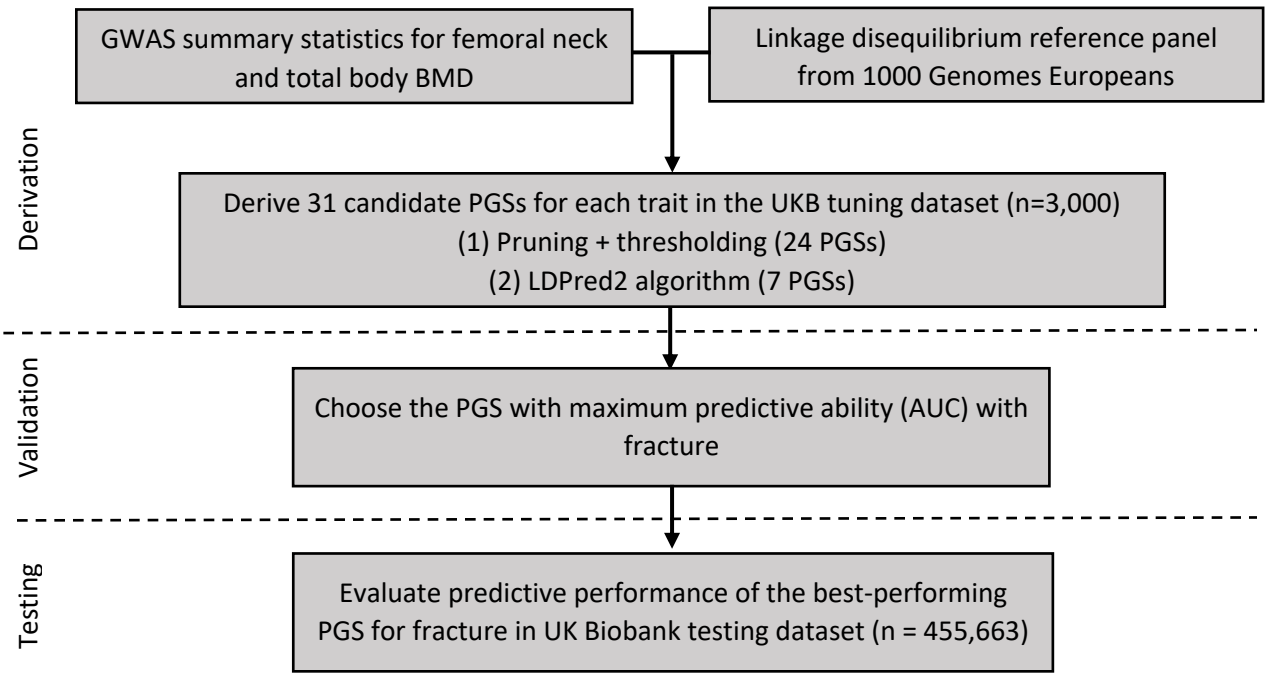
The P+T method PGSs were built using a *p*-value and linkage disequilibrium-driven clumping procedure in PLINK 1.90b. Twenty-four candidate PGSs were identified as having combinations of the *p*-value (1.0, 0.5, 0.05, 5×10^{-4}, 5×10^{-6}, and 5×10^{-8}) and $\boldsymbol{r^2}$ (0.2, 0.4, 0.6, and 0.8) thresholds for each trait.

The LDPred2 computational algorithm was used to generate seven candidate PGSs for each trait. Based on seven hyper-parameter values of ρ (1, 0.3, 0.1, 0.03, 0.01, 0.003, and 0.001), seven sets of candidates PGSs were generated using the LDPred2 computational algorithm grid mode. Each set of PGSs tested a grid of hyper-parameter values, where 102 combinations of hyper-parameters ρ (the proportion of causal variants) and $\boldsymbol{h^2}$ (the SNP heritability) were tuned. For each ρ value, we chose the best model according to the Z-score from the regression of the fracture by the PGS, with age, sex, and BiLEVE/UKB genotyping array and the first four principal components (PCs) being adjusted for. The PGS construction was restricted to the HapMap3 variants only, as LDpred2 suggested (Privé, Arbel, & Vilhjálmsson, 2020a).

Together 31 candidate PGSs were derived. The association between PGS and fracture was further evaluated in odds ratios (OR) per standard deviation of PGS using logistic regression adjusted for age, sex, and BiLEVE/UKB genotyping array and the first four principal

components (PCs). The femoral neck BMD-related PGS ($\boldsymbol{PGS\_FNBMD_{ldpred}}$) and total body-related PGS ($\boldsymbol{PGS\_TBBMD_{ldpred}}$) with the maximum predictive ability (AUC) with fracture were determined to be the best-performing ones and were carried forward into subsequent analyses in the independent UKB testing set. For femoral-neck BMD and total body BMD, $\boldsymbol{\rho}$ thresholds of 0.03 and 0.13, respectively, provided the most optimal discrimination of fracture cases and controls and were chosen to derive the genome-wide PGSs in the UKB testing set for the subsequent analyses. We additionally calculated two previously published femoral neck and total body-related PGSs from Estrada et al. ($\boldsymbol{PGS\_FNBMD_{63}}$) and Xiao et al., ($\boldsymbol{PGS\_TBBMD_{81}}$) in the UKB testing set so as to compare the predictive value of the genome-wide PGS with the "restricted PGS" in assessing fracture risk (Figure 2-1). Since the PGSs were BMD-related, greater PGS is associated with higher BMD and lower fracture risk.

Figure 2-1 Study Design and Workflow



2.3.6 Statistical analysis

Demographic and baseline clinical characteristics of the UKB testing set are presented as mean ± standard deviation (SD) for continuous variables and frequencies (%) for categorical variables. All PGSs were standardized to zero-mean and unit-variance. The primary outcome was an incident fracture that occurred after the baseline visit.

To gauge the potential clinical impact of PGSs, we binned the UKB testing set into 100 groupings based on the percentile of the PGSs and determined the prevalence of fracture within each bin, stratified by sex. The predicted probability of incident fracture based solely on PGSs was also examined by gender. We additionally compared the observed risk gradient with the PGS-predicted risk across percentile bins. For each individual, the 10-year predicted probability of disease was calculated using a simple logistic regression model that includes PGS only. The predicted prevalence of disease within each percentile bin was calculated as the average probability of all individuals within that bin predicted solely by PGS. To illustrate the different cumulative incidences of fracture in individuals with distinct genetic predispositions, we grouped individuals according to different quantile ranges of PGSs: ≤1%, 1-5%, 5-20%, 20-40%, 40-60%, 60-80%, 80-95%, 95-99%, and >99%. The cumulative incidence of fracture by each PGS group was then derived using the cumulative incidence function (CIF), with the competing mortality risk accounted for.

The association between incident fracture risk and each PGS was first assessed using multiple logistic regression models. The discriminatory accuracy of each model was also evaluated using the c-index. Next, we used the Cox proportional hazard modeling to estimate the HRs of PGSs on incident fractures. The Cox proportional hazard model's proportionality assumption was visually inspected beforehand using the Schoenfeld Residual test (SCHOENFELD, 1982a) and

the linearity assumption was checked using the Martingale Residual test (THERNEAU, GRAMBSCH, & FLEMING, 1990a). The UKB testing set satisfied both the proportional hazards and linearity assumptions. Additionally, we examined fracture incidence according to the PGS category in the UKB testing set. We compared the effect of the top percentiles (1%, 5%, 10%, and 20%) with the remaining percentiles (99%, 95%, 90%, and 80%) of each PGS. Using Cox proportional hazard models. The predictive performance of each PGS was also assessed using the C-index. All analyses were adjusted for age, sex, and the first four principal components (PCs).

We also investigated the predictive value of PGS beyond the existing fracture assessment tool. The association between PGS with fracture risk, adjusted for the FRAX risk factors, including age, body weight, height, previous fracture, current smoking, glucocorticoids, and rheumatoid arthritis, was assessed using Cox proportional hazard models. The model with only FRAX risk factors included was set as the base model. In total, five models were formulated as follows: Model 1 – FRAX base model; Model 2 – FRAX + $\boldsymbol{PGS\_FNBMD_{63}}$; Model 3 – FRAX + $\boldsymbol{PGS\_TBBMD_{81}}$; Model 4 – FRAX + $\boldsymbol{PGS\_FNBMD_{ldpred}}$; and Model 5 – FRAX + $\boldsymbol{PGS\_TBBMD_{ldpred}}$. The magnitude of the association between each PGS and fracture risk was assessed by the HRs and its corresponding 95% confidence intervals. In addition, net reclassification improvement (NRI) comparing the nested models was calculated separately for individuals with and without fractures. We designated “high risk” as predicted MOF risk ≥ 20% and “low risk” as predicted MOF risk < 20%, based on the National Osteoporosis Foundation's recommended fixed intervention cutoff. The Integrated discrimination improvement (IDI) was also calculated to incorporate both the direction of change in the calculated risk and the extent of change. All statistical analyses were conducted using R version 4.0.3 software and SAS.

We later discovered a more recent femoral neck BMD-associated GWAS, which reported more SNPs and has better coverage of the genome (Zheng et al., 2015). Therefore, we conducted a sensitivity analysis that tuned and validated a new PGS using summary statistics of this GWAS. The effect of this PGS (PGS_FNnew) on fracture risk was also assessed using both logistic regression and the Cox proportional hazard models, adjusted for age, sex, and the first four PCs.

2.4 Results

2.4.1 Characteristics of the UKB testing set

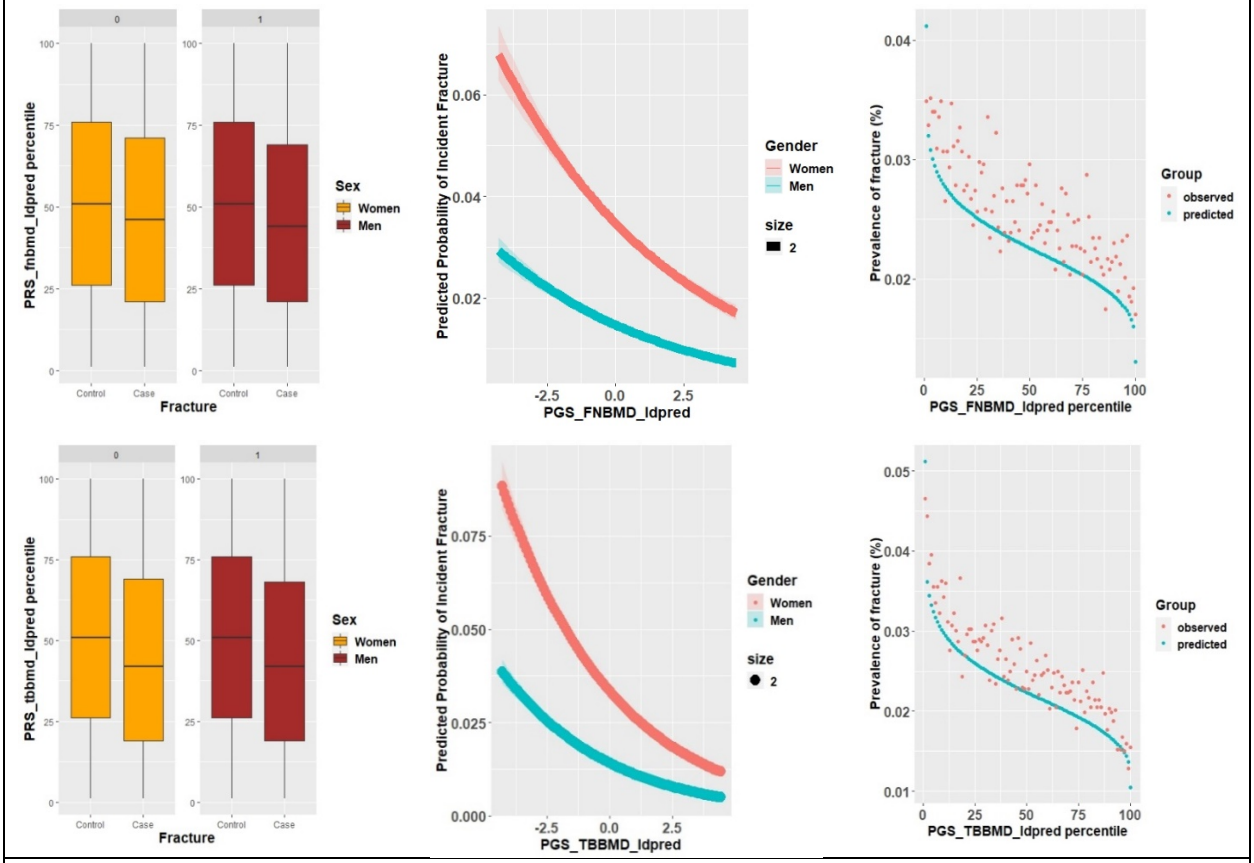
The characteristics of the UKB participants in the testing set (N=455,663) are shown in Appendix C, comprising 17,351 fracture cases and 441,196 non-fracture cases in total. There were 5,720 prevalent fracture cases at the time of recruitment and 11,649 incident cases of fracture during a mean follow-up of 6.2 years. In the UKB testing set, the four PGSs were moderately correlated, with correlation coefficients ranging from -0.03 to 0.43. Separated multiple linear regressions were conducted to examine the association between PGSs and the corresponding measured BMD. As shown in Appendix D, all four PGSs were significantly associated with a higher level of BMD

2.4.2 Fracture risk by PGS groups

In the UKB testing cohort, a lower PGS, which predicts a lower BMD, was associated with higher fracture risk. Our results showed that, for both men and women, the PGS_FNBMD_{63}, PGS_FNBMD_{ldpred}, and PGS_TBBMD_{ldpred} percentile among fracture cases were higher than among healthy controls. The distribution of $\boldsymbol{PGS\_TBBMD_{81}}$; however, it did not show a big difference between fracture cases and non-cases (Figure 2-2A & Appendix E 1A). Similarly, the predicted probability of incident fracture was significantly higher among women than among

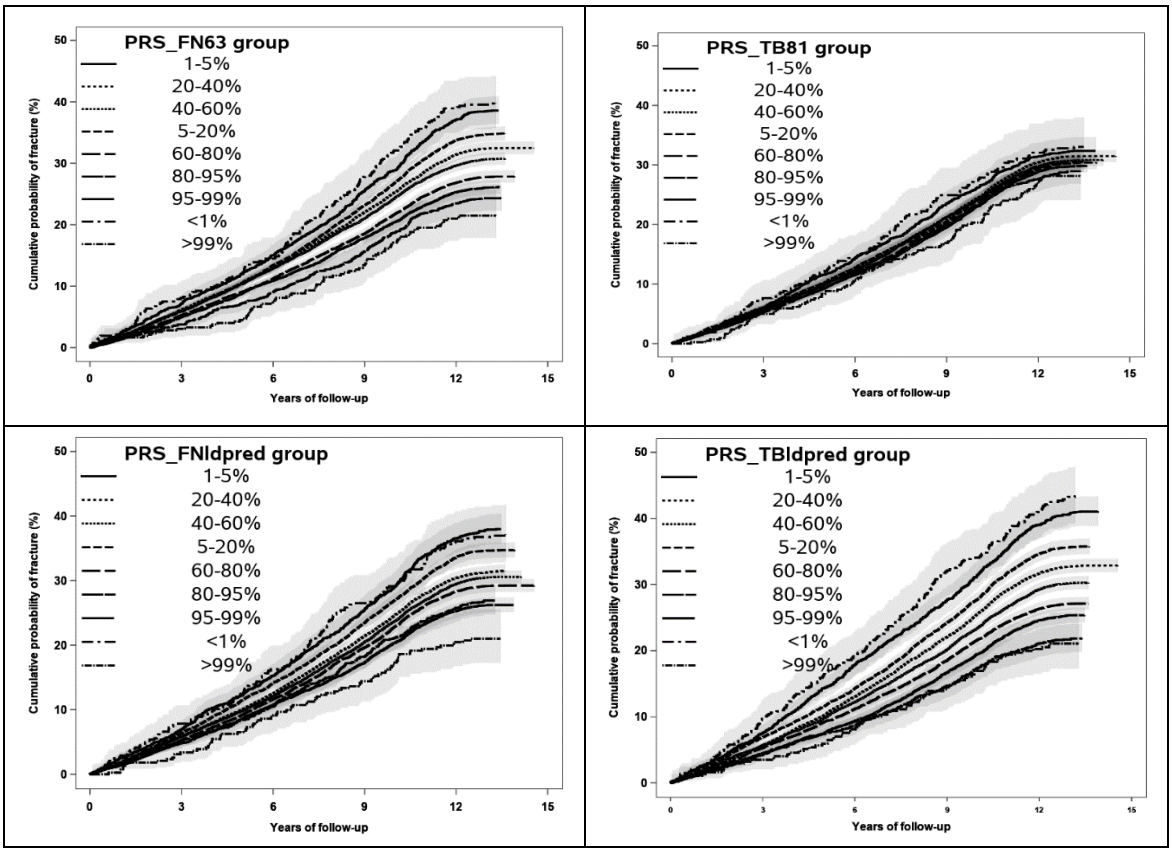
men, and a sharp decrease can be observed in the right tail of the $\boldsymbol{PGS\_FNBMD_{63}}$, $\boldsymbol{PGS\_FNBMD_{ldpred}}$, and $\boldsymbol{PGS\_TBBMD_{ldpred}}$ distributions. Individuals with higher BMD-related PGS have a lower risk of fracture (Figure 2-2B & Appendix E 1B). Based only on the PGSs, the shape of the observed risk gradient was consistent with predicted risk, except for PGS_tbbmd81 (Figure 2-2C & Appendix E 2-1C.). The crude 10-year cumulative fracture incidence by nine PGS groups was shown in Figure 2-3. With competing mortality risk accounted for, significant differences were observed across $\boldsymbol{PGS\_FNBMD_{63}}$, $\boldsymbol{PGS\_FNBMD_{ldpred}}$ and $\boldsymbol{PGS\_TBBMD_{ldpred}}$ groups ($p<0.0001$). The crude fracture incidence was significantly higher among individuals with low PGS (Figure 2-3). Detailed cumulative incidence of fracture by each PGS group is listed in Appendix F.

Figure 2-2 Risk for Incident Fracture According to Genome-wide PGSs.



(A) PGSs percentile among fracture cases versus controls in the UK Biobank testing set. Within each boxplot, the horizontal lines reflect the median, the top and bottom of each box reflect the interquartile range, and the whiskers reflect the maximum and minimum values within each group. (B) Predicted Probability of Incident Fracture by PGSs: Risk gradient for fractures according to the PGS percentiles. 100 groups of the testing dataset were derived according to the percentile of each of the two PGSs. (C) Predicted versus Observed prevalence of incident fracture according to PGS percentiles.

Figure 2-3 Cumulative Incident Function Plot for Fracture According to Decile of the Genome-Wide Polygenic Score (PGS) in UKB Testing Set. Shaded Regions Denote 95% Confidence Intervals.

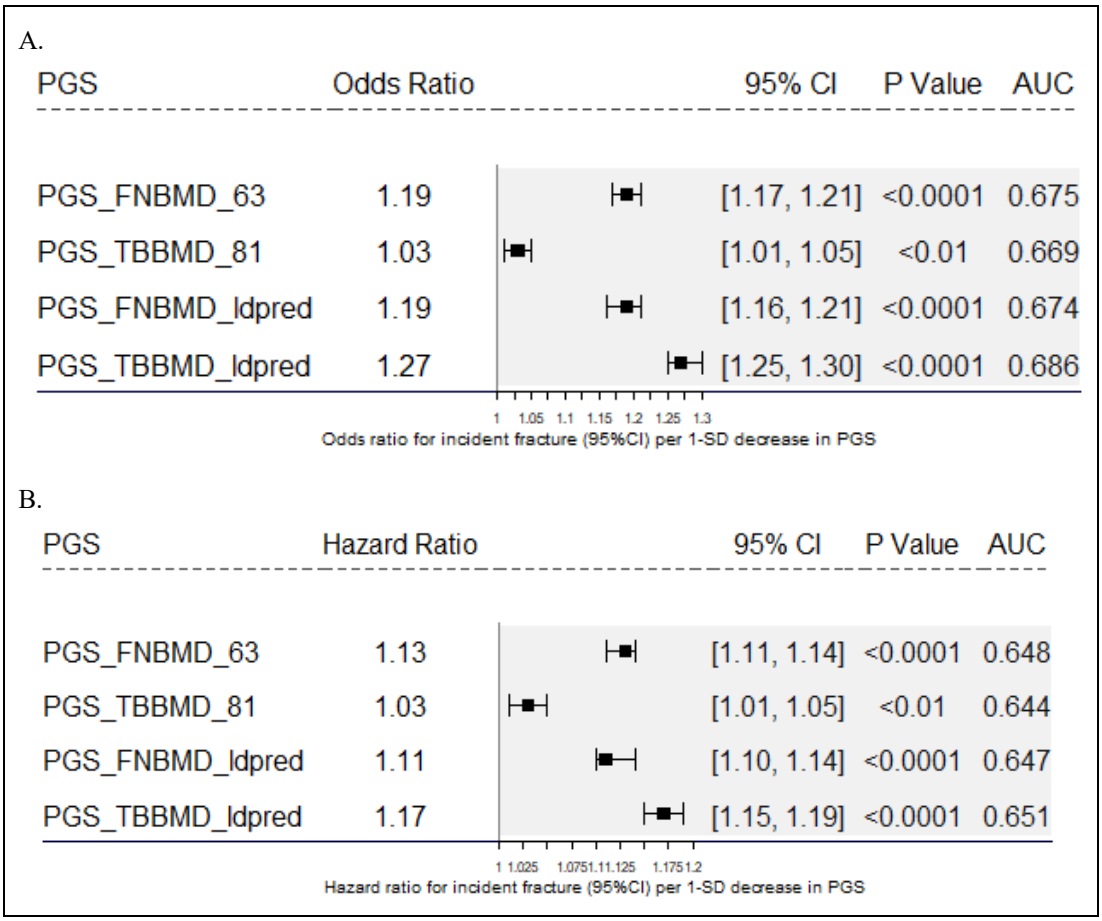


2.4.3 PGSs association with incident fractures

Multiple logistic regression results show that, in the UKB testing set, each of the four GPSs was strongly associated with incident fracture ($p<0.0001$), with an OR ranging from 1.03 to 1.27. A comparison of the genome-wide PGS with previously published PGS from Estrada et al., ($\boldsymbol{PGS\_FNBMD_{63}}$) and Xiao et al. ($\boldsymbol{PGS\_TBBMD_{81}}$) in the UKB testing set is given in Figure 2-4A, showing that the genome-wide PGS of total body BMD had a substantially greater association with fracture risk in terms of OR, whereas the genome-wide PGS of femoral neck

BMD ($\boldsymbol{PGS\_FNBMD_{ldpred}}$) didn't show a significantly higher association with fracture compared to the restricted PGS ($\boldsymbol{PGS\_FNBMD_{63}}$). For total body BMD related PGS, the genome-wide PGS ($\boldsymbol{PGS\_TBBMD_{ldpred}}$) outperformed the restricted PGS ($\boldsymbol{PGS\_TBBMD_{81}}$) with OR estimates per standard deviation decrease at 1.03 (95% CI, 1.01 – 1.05) and 1.27 (95% CI, 1.25 – 1.30) of the $\boldsymbol{PGS\_TBBMD_{81}}$ and $\boldsymbol{PGS\_TBBMD_{ldpred}}$, respectively.

Figure 2-4 Relative Performance of Individual Polygenic Scores (PGS) for Fracture. 4A: Results from Cox Proportional Hazard Models; 4B: Results from Multivariate Logistic Regression Models.



*Separated logistic/Cox proportional hazard regression was conducted for each PGS; each estimate was adjusted for age, sex, and the first four principal components.

The Cox proportional hazard regression results showed attenuated but significant associations between each PGS and fracture risk. For every one-unit decrease of PGSs, the restricted PGS and the genome-wide PGSs were associated with up to 1.13-fold and 1.17-fold increased fracture risk, respectively. Out of four studied PGSs, $\boldsymbol{PGS\_TBBMD_{81}}$ (HR: 1.03; 95%CI 1.01-1.05, p=0.001) had the weakest and the $\boldsymbol{PGS\_TBBMD_{ldpred}}$ (HR: 1.17; 95%CI 1.15-1.19, p<0.0001)

had the strongest association with an incident fracture. Models that include genome-wide PGSs had higher c-indices than models with restricted PGSs (0.651 versus 0.644) (Figure 2-4B).

We also estimated the OR and HR and corresponding 95% CI for individuals in the bottom 1%, 5%, 10%, and 20% of the PGSs, compared with the remaining individuals. Results from Cox proportional hazard regression showed that individuals in the bottom 1% distribution of $\boldsymbol{PGS\_FNBMD_{63}}$, $\boldsymbol{PGS\_FNBMD_{ldpred}}$, and $\boldsymbol{PGS\_TBBMD_{ldpred}}$ had 1.33-, 1.25-, and 1.47-fold increased fracture risk, respectively, compared to their corresponding remaining individuals. In contrast, individuals with extreme $\boldsymbol{PGS\_TBBMD_{81}}$ values did not show a significantly higher risk of fracture. Similar results were observed when applying multiple logistic regression models (Appendix G).

The Cox proportional hazard model showed that, after adjusting for FRAX risk factors available in the UKB testing set, all four PGSs were significantly associated with incident fractures. Out of four PGSs, $\boldsymbol{PGS\_TBBMD_{ldpred}}$ had the strongest association with incident fracture. The HRs of $\boldsymbol{PGS\_FNBMD_{63}}$, $\boldsymbol{PGS\_TBBMD_{81}}$, $\boldsymbol{PGS\_FNBMD_{ldpred}}$, and $\boldsymbol{PGS\_TBBMD_{ldpred}}$ for incident fracture were 1.13 (95% CI, 1.11 – 1.15), 1.03 (95% CI, 1.01 – 1.05), 1.11 (95% CI, 1.09 – 1.14), and 1.16 (95% CI, 1.15 – 1.19), respectively. Compared to the FRAX base model, the association between clinical risk factors and incident fracture risk did not attenuate in all four PGS models (Table 2-1).

The sensitivity analysis showed similar but attenuated results. PGS tuning results show that PGS derived using ldpred2 with $\boldsymbol{\rho}$ thresholds of 0.03 provides the most optimal discrimination of fracture cases and controls and were chosen to derive the genome-wide PGSs in the UKB testing set for the subsequent analyses. The OR and HR of PGS_FNnew for incident fracture were 1.06

(95%CI, 1.05-1.08) and 1.04 (95%CI, 1.03-1.04), respectively. Since the effect of PGS_FNnew on fracture risk is smaller than $\boldsymbol{PGS\_FNBMD_{ldpred}}$, PGS_FNnew was not included in the subsequent analyses.

Table 2-1. Hazard Ratio of Significant Predictive Variables for Incident Fractures in Models with and without PGSs.

Variable	Model 1: FRAX Base Model HR per 1 unit (95% CI)	Model 2: FRAX + $\boldsymbol{PGS\_FNBMD_{63}}$ HR per 1 unit (95% CI)	Model 3: FRAX + $\boldsymbol{PGS\_TBBMD_{81}}$ HR per 1 unit (95% CI)	Model 4: FRAX + $\boldsymbol{PGS\_FNBMD_{ldpred}}$ HR per 1 unit (95% CI)	Model 4: FRAX + $\boldsymbol{PGS\_TBBMD_{ldpred}}$ HR per 1 unit (95% CI)
Age	1.03 (1.02-1.03)	1.03 (1.02 – 1.03)	1.03 (1.02 – 1.03)	1.03 (1.02 – 1.03)	1.03 (1.02 – 1.03)
Sex (women vs. men)	2.83 (2.70 - 2.94)	2.83 (2.70 - 2.94)	2.83 (2.70 – 2.94)	2.83 (2.70 – 2.94)	2.81 (2.63 – 2.94)
Body weight	1.01 (1.01 – 1.01)	1.01 (1.01 – 1.01)	1.01 (1.01 – 1.02)	1.01 (1.01 – 1.02)	1.01 (1.01 – 1.02)
Height	0.98 (0.98 – 0.99)	0.98 (0.98 – 0.99)	0.98 (0.98 – 0.99)	0.98 (0.98 – 0.99)	0.98 (0.98 – 0.99)
Oral glucocorticoid	1.10 (0.87 – 1.22)	1.11 (0.88 – 1.39)	1.10 (0.86 – 1.37)	1.09 (0.87 – 1.39)	1.09 (0.86 – 1.39)
Type 1 diabetes	1.49 (1.30 – 1.69)	1.48 (1.30 – 1.69)	1.48 (1.30 – 1.69)	1.47 (1.28 – 1.69)	1.46 (1.27 – 1.67)
Early menopause	1.02 (0.97 – 1.08)	1.02 (0.97 – 1.07)	1.02 (0.93 – 1.03)	1.02 (0.93 – 1.03)	1.02 (0.93 – 1.03)
Rheumatoid arthritis	1.10 (1.01 – 1.19)	1.10 (1.01 – 1.19)	1.10 (1.01 – 1.19)	1.10 (1.02 – 1.20)	1.10 (1.01 – 1.20)
Current smoking	1.51 (1.43 – 1.59)	1.50 (1.43 – 1.59)	1.50 (1.43 – 1.59)	1.51 (1.43 – 1.59)	1.51 (1.43 – 1.59)
PGS	NA	1.13 (1.11 – 1.15)	1.03 (1.01 – 1.05)	1.11 (1.09 – 1.14)	1.16 (1.15 – 1.19)

2.4.4 Model evaluation

The fracture discrimination ability of PGSs over clinical risk factors was assessed using the concordance index (c-indices) (Appendix H). Compared to the base model, models with PGSs showed moderate improvement in discriminating fracture cases and controls. The $\boldsymbol{PGS\_FNBMD_{63}}$ and the $\boldsymbol{PGS\_TBBMD_{ldpred}}$ improved the discrimination from 0.678 to 0.683 and from 0.678 to 0.686, respectively. In the reclassification analysis, compared to the FRAX base model, the models with $\boldsymbol{PGS\_FNBMD_{63}}$, $\boldsymbol{PGS\_TBBMD_{81}}$, $\boldsymbol{PGS\_FNBMD_{ldpred}}$, and $\boldsymbol{PGS\_TBBMD_{ldpred}}$ improved the reclassification of fracture by 2% (95% CI, 1.5% to 2.4%), 0.2% (95% CI, 0.1% to 0.3%), 1.4% (95% CI, 1.3% to 1.5%), and 2.2% (95% CI, 2.1% to 2.4%), respectively. The $\boldsymbol{PGS\_TBBMD_{ldpred}}$ showed the greatest improvement in terms of reclassification. For the model that included $\boldsymbol{PGS\_TBBMD_{ldpred}}$, 395 individuals were correctly reclassified up to the high-risk group, and 325 individuals who did not experience a fracture were correctly reclassified from the high-risk group to the low-risk group. The continuous NRI showed that improvement in fracture reclassification contributed by $\boldsymbol{PGS\_FNBMD_{63}}$, $\boldsymbol{PGS\_TBBMD_{81}}$, $\boldsymbol{PGS\_FNBMD_{ldpred}}$, and $\boldsymbol{PGS\_TBBMD_{ldpred}}$ were 11.8%, 2.1%, 7.1%, and 13.2%, respectively (Table 2-2).

Table 2-2 Reclassification Table of 10-Year Osteoporotic Fracture Stratified by Event Status. Results of Reclassification Analysis: Percent of Reclassification Compared with FRAX Base Model.

Reclassification										
	Non-fracture Group		Fracture Group		NRI (category)	*p* Value	NRI (continuous)	*p* Value	IDI	*p* Value
	Reclassification down	Reclassification up	Reclassification up	Reclassification down						
PGS_FNBMD_{63}	0.040	0.026	0.010	0.013	0.012 (0.010 to 0.013)	0	0.114 (0.108 to 0.120)	0	0.013 (0.012 to 0.014)	0
PGS_TBBMD_{81}	0.011	0.010	0.004	0.004	0.002 (0.001 to 0.003)	<0.01	0.021 (0.015 to 0.027)	<.01	0.002 (0.001 to 0.023)	0.83
PGS_FNBMD_{ldpred}	0.043	0.028	0.013	0.015	0.014 (0.013 to 0.015)	0	0.071 (0.065 to 0.077)	0	0.014 (0.013 to 0.023）	<0.01
PGS_TBBMD_{ldpred}	0.063	0.034	0.015	0.022	0.022 (0.021 to 0.024)	0	0.132 (0.125 to 0.138)	0	0.032 (0.002 to 0.032）	0.004

*Significant results are in boldface. NRI=net reclassification improvement; IDI=integrated discriminative improvement; 95% confidence intervals are given within brackets.

2.5 Discussion

Early identification of high-risk individuals is crucial in enhancing fragility fracture screening and facilitating preventive interventions (Kanis, 1994b). PGS has the advantage that it can be assessed well before any clinical risk factors emerge. As fragility fracture has a sizable heritable component because of its polygenicity nature, utilizing thousands of genetic variants discovered from GWAS to predict risk holds promise for risk stratification and therefore helps facilitate primary prevention.

Prior studies focused mainly on the predictive ability of PGS derived using genome-wide significant SNPs, resulting in mixed findings. This study systematically derived and validated genome-wide PGS of femoral neck BMD and total body BMD, incorporating information from the entire genome system. To compare the predictive ability of genome-wide PGSs to restricted PGSs, we additionally calculated two previously published PGSs based on 63 femoral neck BMD- and 81 total body BMD-related SNPs, respectively. We quantified the strengths of associations of four PGSs with fracture outcomes in 450,000 UKB participants and demonstrated that PGS accurately predicted striking differences in fracture risk. For the total body BMD, our results showed that the LDpred2 approach, which builds a risk prediction model based on the entire genome, yielded better predictive performance than the approach that includes only 81 variants that reached a genome-wide significance level. However, femoral neck BMD-related PGS calculated using the LDpred2 method showed no improvement over the restricted PGS.

Whether including more SNPs would improve the predictive ability of PGS remains controversial. For many phenotypes, genome-wide PGSs outperform those PGSs calculated by using genome-wide significant variants only, in line with the evidence that much of the genetic predisposition of a disease/trait is explained by the low-level effect SNPs (Speed et al., 2017;

Yang et al., 2010). However, in some cases, including millions of SNPs with negligible effect size in the polygenic score does not affect the predictions (Allegrini et al., 2019; Chung et al., 2019; Fritsche et al., 2019; Khera, A. V. et al., 2018). In a prior study, Khera et al. constructed 30 genome-wide PGSs for five common diseases using up to 7 million SNPs. Results show that genome-wide PGSs had lower c-statistics than PGSs based on genome-wide significant SNPs only (Janssens & Joyner, 2019).

In our study, individuals in the top 1% of total body BMD PGS had an HR of 1.47 compared to the remaining individuals. This level of effects may be sufficient to justify the use of PGSs for clinical screening of individuals in order to detect those in the extreme tail, which may be useful for monitoring and preventive treatment. Several studies have investigated the potential for risk scores based on GWAS-level significant variants in improving fracture risk prediction accuracy and reported weak to no evidence for added value from these scores (Ho-Le, Center, Eisman, Nguyen, & Nguyen, 2017b; Lee, S. H., Lee, Ahn, Kim, Lim, Kim, Cho, Kim, Kim, Kim, Kim, Koh, & Kang, 2013a; Nethander et al., 2020b). More recently, Lu et al. derived a genome-wide PGS (gSOS) of heel ultrasound measurements (speed of sound) using a statistical learning approach (LASSO) and demonstrated that gSOS was more predictive of major osteoporotic fracture and hip fracture than most clinical risk factors. Additionally, they also derived a FRAX-gSOS and demonstrated that it could refine the risk prediction by employing a positive net reclassification index ranging from 0.024 to 0.072 (Lu, Forgetta, Keller-Baruch, Nethander, Bennett, Forest, Bhatnagar, Walters et al., 2021b).

However, as a well-used metric of fracture risk that is incorporated into the FRAX algorithm, the genome-wide PGS for BMD has never been studied. In the current study, we generated more accurate genome-wide PGSs that can possibly capture a larger proportion of total variance in

BMD. BMD-related genome-wide PGSs remained significantly associated with incident fracture risk, even after accounting for FRAX clinical risk factors. Moreover, adding genome-wide PGS to the FRAX clinical risk score has successfully demonstrated significant improvement in predictive accuracy for fracture. The PGS refined risk discrimination and reclassified up to 2% of individuals to a higher or lower fracture risk category. Notably, for total body BMD, in comparison to the restricted PGS, the genome-wide PGS showed significantly better ability in reclassifying individuals who will and will not sustain a fracture.

There are several limitations in the current study worth noting. First, only European ancestry individuals were considered in this study; therefore, the specific PGS calculated here may not have optimal predictive power in other ethnic groups due to different allele frequencies, LD patterns, and effect sizes of common variants across populations of different ethnic backgrounds. Thus, our findings may not generalize to other ethnic groups. Second, due to the limited data availability, we failed to include all 12 clinical risk factors included in FRAX; consequently, a comprehensive evaluation of PGS with complete adjustment was not conducted. Third, the UKB participants were generally younger and healthier than the general population, with a lower incident rate of fracture; this non-random ascertainment is likely to deflate disease prevalence.

2.6 Conclusions

In summary, we constructed two genome-wide PGS for BMD based on the UKB dataset and demonstrated that an efficient PGS estimate enables the identification of strata with up to a 1.5-fold difference in fracture incidence. This finding calls for personalized screening and prevention strategies that incorporate the PGS information into clinical diagnosis, thus considerably increasing the benefits of population-wide screening programs.

Chapter 3 Manuscript 2

"Validation of a genome-wide polygenic score in improving fracture risk assessment beyond the FRAX tool in the Women's Health Initiative Study"

3.1 Abstract

3.1.1 Purposes

Previous study has established two polygenic scores (PGSs) related to femoral neck bone mineral density (BMD) ($\boldsymbol{PGS\_FNBMD_{ldpred}}$) and total body BMD ($\boldsymbol{PGS\_TBBMD_{ldpred}}$) that are associated with fracture risk. However, these findings have not yet been externally validated in an independent cohort. This study aimed to validate the predictive performance of the two established PGSs and to investigate whether adding PGSs to the Fracture Risk Assessment Tool (FRAX) improves the predictive ability of FRAX in identifying women at high risk of major osteoporotic fracture (MOF) and hip fractures (HF).

3.1.2 Methods

The study used the Women's Health Initiative (WHI) cohort of 9,000 postmenopausal women of European ancestry. Cox Proportional Hazard Models were used to assess the association between each PGS and MOF/HF risk. Four models were formulated to investigate the effect of adding PGSs to the FRAX risk factors: (1) Base model: FRAX risk factors; (2) Base model + $\boldsymbol{PGS\_FNBMD_{ldpred}}$; (3) Base model + $\boldsymbol{PGS\_TBBMD_{ldpred}}$; (4) Base model + metaPGS. The

reclassification ability of models with PGS was further assessed using the Net Reclassification Improvement (NRI) and the Integrated discrimination improvement (IDI).

3.1.3 Results

The study found that the PGSs were not significantly associated with MOF or HF after adjusting for FRAX risk factors. The FRAX base model showed moderate discrimination of MOF and HF, with a C-index of 0.623 (95% CI, 0.609 to 0.641) and 0.702 (95% CI, 0.609 to 0.718), respectively. Adding PGSs to the base FRAX model did not improve the ability to discriminate MOF or HF. Reclassification analysis showed that compared to the model without PGS, the model with $\boldsymbol{PGS\_TBBMD_{ldpred}}$ (1.2%, p = 0.04) and metaPGS (1.7%, p = 0.05) improve the reclassification of HF, but not MOF.

3.1.4 Conclusions

The findings suggested that incorporating genetic information into the FRAX tool has minimal improvement in predicting HF risk for elderly Caucasian women. These results highlight the need for further research to identify other factors that may contribute to fracture risk in elderly Caucasian women.

3.2 Introduction

Osteoporosis is a common bone disease that increases predisposition to fractures (Sözen, T., Özışık, & Başaran, 2017b). Worldwide, osteoporotic fractures have become a critical public health issue with a high post-fracture disability and mortality rate, resulting in social and economic burdens (Johnell & Kanis, 2006b). Early detection of the high-risk population could lead to efficacious preventive and therapeutic interventions.

Prior studies have demonstrated the polygenic nature of fractures (Andrew, Antioniades, Scurrah, Macgregor, & Spector, 2005b; Arden & Spector, 1997c; Michaëlsson, K., Melhus, Ferm, Ahlbom, & Pedersen, 2005b; Ralston, S. H. & Uitterlinden, 2010b). The predisposition to osteoporotic fracture is attributable to a complex interaction between genetic and non-genetic factors (Trajanoska, K. & Rivadeneira, 2019b). Many assessment tools have been developed to identify susceptible individuals with a higher propensity to get clinically relevant fractures that merit an intervention (Collins, Mallett, & Altman, 2011; Kanis, Johnell, Oden, Johansson, & McCloskey, 2008c; Nguyen, N. D., Frost, Center, Eisman, & Nguyen, 2008). However, the personalized genetic predisposition of experiencing a fracture was not incorporated into any of those tools. The Fracture Risk Assessment Tool (FRAX) is the most widely used risk stratification tool in North America. FRAX was used to assess the 10-year probability of major osteoporotic fracture (MOF) and hip fracture (HF) on an individual level using 12 clinical risk factors (Kanis et al., 2008c). Nonetheless, the performance of FRAX in terms of fracture discrimination is unsatisfactory (Briot et al., 2014b; Crandall, Schousboe, Morin, Lix, & Leslie, 2019b; Sornay-Rendu, Munoz, Delmas, & Chapurlat, 2010b).

In the past decade, advanced genome-wide association studies (GWAS) have identified millions of genetic variants associated with either fracture or fracture-related traits (Estrada, K., 2012; Medina-Gomez et al., 2018b; Zheng et al., 2015). As a highly heritable (50-85%) skeletal measure (Ralston, Stuart H. & Uitterlinden, 2010) that predicts fracture risk, bone mineral density (BMD) has been comprehensively investigated in several GWASs, with many common genetic variants been discovered (Estrada, K. et al., 2012; Medina-Gomez et al., 2018b; Zheng et al., 2015). Moreover, extensive cohort resources have enabled the genetic prediction of such heritable clinical risk factors from genotypes through polygenic scores (PGS), which capture

information from many single nucleotide polymorphisms (SNPs) assayed from genome-wide genotyping. We previously developed and validated two genome-wide PGSs related to the femoral neck ($\boldsymbol{PGS\_FNBMD_{ldpred}}$) and total body BMD ($\boldsymbol{PGS\_TBBMD_{ldpred}}$) in the UK Biobank (UKB) cohort (Xiao, Xiangxue & Wu, 2022). Both genome-wide PGS was proven to be significantly associated with incident fracture risk, even after accounting for FRAX clinical risk factors (Xiao, Xiangxue & Wu, 2022) However, the UKB participants were generally younger and healthier than the general population. Our prior findings thus lack generalizability outside of the UKB cohort. Also, since not all 12 clinical risk factors in FRAX were available in the UKB, a comprehensive evaluation of PGS with complete adjustment was not conducted.

The objective of this study was twofold: firstly, to conduct a comprehensive validation of the predictive power of two previously established genome-wide PGSs in an external cohort and, secondly, to develop a PGS for BMD by combining the information of both $\boldsymbol{PGS\_FNBMD_{ldpred}}$ and $\boldsymbol{PGS\_TBBMD_{ldpred}}$ using a meta-analytic strategy. The study aimed to assess the PGSs' ability to stratify fracture risk and to determine if combining PGSs with FRAX would enhance the accuracy of identifying individuals at high risk of osteoporotic fracture.

3.3 Methods

3.3.1 Study cohort

The Women's Health Initiative (WHI) study is a nationwide health study aimed at preventing heart disease, breast cancer, and osteoporotic fractures in postmenopausal women. In this study, we used genotype and phenotype data of four WHI sub-studies (the WHI Genomics and Randomized Trials Network (GARNET), the National Heart Lung and Blood Institute (NHLBI),

the Population Architecture using Genomics and Epidemiology (PAGE), and the Women's Health Initiative Memory Study (WHIMS)) acquired through the Database of Genotype and Phenotype (dbGap). As the genetic models were primarily developed and trained using samples of European ancestry, we limited this study to only include Caucasian women to ensure a relatively homogeneous ancestry. Participants who were taking medication that affected BMD (n=0) and participants who did not have complete information regarding FRAX risk factors were excluded from this study (n=797). Overall, the data analysis included 9,203 eligible women.

3.3.2 Ethics approval

This study was approved by the Database of Genotype and Phenotype (dbGap) (https ://www.ncbi.nlm.nih.gov/proje cts/gap/cgi-bin/study .cgi?study id=phs00 0200.v12.p3) and the institutional review board at the University of Nevada, Las Vegas. The data was fully anonymized before we accessed them, and UNLV IRB waived the informed consent.

3.3.3 Fracture and outcome ascertainment

The primary outcome of this study was MOF, which were defined as a composite of hip, humerus, forearm, and clinical vertebral fractures in accordance with the FRAX definition. The follow-up time for WHI participants was calculated from the date of their baseline visit until the occurrence of the first fracture or until the subject's death. Self-reported fractures were identified by questionnaires. People who did not experience a fracture or death were followed up until the end of the WHI main study, was 12 years after enrollment.

3.3.4 Clinical risk factors ascertainment

Information regarding age, race, exercise, smoking status, alcohol intake, previous fragility fractures, familial history of fracture, frequency of falls, medication use were collected at

baseline using a standard medical questionnaire. Heavy drinking was defined as consume more than three alcoholic drinks per day. Smoking status was categorized following the American Heart Association (AHA) guidelines as “never”, “past”, and “current”. Height was measured in centimeters in standing position, and weight was measured in kilograms using a balance beam.

3.3.5 Genotype imputation

The genotype data used in this study were obtained from blood samples and acquired through dbGap. Genotyping was performed using either the Illumina (Illumina, San Diego, California) or Affymetrix 6.0 Array Set Platform (Affymetrix, Santa Clara, California). Genotype imputation was carried out using the Sanger Imputation Server, employing the Haplotype Reference Consortium (HRC) reference panel and the Positional Burrows-Wheeler Transform (PBWT) imputation algorithm.

3.3.6 Polygenic scores

$\boldsymbol{PGS\_FNBMD_{ldpred}}$ and $\boldsymbol{PGS\_TBBMD_{ldpred}}$ were quantified using LDpred2 with optimal hyperparameters determined previously (Xiao, Xiangxue & Wu, 2022). For femoral neck BMD and total body BMD, $\boldsymbol{\rho}$ thresholds of 0.03 and 0.13, respectively, were used to derive the genome-wide PGSs for each individual in the WHI cohort. Since the PGSs were BMD-related, greater PGS is projected to be associated with higher BMD and lower fracture risk.

To construct the metaPGS for BMD, we used the existing $\boldsymbol{PGS\_FNBMD_{ldpred}}$ and $\boldsymbol{PGS\_TBBMD_{ldpred}}$ scores. The metaPGS was calculated as follows:

$$\boldsymbol{PGS_i^{meta}} = \frac{\boldsymbol{\beta_1 Z_{i1} + \beta_2 Z_{i2}}}{\sqrt{\boldsymbol{\beta_1^2 + \beta_2^2 + 2\beta_1\beta_2\rho_{1,2}}}}$$

where $\boldsymbol{Z_{i1}}$, $\boldsymbol{Z_{i2}}$, are the standardized $\boldsymbol{PGS\_FNBMD_{ldpred}}$ and $\boldsymbol{PGS\_TBBMD_{ldpred}}$ for the $\boldsymbol{i}$th individual, respectively. $\boldsymbol{\beta_1}$ and $\boldsymbol{\beta_2}$ are the univariate log odds ratios for each score (estimated using logistic regressions), and $\boldsymbol{\rho_{1,2}}$ is the Pearson correlation between the $\boldsymbol{i}$th and $\boldsymbol{j}$th scores.

3.3.7 Statistical analysis

The $\boldsymbol{PGS\_FNBMD_{ldpred}}$ and $\boldsymbol{PGS\_TBBMD_{ldpred}}$ were standardized with a mean of zero and a standard deviation (SD) of one to enable a standardized comparison of effects. The study categorized participants into three groups based on the percentile distribution (≤10%, 10-90%, and >90%) of each PGS to show the cumulative fracture incidence in individuals with distinct genetic profiles. The observed 10-year incidence of MOF by PGS groups was calculated using the cumulative incidence function (CIF), accounting for the competing mortality risk.

To investigate whether adding PGS would improve the predictive ability of FRAX, we formulated four models: (1) Base model: FRAX risk factors; (2) Base model + $\boldsymbol{PGS\_FNBMD_{ldpred}}$; (3) Base model + $\boldsymbol{PGS\_TBBMD_{ldpred}}$; (4) Base model + metaPGS. The magnitude of the association between each PGS and MOF/HF risk was assessed using hazard ratios (HRs) and corresponding 95% confidence intervals estimated from the Cox Proportional Hazard Models. Model comparison was performed using the likelihood ratio test. The Cox proportional hazard model's proportionality assumption was visually inspected and examined using the Schoenfeld residual test (SCHOENFELD, 1982b) The linearity assumption was checked using the martingale residual test (THERNEAU, GRAMBSCH, & FLEMING, 1990b). All three PGSs ($\boldsymbol{PGS\_FNBMD_{ldpred}}$, $\boldsymbol{PGS\_TBBMD_{ldpred}}$, and metaPGS) were evaluated both as continuous and categorical variables in the survival analyses.

The performance of four models in identifying individuals at risk of sustaining a fracture was evaluated using the Area Under the Curve (AUC) and tested for statistical significance using the DeLong test (DeLong, DeLong, & Clarke-Pearson, 1988). The Net Reclassification Improvement (NRI) was used to assess the reclassification ability of each model by estimating the predicted risk of fracture for each individual and categorizing them into three risk groups. High risk was defined as a predicted MOF risk ≥ 20% (HF risk ≥ 3%), while low risk was defined as a predicted MOF risk < 20% (HF risk < 3%), based on the National Osteoporosis Foundation's recommended intervention cutoff (Cosman et al., 2014b). The NRI was used to determine how well the models with PGSs reclassified subjects compared to the base FRAX model. The Integrated discrimination improvement (IDI) was used to measure the direction and extent of the change in the predicted risk. Statistical analyses were conducted in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA).

3.4 Results

3.4.1 Sample characteristics

The study analyzed a total of 9,203 women who were followed for an average of 12 years. Out of these, 1,255 (13.6%) women sustained at least one MOF during the follow-up period, with 600 (6.5%) experiencing HF. The average duration of follow-up for women who experienced at least one MOF was 6.7 years. The baseline characteristics of the participants were compared between fracture status groups and presented in Table 3-1. Older age, lower body mass index and had higher alcohol consumption were associated with a higher likelihood of fracture. Participants who experienced fractures also had a higher prevalence of prior fractures, family history of HFs,

and falls in the past 12 months. The distribution of $\boldsymbol{PGS\_FNBMD_{ldpred}}$, $\boldsymbol{PGS\_TBBMD_{ldpred}}$, and metaPGS in the WHI cohort was generally normal.

Table 3-1 Characteristics of 9,203 Women Stratified by Major Osteoporosis Fracture (MOF) and Hip Fracture (HF) Status

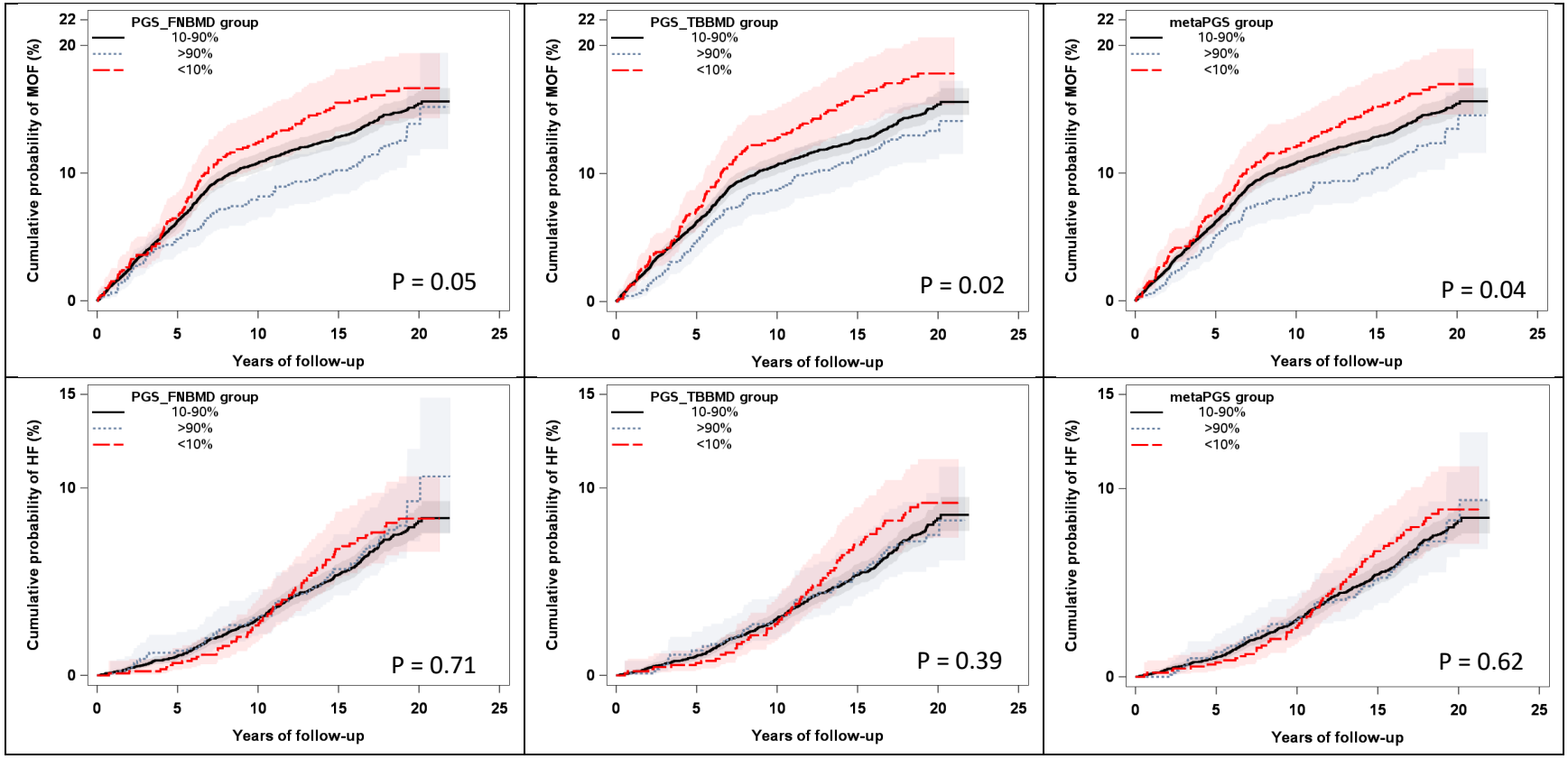
	Subjects without fracture (N=7,948)	Subjects with MOF (N=1,255)	P value	Subjects with HF (N=600)	P value
Age (year), mean (SD)	66.96 ± 6.40	69.07 ± 5.74	<0.01	70.18 ± 5.00	<0.01
Weight (kg), mean (SD)	75.62 ± 15.79	73.24 ± 15.11	<0.01	72.07 ± 15.23	<0.01
Height (cm), mean(SD)	161.36 ± 5.96	161.40 ± 6.10	0.81	161.76 ± 6.13	0.10
Body mass index (kg/m^2), mean (SD)	29.00 ± 5.79	28.10 ± 5.59	<0.01	27.50 ± 5.42	<0.01
Smoking, n (%)					
Never	4,033 (50.74%)	651 (51.87%)	0.70	323 (53.83%)	0.31
Past	3,209 (40.37%)	491 (39.12%)		230 (38.33%)	
Current	706 (8.88%)	113 (9.00%)		47 (7.83%)	
≥ 3 alcohol drinks per day, n (%)					
Yes	119 (1.49%)	22 (1.75%)	0.46	9 (1.50%)	0.99
No	7,829 (98.50%)	1,233 (98.25%)		591 (98.50%)	
Rheumatoid arthritis, n (%)					
Yes	376 (4.73%)	72 (5.74%)	0.14	43 (7.17%)	0.01
No	7,572 (95.27%)	1,183 (94.26%)		557 (92.83%)	
Previous fragility fracture, n (%)					
Yes	3,454 (43.46%)	697 (55.54%)	<0.01	330 (55.00%)	<0.001
No	4,494 (56.54%)	558 (44.46%)		270 (45.00%)	
Familial history of fracture, n (%)					
Yes	1,150 (14.47%)	230 (18.33%)	<0.01	119 (19.83%)	<0.01
No	6,798 (85.53%)	1025 (81.67%)		481 (80.17%)	
$\boldsymbol{PGS\_FNBMD_{ldpred}}$, mean (SD)	0.08 ± 0.80	0.03 ± 0.81	0.04	0.04 ± 0.82	0.30
$\boldsymbol{PGS\_TBBMD_{ldpred}}$, mean (SD)	-0.62 ± 0.79	-0.68 ± 0.79	0.02	-0.69 ± 0.79	0.06
MetaPGS, mean (SD)	0.32 ± 0.81	0.26 ± 0.80	<0.01	0.27 ± 0.80	0.12

3.4.2 Crude 10-year cumulative incidence by PGSs

Figure 3-1 presents the crude 10-year cumulative incidence of MOF and HF according to three PGS groups. After accounting for competing mortality, there were borderline significant differences observed across the group of $\boldsymbol{PGS\_FNBMD_{ldpred}}$ for MOF (p = 0.05), but not for HF (p = 0.71). Similar results were observed across the groups of $\boldsymbol{PGS\_TBBMD_{ldpred}}$ (MOF:

p=0.02) and metaPGS (MOF: p=0.04). Individuals with lower $\boldsymbol{PGS\_FNBMD_{ldpred}}$, $\boldsymbol{PGS\_TBBMD_{ldpred}}$, and metaPGS had higher incidences of MOF and HF

Figure 3-1 Cumulative Incident Function Plot for Fracture According to Decile of the Genome-Wide Polygenic Score (PGS) in WHI. Shaded Regions Denote 95% Confidence Intervals.



*Cumulative Incident for MOF and HF according to the percentile distribution (10%, 10-90%, and >90%) of $\boldsymbol{PGS\_FNBMD_{ldpred}}$, $\boldsymbol{PGS\_TBBMD_{ldpred}}$, and metaPGS, respectively.

3.4.3 Association between PGSs and fracture

The Cox Proportional Hazard model showed that when treating PGSs as continuous variables, all $\boldsymbol{PGS\_FNBMD_{ldpred}}$, $\boldsymbol{PGS\_TBBMD_{ldpred}}$, and metaPGS were not significantly associated with MOF or HF after adjusting for FRAX risk factors (Tables 3-2 & 3-3). We next used PGS groups and included PGS in Cox regression models as categorical variables. The results were similar. Individuals in the top 10% of metaPGS distribution had no increased MOF risk compared with the bottom 10% of the individuals (HR: 0.84, 95% CI, 0.65 – 1.10). Compared to the women in the high $\boldsymbol{PGS\_FNBMD_{ldpred}}$, $\boldsymbol{PGS\_TBBMD_{ldpred}}$, and metaPGS groups, women in the low PGS groups did not show a significantly higher risk of MOF (Table 3-4). Similar findings with HF outcomes were also observed (Table 3-5).

Table 3-2 Results of Cox Proportional Hazard Regression Analyzing the Effect of Baseline Variables on Major Osteoporosis Fracture (MOF) the Women's Health Initiative (WHI) Cohort.

Variable	Model 1: FRAX Base Model HR (95% CI) in MOF	Model 2: FRAX + $\boldsymbol{PGS\_FNBMD_{ldpred}}$ HR (95% CI) in MOF	Model 3: FRAX + $\boldsymbol{PGS\_TBBMD_{ldpred}}$ HR (95% CI) in MOF	Model 4: FRAX + **metaPGS** HR (95% CI) in MOF
Age	1.07 (1.06 – 1.08)	1.07 (1.06 – 1.08)	1.07 (1.06 – 1.08)	1.07 (1.06 – 1.08)
Body weight	0.99 (0.99 – 1.00)	0.99 (0.99 – 1.00)	0.99 (0.99 – 1.00)	0.99 (0.99 – 1.00)
Height	1.02 (1.01 – 1.03)	1.02 (1.01 – 1.03)	1.02 (1.01 – 1.03)	1.02 (1.01 – 1.03)
Previous osteoporotic fracture	1.51 (1.34 – 1.70)	1.50 (1.33 – 1.69)	1.51 (1.34 – 1.70)	1.51 (1.34 – 1.70)
Parental history of hip fracture	1.22 (1.05 – 1.42)	1.22 (1.05 – 1.42)	1.22 (1.05 – 1.43)	1.22 (1.05 – 1.42)
Rheumatoid arthritis	1.24 (0.96 – 1.60)	1.24 (0.96 – 1.60)	1.24 (0.96 – 1.60)	1.24 (0.96 – 1.60)
Current smoking	1.43 (1.15 – 1.77)	1.43 (1.15 – 1.77)	1.42 (1.15 – 1.77)	1.43 (1.15 – 1.77)
Daily drinking > 3	1.09 (0.69 – 1.72)	1.09 (0.69 – 1.72)	1.09 (0.69 – 1.73)	1.09 (0.69 – 1.72)
Secondary osteoporosis	1.03 (0.90 – 1.18)	1.03 (0.90 – 1.18)	1.03 (0.90 – 1.17)	1.03 (0.90 – 1.18)
PGS	NA	0.96 (0.90 – 1.04)	1.02 (0.95 – 1.10)	1.01 (0.94 – 1.09)

*Significant results are in boldface

Table 3-3 Results of Cox Proportional Hazard Regression Analyzing the Effect of Baseline Variables on Hip Fracture (HF) in the Women's Health Initiative (WHI) Cohort.

Variable	Model 1: FRAX Base Model HR (95% CI) in HF	Model 2: FRAX + $\boldsymbol{PGS\_FNBMD_{ldpred}}$ HR (95% CI) in HF	Model 3: FRAX + $\boldsymbol{PGS\_TBBMD_{ldpred}}$ HR (95% CI) in HF	Model 4: FRAX + $\boldsymbol{metaPGS}$ HR (95% CI) in HF
Age	1.12 (1.11 – 1.14)	1.12 (1.11 – 1.14)	1.13 (1.11 – 1.15)	1.13 (1.11 – 1.14)
Body weight	0.99 (0.98 – 1.00)	0.99 (0.98 – 1.00)	0.99 (0.98 – 1.00)	0.99 (0.98 – 1.00)
Height	1.04 (1.02 – 1.05)	1.04 (1.02 – 1.05)	1.04 (1.02 – 1.05)	1.04 (1.02 – 1.05)
Previous osteoporotic fracture	1.45 (1.22 – 1.71)	1.45 (1.22 – 1.71)	1.45 (1.22 – 1.72)	1.45 (1.22 – 1.71)
Parental history of hip fracture	1.30 (1.06 – 1.60)	1.30 (1.06 – 1.60)	1.31 (1.06 – 1.61)	1.30 (1.06 – 1.61)
Rheumatoid arthritis	1.72 (1.25 – 2.38)	1.72 (1.25 – 2.38)	1.72 (1.25 – 2.38)	1.72 (1.25 – 2.38)
Current smoking	1.56 (1.13 – 2.15)	1.56 (1.13 – 2.15)	1.55 (1.13 – 2.14)	1.56 (1.13 – 2.14)
Daily drinking > 3	0.85 (0.42 – 1.70)	0.85 (0.42 – 1.71)	0.85 (0.42 – 1.72)	0.85 (0.42 – 1.71)
Secondary osteoporosis	1.13 (0.94– 1.36)	1.13 (0.94 – 1.36)	1.12 (0.93 – 1.35)	1.13 (0.93 – 1.35)
PGS	NA	1.01 (0.91 – 1.12)	1.09 (0.98 – 1.21)	0.96 (0.86 – 1.06)

*Significant results are in boldface

Table 3-4 Hazard Ratio for Major Osteoporotic Fractures (MOF) in low vs. high PGS groups: Results of Cox Proportional Hazard Models adjusted for FRAX clinical risk factors.

Variable	Model 1: FRAX Base Model HR (95% CI) in MOF	Model 2: FRAX + $\boldsymbol{PGS\_FNBMD_{ldpred}}$ HR (95% CI) in MOF	Model 3: FRAX + $\boldsymbol{PGS\_TBBMD_{ldpred}}$ HR (95% CI) in MOF	Model 4: FRAX + **metaPGS** HR (95% CI) in MOF
Age	1.07 (1.06 – 1.08)	1.07 (1.06 – 1.08)	1.07 (1.06 – 1.08)	1.07 (1.05 – 1.08)
Body weight	0.99 (0.99 – 1.00)	0.99 (0.99 – 1.00)	0.99 (0.99 – 1.00)	0.99 (0.99 – 1.00)
Height	1.02 (1.01 – 1.03)	1.02 (1.01 – 1.03)	1.01 (1.00 – 1.03)	1.02 (1.01 – 1.03)
Previous osteoporotic fracture	1.51 (1.34 – 1.70)	1.50 (1.33 – 1.69)	1.52 (1.35 – 1.71)	1.50 (1.33 – 1.69)
Parental history of hip fracture	1.22 (1.05 – 1.42)	1.22 (1.05 – 1.42)	1.22 (1.05 – 1.42)	1.22 (1.05 – 1.42)
Rheumatoid arthritis	1.24 (0.96 – 1.60)	1.24 (0.96 – 1.60)	1.24 (0.96 – 1.60)	1.24 (0.96 – 1.60)
Current smoking	1.43 (1.15 – 1.77)	1.43 (1.15 – 1.77)	1.40 (1.13 – 1.74)	1.43 (1.15 – 1.78)
Daily drinking > 3	1.09 (0.69 – 1.72)	1.09 (0.69 – 1.72)	1.10 (0.69 – 1.73)	1.09 (0.69 – 1.71)
Secondary osteoporosis	1.03 (0.90 – 1.18)	1.03 (0.90 – 1.18)	1.04 (0.91 – 1.18)	1.03 (0.90 – 1.18)
PGS				
<10%	NA	Ref.	Ref.	Ref.
10 – 90%	NA	0.92 (0.77 – 1.11)	0.88 (0.74 – 1.04)	0.93 (0.78 – 1.12)
>90%	NA	0.84 (0.64 – 1.09)	0.85 (0.66 – 1.10)	0.84 (0.65 – 1.10)

*Significant results are in boldface

Table 3-5 Hazard Ratio for Hip Fractures (HF) in low vs. high PGS groups: Results of Cox Proportional Hazard Models adjusted for FRAX clinical risk factors.

Variable	Model 1: FRAX Base Model HR (95% CI) in HF	Model 2: FRAX + $\boldsymbol{PGS\_FNBMD_{ldpred}}$ HR (95% CI) in HF	Model 3: FRAX + $\boldsymbol{PGS\_TBBMD_{ldpred}}$ HR (95% CI) in HF	Model 4: FRAX + $\boldsymbol{metaPGS}$ HR (95% CI) in HF
Age	1.12 (1.11 – 1.14)	1.12 (1.11 – 1.14)	1.12 (1.11 – 1.14)	1.12 (1.11 – 1.14)
Body weight	0.99 (0.98 – 1.00)	0.99 (0.98 – 1.00)	0.99 (0.98 – 1.00)	0.99 (0.98 – 1.00)
Height	1.04 (1.02 – 1.05)	1.04 (1.02 – 1.05)	1.04 (1.02 – 1.05)	1.04 (1.02 – 1.05)
Previous osteoporotic fracture	1.45 (1.22 – 1.71)	1.45 (1.23 – 1.72)	1.45 (1.22 – 1.71)	1.45 (1.22 – 1.71)
Parental history of hip fracture	1.30 (1.06 – 1.60)	1.30 (1.06 – 1.61)	1.30 (1.05 – 1.60)	1.30 (1.06 – 1.60)
Rheumatoid arthritis	1.72 (1.25 – 2.38)	1.72 (1.25 – 2.38)	1.72 (1.25 – 2.38)	1.72 (1.25 – 2.38)
Current smoking	1.56 (1.13 – 2.15)	1.56 (1.14 – 2.15)	1.56 (1.13 – 2.14)	1.56 (1.13 – 2.15)
Daily drinking > 3	0.85 (0.42 – 1.70)	0.86 (0.42 – 1.72)	0.85 (0.42 – 1.70)	0.85 (0.42 – 1.71)
Secondary osteoporosis	1.13 (0.94– 1.36)	1.13 (0.94 – 1.36)	1.13 (0.94 – 1.36)	1.13 (0.94 – 1.36)
PGS				
<10%	NA	Ref.	Ref.	Ref.
10 – 90%	NA	1.02 (0.78 – 1.33)	0.99 (0.77 – 1.27)	1.02 (0.79 – 1.31)
>90%	NA	1.23 (0.86 – 1.75)	1.07 (0.74 – 1.54)	1.12 (0.78 – 1.60)

*Significant results are in boldface

3.4.4 Model evaluation

To evaluate the ability of PGS_FNBMD_{ldpred}, PGS_TBBMD_{ldpred}, and metaPGS to discriminate fractures over FRAX, the study used the concordance index (C-index), as shown in Table 3-6. The FRAX base model showed moderate discrimination of MOF and HF, with C-index values of 0.623 (95% CI, 0.609 to 0.641) and 0.702 (95% CI, 0.609 to 0.718), respectively. However, no improvement was observed in discriminating MOF and HF when PGSs were added to the base FRAX model.

Table 3-6 Concordance index (and 95% confidence interval) of predicted and observed fracture risk for the model with and without PGS

	Model 1: FRAX Base Model HR (95% CI) in HF	Model 2: FRAX + $\boldsymbol{PGS\_FNBMD_{ldpred}}$ HR (95% CI) in HF		Model 3: FRAX + $\boldsymbol{PGS\_TBBMD_{ldpred}}$ HR (95% CI) in HF		Model 4: FRAX + $\boldsymbol{metaPGS}$ HR (95% CI) in HF	
	C-index	C-index	p-value	C-index	p-value	C-index	p-value
MOF	0.623 (0.609 – 0.641)	0.623 (0.608 – 0.641)	0.72	0.623 (0.609 – 0.641)	0.60	0.623 (0.602 – 0.641)	0.92
Hip fracture	0.701 (0.609 – 0.718)	0.702 (0.609 – 0.718)	0.98	0.702 (0.609 – 0.723)	0.33	0.702 (0.611 – 0.785)	0.63

In the reclassification analysis, compared to the FRAX base model, models with $\boldsymbol{PGS\_FNBMD_{ldpred}}$ (0.37%, p = 0.33), $\boldsymbol{PGS\_TBBMD_{ldpred}}$ (0.5%, p = 0.14), or metaPGS (0.05%, p = 0.99) did not improve the reclassification of MOF (Table 3-7). The model incorporated $\boldsymbol{PGS\_FNBMD_{ldpred}}$ correctly reclassified five individuals (0.45%) to the high-risk group, and eighteen (0.27%) individuals who did not experience a MOF were correctly reclassified to the low-risk group. For the model including metaPGS, two (0.18%) individuals were correctly reclassified to the high-risk group, and seven (0.11%) women who did not experience a MOF were correctly reclassified to the low-risk group. The continuous NRI revealed that the improvement in MOF reclassification contributed by $\boldsymbol{PGS\_FNBMD_{ldpred}}$, $\boldsymbol{PGS\_TBBMD_{ldpred}}$, and metaPGS overall were 0.63% (p = 0.87), 0.88% (p = 0.81), and 1.54% (p=0.68), respectively. Better reclassification provided by PGSs was observed in HF prediction. $\boldsymbol{PGS\_TBBMD_{ldpred}}$ and metaPGS improved the reclassification of HF significantly by 1.2% (p = 0.04) and 1.7% (p=0.05). In the context of HF prediction, the FRAX+metaPGS model correctly reclassified 6 out of 600 (1.1%) participants with HF upward to the high-risk group, and 58 out of 8,603 (0.8%) women who did not experience HF were correctly reclassified downward to the low-risk group. Additionally, the inclusion of $\boldsymbol{PGS\_TBBMD_{ldpred}}$ to the base FRAX model let to a significant increase in IDI of 1.93% (p=0.01) for predicting HF. However,

the overall improvement in fracture event reclassification provided by the PGSs models was minimal.

Table 3-7 Reclassification table of 10-year MOF and HF stratified by event status.

Reclassification										
	Non-fracture group		Fracture group		NRI (category)	*p*	NRI (continuous)	*p*	IDI	*p*
	Reclassification down	Reclassification up	Reclassification up	Reclassification down						
$\boldsymbol{PGS\_FNBMD_{ldpred}}$										
MOF	18 (0.27%)	19 (0.29%)	5 (0.45%)	7 (0.64%)	0.37%	0.33	0.63%	0.87	0.49%	0.08
HF	13 (0.18%)	25 (0.35%)	1 (0.18%)	0 (0%)	-0.17%	0.42	-1.11%	0.87	0.10%	0.76
$\boldsymbol{PGS\_TBBMD_{ldpred}}$										
MOF	9 (0.14%)	5 (0.1%)	6 (0.54%)	4 (0.36%)	0.5%	0.14	0.88%	0.81	0.14%	0.69
HF	102 (1.42%)	113 (1.57%)	8 (1.45%)	6 (1.09%)	1.2%	0.04	6.25%	0.36	1.93%	0.01
metaPGS										
MOF	7 (0.11%)	9 (0.14%)	2 (0.18%)	3 (0.27%)	0.05%	0.99	1.54%	0.68	0.06%	0.46
HF	58 (0.8%)	62 (0.8%)	6 (1.1%)	3 (0.5%)	1.7%	0.05	1.48%	0.83	0.76%	0.06

*Significant results are in boldface. NRI=net reclassification improvement; IDI=integrated discriminative improvement

3.5 Discussion

Implementing individual-level genome-wide PGSs summarizing the underlying genetic predisposition of certain diseases in the clinical setting holds excellent promise. Previously, we developed and internally validated two genome-wide BMD-related PGSs using data from the UKB cohort (Xiao, Xiangxue & Wu, 2022). Our findings indicated that both PGSs were significantly associated with incident fractures, even after being adjusted for FRAX clinical risk factors (Xiao, Xiangxue & Wu, 2022). In the current study, we replicated the two BMD-associated PGSs from previous work (Xiao, Xiangxue & Wu, 2022) and additionally derived a third PGS -- metaPGS combining the effect of the two established PGSs and evaluated their predictive effect in an independent WHI postmenopausal women sample. We examined whether the PGSs could stratify individuals into different risk strata within this external validation cohort and the predictive ability of each PGS beyond the FRAX tool.

Our findings showed that both the BMD-related genome-wide PGSs and the metaPGS did not perform as well and were not significantly associated with fractures in the WHI cohort. Moreover, adding genetic information to the FRAX tool was associated with minimal improvements in predicted probabilities for elderly Caucasian women only HF. These findings were in discordance with our previous research findings (Xiao, Xiangxue & Wu, 2022), of which PGSs calculated based on genome-wide significant loci showed significant association with fractures and provided minor improvement of fracture prediction beyond the base model consisting of convention clinical risk factors. Previous studies have produced mixed results regarding the effectiveness of polygenic scores in improving fracture prediction accuracy. For example, Thao et al. reported that genetic profiling of 63 BMD-related genetic variants could enhance fracture prediction performance when compared to the Garvan fracture risk calculator

(Ho-Le, Center, Eisman, Nguyen, & Nguyen, 2017c). Our prior work demonstrated that incorporating genetic information from 81 BMD-related genetic variants could improve fracture prediction performance beyond FRAXA (Xiao, Xiangxue & Wu, 2021). A more recent study generated and validated a genome-wide PGS for speed of sound (SOS) also reported a consistent association with fracture risk (Lu et al., 2021). However, Eriksson and colleagues reported only minor improvements in fracture prediction when adding a PGS to a base model consisting of age, height, and weight (Eriksson, Joel et al., 2015)

The performance of PGS in different cohorts is affected by many factors. The PGSs may have been overfitted to the training sample, meaning that it is too closely tied to the specific individuals and variants used in the training sample. This inconsistency can result in poor performance when applied to a validation sample. Compared to the UK Biobank genotype data, WHI genotyping data has fewer SNPs and less genome coverage. The hyper-parameters tuned in UKB might not be as optimal in WHI due to heterogeneity between the training and testing cohorts. Also, the allele frequencies of SNPs will vary by population, together with the causal variants and their effect sizes (Martin et al., 2017; Martin et al., 2019). Moreover, genotyping imputation is one way to introduce variability in calculated PGSs at the individual level without changing the underlying genetic model (Chen, S. et al., 2020). The UKB carried out imputation on the genotype data using SHAPEIT3 and IMPUTE4, whereas the WHI was imputed using Positional Burrows-Wheeler Transform (PBWT) imputation algorithm. Lastly, while genetics plays a role in determining traits and conditions, other factors such as the environment, lifestyle, and sociodemographic characteristics can also influence the expression of these traits. So, even individuals with similar ancestry may have different risks for certain conditions based on these other factors, affecting the accuracy of genetic risk predictions. (Mostafavi et al., 2020).

This study comprehensively validated the predictive power of two previously established genome-wide PGSs, as well as a metaPGS that combined information from these two PGSs. Additionally, we assessed the ability of PGSs to stratify fracture risk and to determine if combining PGSs with FRAX would enhance the accuracy of identifying individuals at high risk of osteoporotic fracture. It is essential to acknowledge the limitations of this study. First, the sample size of current study is relatively small to replicate findings discovered in a cohort of half million (UKB); Second, fracture risk of WHI may not be fully captured by the PGS calculated using the hyper-parameters derived in other cohorts. Finally, our study only included individuals of European ancestry, which may limit the generalizability of our findings.

3.6 Conclusions

Early detection of high-risk individuals could lead to efficacious preventive and therapeutic interventions. However, based on the hyper-parameters derived in the UKB, we could not replicate our prior findings in this external independent WHI cohort. The two BMD-related genome-wide PGSs and the metaPGS were not significantly associated with fractures in the WHI cohort. Adding genetic information to the FRAX tool was associated with minimal improvements in predicted hip fracture probabilities among elderly Caucasian women.

Chapter 4 Manuscript 3

"Enhanced Fracture Risk Prediction: A Novel Multi-Trait Genetic Approach Integrating Polygenic Scores of Fracture-Related Traits"

4.1 Abstract

4.1.1 Purposes

Current polygenic scores (PGS) have limited predictive power for fracture risk. To improve genetic prediction, we developed and evaluated a novel approach called metaPGS, which combines genetic information from multiple fracture-related traits.

4.1.2 Methods

We first derived individual PGS from genome-wide association studies of 16 fracture-related traits. Then, we employed an elastic-net logistic regression model to examine the association between the 16 PGSs and fractures while controlling for covariates such as age, sex, and the first four principal components. The optimal metaPGS model was chosen based on the highest area under the receiving-operating characteristic curve (AUC). The metaPGS was constructed by combining the 11 most significant individual PGSs selected using the Elastic regularized regression model. We evaluated the predictive power of the metaPGS alone and in combination with clinical risk factors recommended by guidelines. The ability of the models to reclassify fracture risk was also assessed using Net Reclassification Improvement (NRI) and Integrated Discrimination Improvement (IDI).

4.1.3 Results

The metaPGS had a significant association with incident fractures (HR: 1.22, 95% CI: 1.19 - 1.27 per standard deviation of metaPGS), which was stronger than previously developed bone mineral density (BMD)-related individual PGSs. The metaPGS had comparable predictive power to established risk factors such as age, body weight, and early menopause. The association between the metaPGS and incident fractures remained significant after adjusting for clinical risk factors, indicating added predictive value beyond established clinical risk factors. Adding the metaPGS to the FRAX model improved the discrimination of fractures from non-fracture cases.

4.1.4 Conclusions

The metaPGS is a promising approach for stratifying fracture risk in the European population, improving fracture risk prediction by combining genetic information from multiple fracture-related traits. Our findings support the potential clinical utility of the metaPGS for fracture risk assessment and personalized prevention strategies.

4.2 Introduction

Osteoporosis is a prevalent skeletal disorder characterized by decreased bone mineral density (BMD) and increased susceptibility to fractures, especially in the hip, spine, and wrist (Sözen, Tümay, Özışık, & Başaran, 2017b). Osteoporotic fractures can lead to significant morbidity, mortality, and healthcare expenses (Nazrun, Tzar, Mokhtar, & Mohamed, 2014), with an estimated 2 million cases and $19 billion in costs annually in the United States alone (Singer et al., 2015; Wright et al., 2014). Given the global aging population, the incidence of osteoporosis is projected to increase (Reginster & Burlet, 2006), underscoring the importance of early identification of individuals at high risk of primary fractures.

The risk of osteoporotic fracture has a high heritability, with genetic liability up to 46% (Sigurdsson, Halldorsson, Styrkarsdottir, Kristjansson, & Stefansson, 2008). Genetic factors substantially contribute to fracture risk (Zhu, Bai, & Zheng, 2021). Genome-wide association studies (GWAS) over the past decade have identified single nucleotide polymorphisms (SNPs) associated with bone strength-related traits (Zhu et al., 2021). Around 15 genomic loci and thousands of SNPs be robustly associated with fractures (Trajanoska, K. et al., 2018), and many more genetic associations have been reported for fracture-related traits/risk factors (Jiang et al., 2018; Matteini et al., 2016; Zillikens et al., 2017).

Bone mineral density (BMD) is the most critical predictor of osteoporosis and fracture (Kanis, 1994a). Polygenic score (PGS) derived from GWAS summary statistics for BMD has been used to quantify an individual's genetic liability to fractures (Ho-Le et al., 2017c; Lee, S. H., Lee, Ahn, Kim, Lim, Kim, Cho, Kim, Kim, Kim, Kim, Koh, & Kang, 2013b; Lu, Forgetta, Keller-Baruch, Nethander, Bennett, Forest, Bhatnagar, Walters et al., 2021a; Mitchell, J. A. et al., 2016; Xiao, Xiangxue & Wu, 2021). Previous studies have highlighted the potential of BMD-related PGS for risk prediction of fracture (Ho-Le et al., 2017c; Mitchell, J. A. et al., 2016). Nevertheless, the clinical utility of PGS in fracture prediction is limited, with a marginal additive effect of PGS on clinical factors.

A multi-PGS extension, metaPGS, has been developed to improve predictive performance by combining multiple PGSs into one score (Inouye et al., 2018). It has been applied to many other complex diseases and was proven to significantly increase the predictive accuracy of coronary artery disease (Inouye et al., 2018), ischemic stroke (Abraham et al., 2019), type 2 diabetes (Chen, X. et al., 2021), and breast cancer (Läll et al., 2019). In fracture prediction, an individual's estimated genetic propensity was typically derived based on the GWAS summary statistics of a

single trait, BMD. Considering that fragility fracture is a multifactorial disease influenced by various physiological factors beyond BMD (Clifford J. Rosen,), PGS depending on only one trait may not be sufficient to capture the genetic components of fracture. If a particular disease/trait is causally involved in the etiology of fracture, the PGS for that disease/trait as a genetic proxy should predict fracture occurrence, and a metaPGS may be particularly useful in fracture prediction. Integrating genetic information of multiple fracture-related traits into metaPGS can improve predictive accuracy.

Therefore, this study aimed to develop and validate a multi-trait metaPGS to integrate genetic information of multiple fracture-related traits to improve predictive accuracy. To evaluate the predictive value of metaPGS beyond the currently available fracture prediction tool, we examined the potential clinical use of metaPGS beyond the existing Fracture risk assessment tool (FRAX), an algorithm predicting 10-year probabilities of major osteoporotic fracture (MOF) and hip fracture (HF) based on 12 clinical risk factors (Kanis et al., 2008b). By improving the accuracy of genetic risk prediction for osteoporotic fractures, metaPGS could aid in identifying high-risk individuals and implementing preventive measures.

4.3 Methods

4.3.1 Study cohort

The UK Biobank (UKB) is a large-scale population-based observational study comprising 502,617 individuals aged between 40-69 years, recruited from the United Kingdom between 2006 and 2010 (Sudlow, C. et al., 2015). A standardized socio-demographic questionnaire, medical history, and other lifestyle factors were collected at recruitment. Individual records were linked to the Hospital Episode Statistics (HES) records and the national death and cancer

registries as the underlying genetic models were developed and trained primarily using European ancestry samples, including individuals of white British ancestry in the current study, allowed for a better representation of the genetic architecture in that population and resulted in more accurate predictions. Thus, the current study only included individuals of white British ancestry to examine a relatively homogeneous group.

4.3.2 Fracture events ascertaining

Fracture cases were identified using the baseline questionnaire of self-reported fracture incidents fractures within the past five years. Hospital Episodes Statistics linked through NHS Digital, with a hospital-based fracture diagnosis irrespective of mechanism within the primary or secondary diagnosis field. All the incident fracture cases were identified through the hospital episode statistics. Fractures of the skull, face, hands, and feet, pathological fractures due to malignancy, atypical femoral fractures, and periprosthetic and healed fractures were excluded from the analysis. Based on the date of the ICD-10 record, fractures sustained after the initial assessment visit was defined as incident cases (N=13,623).

4.3.3 Data processing and quality control

A total of 488,251 participants were genotyped using Affymetrix arrays (Bycroft, C. et al., 2018). The genotype data were quality controlled and additionally imputed using the Haplotype Reference Consortium (HRC) (McCarthy et al., 2016b) and the UK10K haplotype resources, yielding a total of 96 million imputed variants. SNPs with minor allele frequency less than 0.1% and SNPs that are missing in a high fraction of subjects (>0.01), Hardy-Weinberg equilibrium p value > $1*\mathbf{10^{-6}}$. Individuals with a high rate of genotype missingness (> 0.01) were excluded

from PGS construction. A total of 450,395 individuals and 11.5 million variants passed the quality control standards and remained for subsequent analysis.

4.3.4 Individual PGS tuning

GWAS summary statistics were available for 16 complex traits/diseases related to fracture risk. PGSs were generated with the estimated effect sizes from the most recent literature on large GWAS (Appendix I). To minimize the risk of over-fitting due to overlapping samples between the GWAS discovery set and the UKB validation set, the selected GWAS did not include UKB samples. GWASs for femoral neck BMD (Estrada, K. et al., 2012), total body BMD (Medina-Gomez et al., 2018b), hand grip strength (HGS) (Matteini et al., 2016), appendicular lean mass (ALM) (Zillikens et al., 2017), whole body lean mass (WBLM) (Zillikens et al., 2017), vitamin D (VD) (Jiang et al., 2018), serum calcium concentration (SCC) (O'Seaghdha et al., 2013), homocysteine (HC) (van Meurs et al., 2013), thyroid stimulating hormone level (TSH) (Teumer et al., 2018), fasting glucose (FG) (Lagou et al., 2021), fasting insulin (FI) (Lagou et al., 2021), type 1 diabetes (T1D) (Robertson et al., 2021), type 2 diabetes (T2D) (Vujkovic et al., 2020), rheumatoid arthritis (RA) (Ha, Bae, & Kim, 2021), inflammatory bowel disease (IBD) (Liu et al., 2015a), hip bone size (HBS) (Styrkarsdottir et al., 2019), and coronary artery disease (CAD) (Nikpay et al., 2015) were selected for individual PGS derivation.

We randomly selected 1000 fracture cases and 2000 non-fracture cases for individual PGS tuning. Based on GWAS summary statistics of 16 fracture-related phenotypes and a linkage disequilibrium reference panel of 503 European samples from 1000 Genomes (phase 3, version 5), a set of candidate PGSs were derived for each phenotype/trait using the Pruning and Thresholding (P+T) method and the LDPred2 computational algorithm (Privé, Arbel, & Vilhjálmsson, 2020b).

Using the P+T method, twenty-four candidate PGSs were calculated with combinations of *P* value (1.0, 0.5, 0.05, 5×10^{-4}, 5×10^{-6}, and 5×10^{-8}) and $\boldsymbol{r^2}$ (0.2, 0.4, 0.6, and 0.8) thresholds for each trait. The LDPred2 computational algorithm grid mode was used to generate seven candidate PGSs based on seven hyper-parameter values of ρ (1, 0.3, 0.1, 0.03, 0.01, 0.003, and 0.001). The PGS construction was restricted to the HapMap3 variants only, as LDpred2 suggested.

For each of the 16 phenotypes, 31 candidate PGS were derived for each individual in the UKB tuning set. As the risk of fractures increases with age due to the weakening of bones, and women are at higher risk for osteoporosis-related fractures than men, the association between each PGS and the fracture was further evaluated in terms of odds ratios (OR) per standard deviation of PGS using logistic regression adjusted for age, sex, and BiLEVE/UKB genotyping array and the first four principal components (PCs). The most optimal model for the largest magnitude odds ratio was selected as the one representative PGS for each trait and carried forward into subsequent analyses.

4.3.5 Derivation of the metaPGS

Each representative PGS determined from the previous step was standardized to have a zero mean and unit standard deviation. We then split the remaining UKB European ancestry dataset into a training set (n=135, 119) and a testing set (n=315,276). Using the UKB training set, we employed elastic-net logistic regression (Zou & Hastie, 2005) to model the association between the 16 PGSs and fracture, adjusting for age, sex, and the first four PCs. A range of models with different penalties was evaluated using 10-fold cross-validation. In terms of the highest area under the receiving-operating characteristic curve (AUC), the best model was selected as the

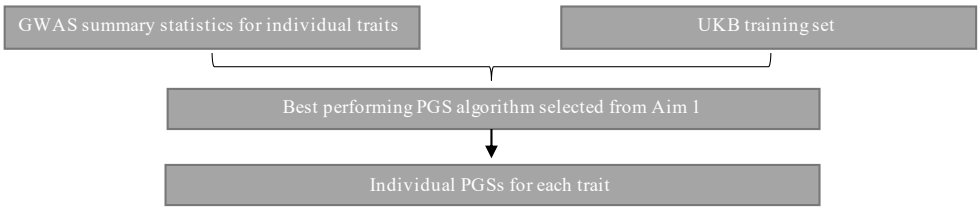
final model to generate metaPGS and held fixed for validation in the UKB testing set. The metaPGS was calculated using a weighted average of the standardized individual PGSs:

$$\boldsymbol{PGS_i^{meta}} = \frac{\boldsymbol{\alpha_1 PGS_{i1}} + \cdots + \boldsymbol{\alpha_{16} PGS_{i16}}}{\boldsymbol{\alpha_1} + \cdots + \boldsymbol{\alpha_{16}}}$$

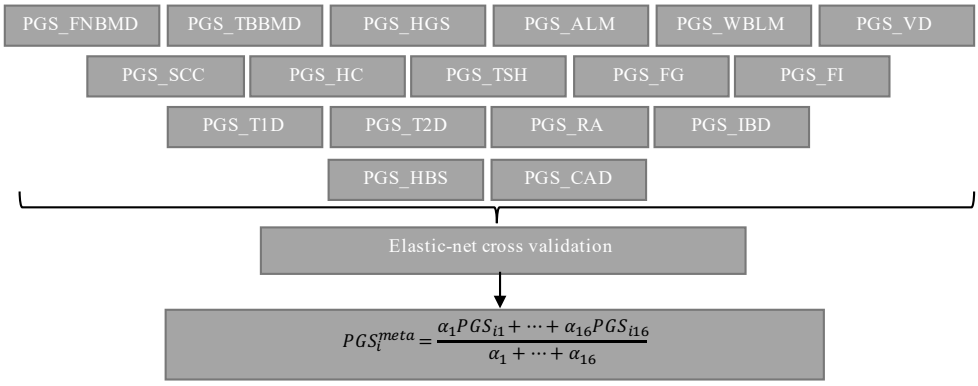
where PGS_{i1},…, PGS_{i16} are the 16 zero mean and unit variance standardized PGSs for the i_{th} individual; α_1,…, α_{19} are the coefficients (log odds ratio) for each of the 16 PGSs (Figure 4-1.)

Figure 4-1 Study design and workflow. a. Derivation of individual PGSs in the UKB training set (n=135,119) using GWAS summary statistics for individual traits. b. The metaPGS for fracture was then derived by integrating individual PGSs using the elastic-net cross-validation. c. Validation of the metaPGS for fracture will be performed in the UKB validation set (n=315,276).

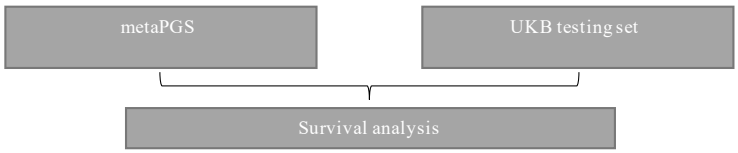
a. Derivation of individual PGSs



b. Derivation of metaPGS for fracture



c. Validation of the metaPGS



PGS, polygenic score; FNBMD, femoral neck bone mineral density; TBBMD, total body bone mineral density; HGS, hand grip strength; ALM, appendicular lean mass; WBLM, whole body lean mass; VD, vitamin D; SCC, serum calcium concentration; HC, homocysteine; TSH, thyroid stimulating hormone level; FG, fasting glucose; FI, fasting insulin; T1D, type 1 diabetes; T2D, type 2 diabetes; RA, rheumatoid arthritis; IBD, inflammatory bowel disease; HBS, hip bone size; CAD, coronary artery disease.

4.3.6 Statistical analysis

The demographic and clinical characteristics of the UKB testing set were described using mean and standard deviation (SD) for continuous variables and the frequency and percent for categorical variables. The primary outcome of this study was incident fractures. All PGSs in the UKB testing set were standardized to facilitate interpretability to have unit variance. To illustrate the different cumulative incidences of fracture in individuals with distinct genetic predispositions, we grouped individuals according to different quantile ranges of metaPGS: ≤1%, 1-5%, 5-20%, 20-40%, 40-60%, 60-80%, 80-95%, 95-99%, and >99%. The cumulative incidence of fracture by metaPGS groups was then derived using the cumulative incidence function (CIF), with the competing mortality risk accounted for.

The separate prediction of each of the 16 trait-specific PGSs was examined by fitting a series of simple logistic regression models. To account for multiple testing across the individual PGSs tested in separate logistic regression models (single-PGS models), we used 10,000 permutations to find the significance threshold to control the false discovery rate P values. Using the UKB training set, we employed elastic-net logistic regression (Zou & Hastie, 2005) to model the association between the 16 PGSs and fracture, adjusting for age, sex, and the first four PCs. Based on significant individual PGSs selected from the Elastic regularized regression model, metaPGS was derived for each individual in the UKB testing set. Two previously developed BMD-related PGSs (PGS_FNBMD (Ho-Le et al., 2017c) and PGS_TBBMD (Xiao, Xiangxue & Wu, 2021)) were also included in the subsequent analysis for comparison purposes.

All scores (PGS_FNBMD, PGS_TBBMD, and metaPGS) were evaluated using logistic regression and Cox proportional hazard regression. C-indexes were derived for the logistic regression, as well as for the Cox models using age as the time scale. Additionally, we examined

the fracture incidence according to the PGS category in the UKB testing set. We compared the effect of top percentiles (1%, 5%, 10%, and 20%) with the remaining percentiles (99%, 95%, 90%, and 80%) of each PGS using Cox proportional hazard models. All regression models were controlled for age, sex, and the first four PCs.

We also investigated the predictive value of metaPGS beyond the existing fracture assessment tool and compared its performance with two previously developed BMD-related PGSs (PGS_FNBMD (Ho-Le et al., 2017c) and PGS_TBBMD (Xiao, Xiangxue & Wu, 2021)). The association between each PGS with fracture risk, adjusted for the FRAX risk factors, including age, body weight, height, previous fracture, current smoking, glucocorticoids, and rheumatoid arthritis, was assessed using Cox proportional hazard models. The model with only FRAX risk factors was set as the base model. Four models were formulated: 1) Model 1 – FRAX base model; 2) Model 2 – FRAX + $\boldsymbol{PGS\_FNBMD}$; 3) Model 3 – FRAX + $\boldsymbol{PGS\_TBBMD}$; and 4) Model 4 – FRAX + $\boldsymbol{metaPGS}$. The magnitude of the association between each PGS and fracture risk was assessed by the hazard ratio and its corresponding 95% confidence intervals.

In addition, net reclassification improvement (NRI) was adopted to compare the reclassification ability of the models with PGSs to those without PGS. We designated "high risk" as the predicted MOF risk ≥ 20% and "low risk" as the predicted MOF risk < 20%, based on the National Osteoporosis Foundation's recommended fixed intervention cutoff (Cosman et al., 2014c). The Integrated discrimination improvement (IDI) was also calculated to incorporate both the direction of change in the calculated risk and the extent of change. All statistical analyses were conducted using R version 4.0.3 software and SAS 9.4 (SAS Institute, Inc., Cary, NC, USA).

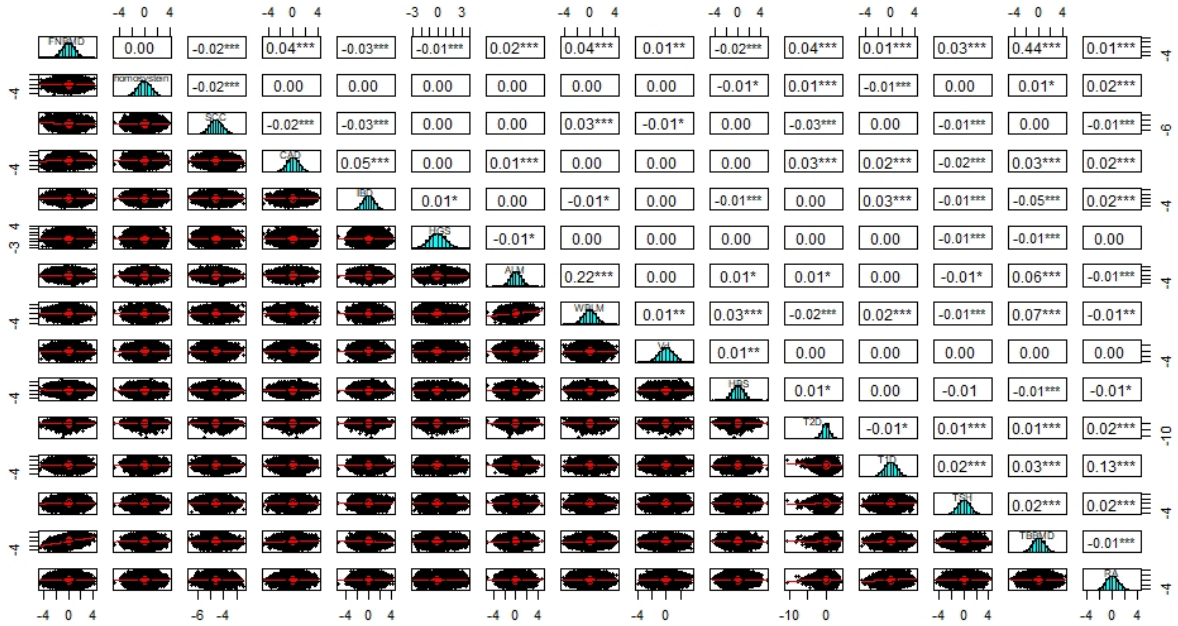
4.4 Results

The characteristics of the UKB testing set are shown in Table 4-1. The overall UKB testing set consists of 315,276 individuals, of which 8,787 were incident fracture cases and 306,489 were non-fracture cases. Figure 4-2 shows correlations between 16 individual PGSs, with strong correlations observed between HC and SCC, SCC and CAD, CAD and IBD, ALM and WBLM, T1D and TSH, TSH and TBBMD, TBBMD, and RA. The metaPGS was derived based on 11 significant individual PGSs selected from the Elastic regularized regression model (model weights are shown in Figure 4-3).

Table 4-1 Participant Characteristics of the UK Biobank validation cohort (N=315,279).

	UKB (N=315,279)	Fracture (n=8,787) (2.79%)	Non-fracture (n=306,489) (97.21%)
Age at assessment, yrs.	56.77 ± 8.02	59.86 ± 7.20	56.68 ± 8.02
Height (cm)	168.67 ± 9.25	166.16 ± 8.85	168.75 ± 9.25
Weight (kg)	78.17 ± 15.90	74.05 ± 15.31	78.29 ± 15.91
Body mass index (BMI) ($\boldsymbol{kg/m^2}$)	27.40 ± 4.77	26.77 ± 4.89	27.41 ± 4.76
Current smoker	32,885 (10.43%)	1,022 (11.63%)	31,863 (10.04%)
Fractures in the past 5 years	47,576 (10.44%)	19,262 (9.25%)	28,314 (11.44%)
Oral glucocorticoid user	1,628 (0.52%)	48 (0.55%)	1,580 (0.52%)
Rheumatoid arthritis	7,524 (2.39%)	403 (4.59%)	7,121 (2.23%)
Type 1 diabetes	2,971 (0.94%)	142 (1.62%)	2,829 (0.92%)
Menopause before age 45 years	24,631 (7.81%)	1,421 (14.12%)	23,390 (7.63%)

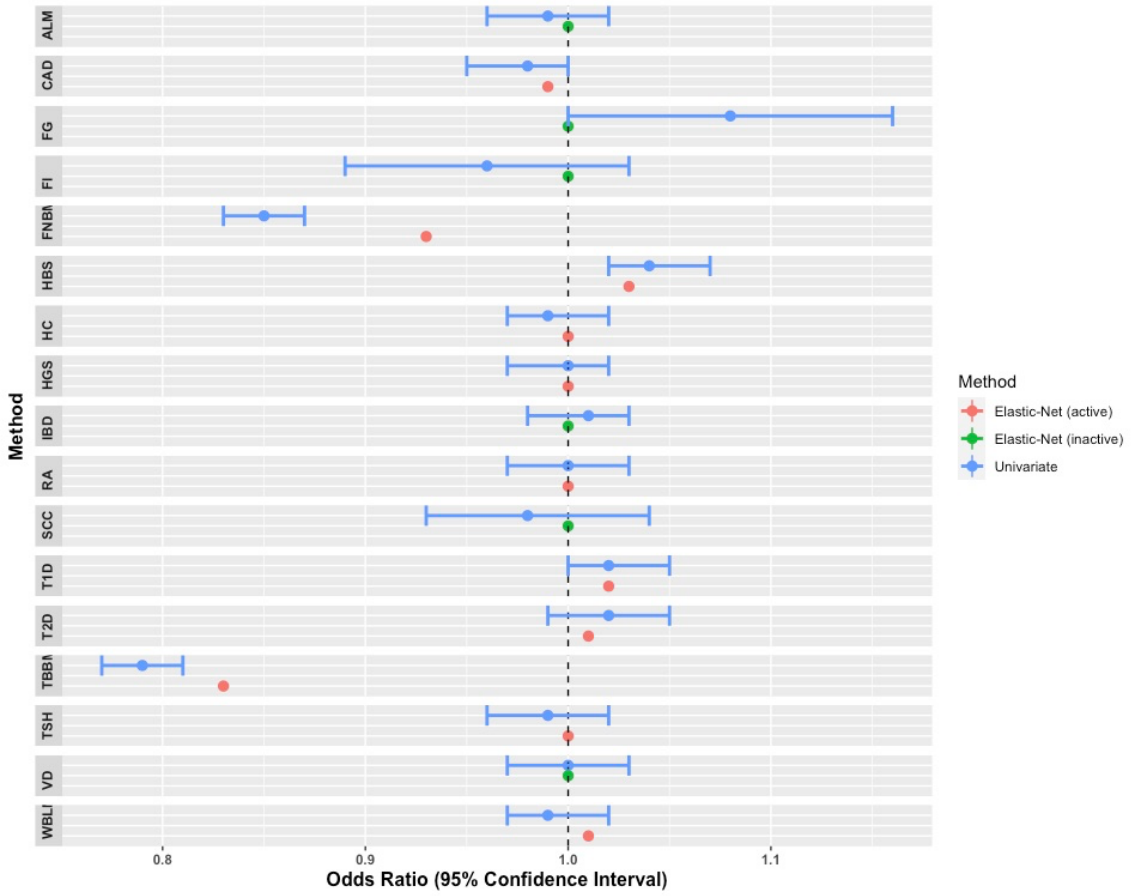
Figure 4-2 Correlations between 16 trait-specific individual PGSs in the UKB derivation set.



$*\mathbf{p < 0.05}$, $**\ \mathbf{p < 10^{-3}}$, $***\ \mathbf{p < 10^{-10}}$

Pearson correlation coefficients and p-values for each pair of PGSs. PGS, polygenic score; FNBMD, femoral neck bone mineral density; TBBMD, total body bone mineral density; HGS, hand grip strength; ALM, appendicular lean mass; WBLM, whole body lean mass; VD, vitamin D; SCC, serum calcium concentration; HC, homocysteine; TSH, thyroid stimulating hormone level; FG, fasting glucose; FI, fasting insulin; T1D, type 1 diabetes; T2D, type 2 diabetes; RA, rheumatoid arthritis; IBD, inflammatory bowel disease; HBS, hip bone size; CAD, coronary artery disease.

Figure 4-3 Associations of 16 trait-specific PGSs with the fracture outcome in the UKB derivation set.



Estimates per standard deviation increase of each individual PGS evaluated in logistic regression (univariate) and elastic-net logistic regression adjusted for age and sex. 'inactive' indicates that the elastic-net estimated odds ratio was negligible (between 0.999 and 1.001, shown as a blue dot). CI, confidence interval.

We assessed the crude 10-year cumulative fracture incidence by nine PGS groups (Figure 4-4). With competing mortality risk accounted for, significant differences in the 10-year risk of fracture were observed across metaPGS deciles ($p<0.0001$). The top and bottom 1% of the metaPGS showed a substantial difference in the cumulative fracture incidence. A comparison of the metaPGS with its individual components (PGS_FNBMD and PGS_TBBMD) is shown in Figure 4-5. Results show that metaPGS had a greater association with fracture risk compared to

the two individual PGSs. All three PGSs were strongly associated with incident fracture ($p<0.0001$), with an odds ratio (OR) ranging from 1.15 to 1.35. The metaPGS was associated with an incident fracture with a hazard ratio (HR) of 1.22 (95% CI 1.19 – 1.27) per standard deviation of metaPGS, which was stronger than PGS_FNBMD (HR=1.10, 95% CI 1.08 – 1.12) and PGS_TBBMD (HR=1.15, 95% CI 1.12 – 1.18) (Figure 4-5.). Using cox proportional hazard models, we also assessed the HRs for the top 1%, 5%, 10%, and 20% decile vs. the remaining percentiles of the PGSs. The results showed that the bottom 1% of the population had a 1.36-fold (95% CI: 1.15-1.61) increased fracture risk than the remaining population (Table 4-2).

Figure 4-4 Cumulative Incident Function Plot for Fracture According to Decile of the metaPGS in UKB Testing Set. Shaded Regions Denote 95% Confidence Intervals.

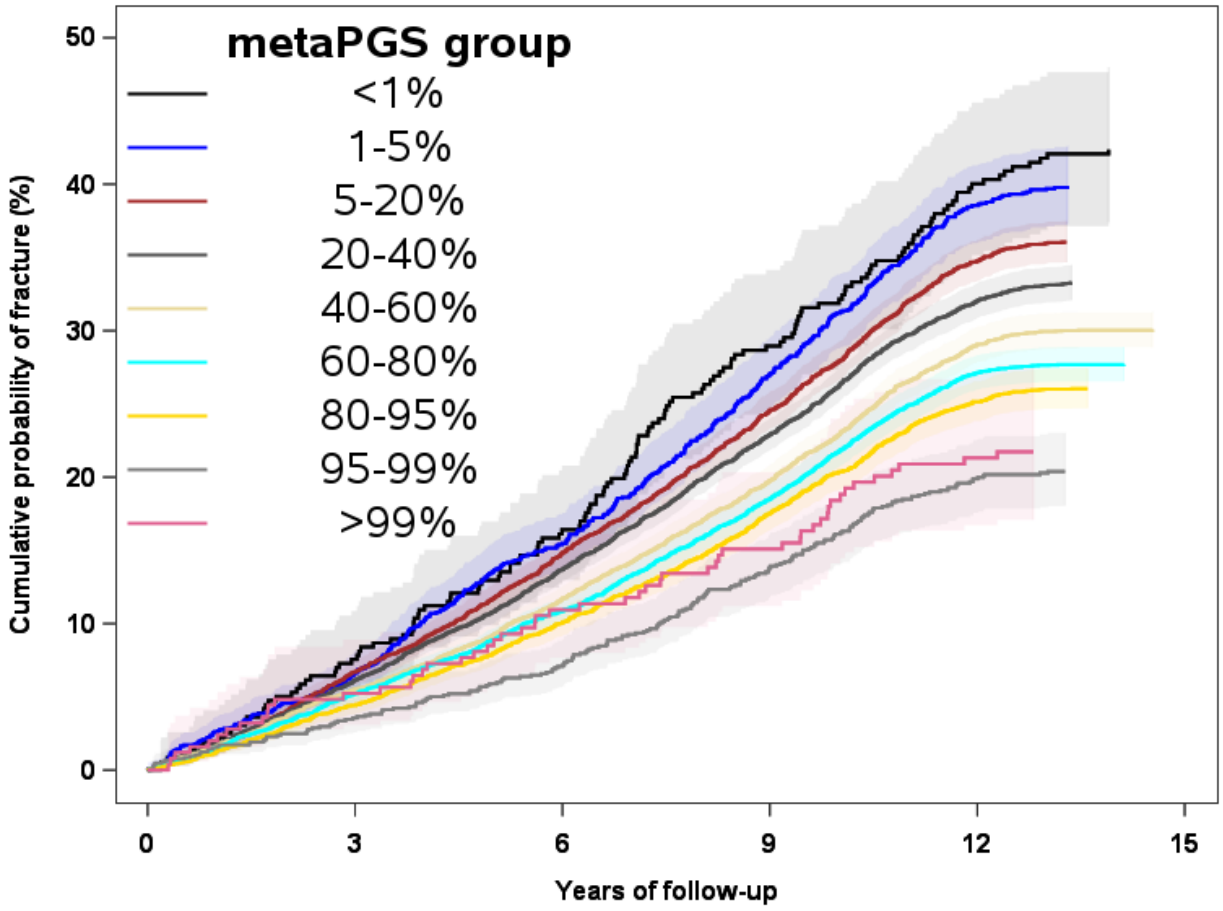


Figure 4-5 Relative Performance of PGS_FNBMD, PGS_TBBMD, and metaPGS for fracture. 5A: Results from Cox Proportional Hazard Models; 5B: Results from Multivariate Logistic Regression Models.

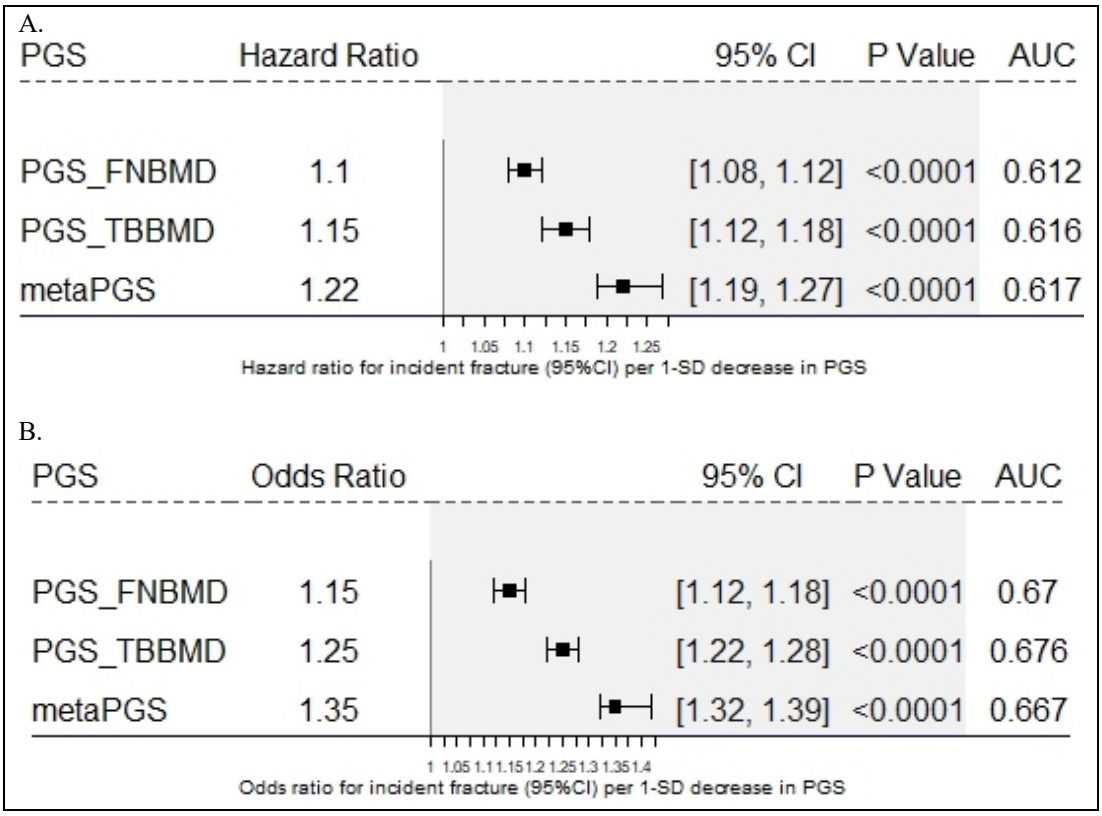


Table 4-2 Hazard Ratios (HR) and 95% Confidence Intervals (CI) for Incident Fracture Per 1 SD Decrease in PGS_FNBMD, PGS_FNBMD, and metaPGS, respectively: Results from Cox Proportional Hazard Models for The UKB Validation Cohort.

		PGS_FNBMD	PGS_TBBMD	metaPGS
High PGS definition	Reference group	HR (95% CI)	HR (95% CI)	HR (95% CI)
Bottom 20% of distribution	Remaining 80%	1.20 (1.14 – 1.25)	1.25 (1.19– 1.32)	1.27 (1.20 – 1.33)
Bottom 10% of distribution	Remaining 90%	1.20 (1.12 – 1.28)	1.29 (1.22 – 1.37)	1.30 (1.23 – 1.39)
Bottom 5% of distribution	Remaining 95%	1.33 (1.22 – 1.45)	1.32 (1.22 – 1.43)	1.34 (1.23 – 1.45)
Bottom 1% of distribution	Remaining 99%	1.31 (1.08 – 1.59)	1.46 (1.25 – 1.72)	1.36 (1.15 – 1.61)

The clinical utility of a PGS depends on its performance in combination with established risk factors and genetic risk models. We next evaluated the predictive value of metaPGS while adjusting for established risk factors. We examined seven FRAX risk factors available in the UKB data. As expected, established risk factors were positively associated with incident fracture, current smoking, and sex being the strongest risk factors (Table 4-3). Adjusting for these risk factors only modestly attenuated the association of the metaPGS with incident fracture. The metaPGS had the strongest association with incident fracture. The HRs of PGS_FNBMD, PGS_TBBMD, and metaPGS for incident fracture were 1.09 (95% CI, 1.08– 1.11), 1.15 (95% CI, 1.14 – 1.18), and 1.21 (95% CI, 1.18– 1.25), respectively. Compared to the FRAX base model, the association between clinical risk factors and incident fracture risk did not attenuate in all four PGS models.

Table 4-3 Hazard Ratio for the hazard function for significant predictive variables for incident fractures in the FRAX model and FRAX models with PGS_FNBMD, PGS_TBBMD, and metaPGS.

Variable	Model 1: FRAX Base Model HR per 1 unit (95% CI)	Model 2: FRAX + PGS_FNBMD HR per 1 unit (95% CI)	Model 3: FRAX + PGS_TBBMD HR per 1 unit (95% CI)	Model 4: FRAX + metaPGS HR per 1 unit (95% CI)
Age	0.97 (0.96 – 0.97)	0.97 (0.96 – 0.97)	0.97 (0.96 – 0.97)	0.97 (0.96 – 0.97)
Sex (women vs. men)	1.57 (1.49 – 1.68)	1.57 (1.49 – 1.68)	1.56 (1.49 – 1.68)	1.56 (1.49 – 1.68)
Body weight	0.98 (0.98 – 0.99)	0.98 (0.98 – 0.99)	0.98 (0.98 – 0.99)	0.98 (0.98 – 0.99)
Height	1.00 (0.99 – 1.01)	1.00 (0.99 – 1.00)	1.00 (0.99 – 1.00)	1.00 (0.99 – 1.00)
Oral glucocorticoid	0.93 (0.70 – 1.24)	0.93 (0.70 – 1.24)	0.93 (0.70 – 1.23)	0.92 (0.69 – 1.23)
Type 1 diabetes	0.63 (0.53 – 0.74)	0.63 (0.53 – 0.75)	0.64 (0.54 – 0.76)	0.64 (0.54 – 0.76)
Early menopause	1.17 (1.10 – 1.25)	1.17 (1.10 – 1.24)	1.17 (1.10 – 1.24)	1.17 (1.10 – 1.24)
Rheumatoid arthritis	0.95 (0.86 – 1.05)	0.95 (0.86 – 1.05)	0.95 (0.86 – 1.05)	0.95 (0.86 – 1.06)
Current smoking	1.66 (1.56 – 1.79)	1.66 (1.56 – 1.79)	1.66 (1.56 – 1.79)	1.66 (1.56 – 1.79)
PGS	NA	1.09 (1.08 – 1.11)	1.15 (1.14 – 1.18)	1.21 (1.18 – 1.25)

In the reclassification analysis, compared to the FRAX base model, the models with PGS_FNBMD, PGS_TBBMD, and metaPGS improved the reclassification of fracture by 0.9% (95% CI, 0.04% to 1.58%), 1.36% (95% CI, 0.52% to 2.19%), and 1.41% (95% CI, 0.58% to 2.24%), respectively (Table 4-4). Moreover, the metaPGS showed the greatest improvement in terms of reclassification. For the model that included metaPGS, 13799 (6.9%) individuals were correctly reclassified up to the high-risk group, and 13530 (4.3%) individuals who did not experience a fracture were correctly reclassified from the high-risk group to the low-risk group. The continuous NRI showed that improvement in fracture reclassification contributed by PGS_FNBMD, PGS_TBBMD, and metaPGS were 10.1%, 15.9%, and 16.8%, respectively.

Table 4-4 Reclassification table of 10-Year Osteoporotic Fracture Stratified by Event Status. Results of Reclassification Analysis: Percent of reclassification compared with FRAX base model.

Reclassification										
	Non-fracture group		Fracture group		NRI (category)	*p*	NRI (continuous)	*p*	IDI	*p*
	Reclassification down	Reclassification up	Reclassification up	Reclassification down						
$\boldsymbol{PGS\_FNBMD_{ldpred}}$	2.8%	2.8%	4.4%	3.5%	0.92% (0.25% to 1.58%)	0.007	10.08% (7.71% to 12.45%)	<0.001	0.04% (0.01% to 0.11%)	0.16
$\boldsymbol{PGS\_TBBMD_{ldpred}}$	4.4%	4.4%	7.0%	5.6%	1.36% (0.52% to 2.19%)	0.002	15.88% (13.51% to 18.25%)	<0.001	0.11% (0.04% to 0.11%)	<0.001
metaPGS	4.3%	4.3%	6.9%	5.4%	1.41% (0.58% to 2.24%)	<0.001	16.82% (14.46% to 19.19%)	<0.001	0.12% (0.04% to 0.11%)	<0.001

*Significant results are in boldface.
NRI=net reclassification improvement; IDI=integrated discriminative improvement; 95% confidence intervals are given

4.5 Discussion

The present study developed and evaluated a novel metaPGS for fracture risk prediction by combining genetic information from multiple fracture-related traits. The ability of the metaPGS to predict fracture risk was evaluated alone and in combination with the clinical risk score recommended by guidelines. The metaPGS demonstrated a significant association with incident fractures, with a hazard ratio of 1.22 per standard deviation of metaPGS, which was significantly more potent than previously established BMD-related individual PGSs. The predictive power of the metaPGS was comparable to established risk factors such as age, body weight, and early menopause. Adding the metaPGS to the existing FRAX model improved the discrimination of fractures from non-fracture cases, suggesting that the metaPGS can help stratify fracture risk in the European population and for developing personalized prevention strategies.

Our study contributes to the use of genomic information to stratify individuals for fracture risk. Pleiotropy, a phenomenon in which a single gene or genetic variant influences multiple traits or diseases, has been well-documented in previous research (Bulik-Sullivan et al., 2015). Since genetic variants can affect multiple traits simultaneously, independent PGSs for fracture risk are expected to overlap significantly. To overcome this challenge, we employed elastic net regularized regression to combine multiple PGSs and estimate their contributions to fracture risk prediction while minimizing collinearity. The resulting metaPGS combines genetic information from 11 of 16 bone-related traits and disorders, resulting in a robust and strongly associated predictor of fracture risk.

Compared to existing individual PGSs, the new metaPGS showed a more significant association with fracture and a more remarkable risk discrimination ability. Moreover, the metaPGS has comparable predictive power to some established risk factors. By combining metaPGS with the

current fracture risk assessment tool, our findings suggested added value of metaPGS beyond established clinical risk factors. The predictive ability of metaPGS was largely independent of established risk factors for fracture, implying that the metaPGS captured residual risk not quantified by the established risk factors. In addition, the results of reclassification analyses indicated that adding metaPGS to the FRAX model improved discriminating fractures and non-fractures cases, and its performance in fracture risk reclassification is better than the two previously developed BMD-related PGSs (Xiao, Xiangxue & Wu, 2023).

There are several limitations worth mentioning. The predictive performance of the metaPGS for fracture is limited if compared with some diseases, such as CAD (Inouye et al., 2018). The reasons could be that fragility fracture is more heterogeneous than other diseases and that the GWAS sample size for mechanistically defined fracture is also limited. The sample size of older individuals (>75 years) in the UKB is relatively small, limiting our ability to model fracture risk in the age strata where most events occur. Furthermore, the duration of follow-up in UKB is relatively limited, and because of the limited covariates available in the UKB, we could not assess the predictive value of the metaPGS beyond the full FRAX model. Fourth, since the metaPGS was derived and tested in individuals of European ancestry, it may not have equivalent predictive power for other ethnic groups due to variations in allele frequencies, linkage disequilibrium patterns, and effect sizes of common polymorphisms across different ancestries. Lastly, since a family history of fracture was not available in the UKB, we could not examine whether the association of the metaPGS with fracture risk would be affected by family history.

Our study developed and evaluated a novel approach for fracture risk prediction, the metaPGS, which combines genetic information from multiple fracture-related traits. Despite challenges in phenotypic heterogeneity and GWAS power, our study presents a powerful fracture genomic risk

score to date and assesses its potential for risk stratification in the context of established risk factors and clinical guidelines. The metaPGS provides added value to established clinical risk factors and has potential clinical utility for personalized prevention strategies. Future studies should validate the metaPGS in other populations and evaluate its clinical utility. The metaPGS is a promising approach for fracture risk prediction that overcomes the limitations of single PGSs and represents a significant step towards using genomic information to help stratify individuals for fracture risk.

Chapter 5 Conclusions

With an overall objective to leverage the predictive power of genetic information in fracture risk assessment, this dissertation included three distinct but ancillary studies following the multiple-paper dissertation format. The first and third studies used data from the UKB data, and the second used data from the WHI study.

The first study highlighted the importance of early identification of individuals at high risk of fragility fracture using PGS. PGS can be assessed before clinical risk factors emerge, and employing thousands of genetic variants discovered from GWAS can help with risk stratification and primary prevention. The study developed and validated genome-wide PGS for femoral neck BMD and total body BMD and compared their predictive ability to restricted PGS based on a limited number of SNPs. The results showed that genome-wide PGS accurately predicted fracture risk, and the LDpred2 approach, which includes the entire genome, had better predictive performance than the approach that only included genome-wide significant variants. However, the femoral neck BMD-related PGS showed no improvement over the restricted PGS. The study suggests that PGS can be useful for the clinical screening of individuals in order to detect those at high risk, which may be useful for monitoring and preventive treatment. Moreover, adding genome-wide PGS to the FRAX clinical risk score improved predictive accuracy for fracture and successfully reclassified up to 2% of individuals to a higher or lower risk category. However, there are limitations to the study, such as the limited data availability and the inclusion of only European ancestry individuals.

The second study discussed the implementation of genome-wide PGSs in the clinical setting for predicting the risk of certain diseases. Specifically, the paper focuses on the development and validation of three PGSs related to BMD using data from the UKB cohort. The paper then

evaluates the performance of these PGSs in an independent validation cohort of postmenopausal women from the WHI and compares their predictive ability to the FRAX, which uses clinical risk factors to predict fracture risk. The study's findings show that the BMD-related PGSs and the metaPGS did not perform as well in the WHI cohort and were not significantly associated with fractures. Furthermore, adding genetic information to the FRAX tool only led to minimal improvements in predicting hip fracture probabilities among elderly Caucasian women. The study suggests that the performance of PGSs in different cohorts can be affected by factors such as overfitting, differences in genotyping data, allele frequencies, and environmental and lifestyle factors that can influence the expression of certain traits and conditions. Overall, the study highlights the importance of validating PGSs in independent cohorts to assess their generalizability and potential for clinical use. The findings suggest that PGSs may not provide significant improvements in fracture risk prediction beyond conventional clinical risk factors, but further research is needed to explore their potential utility in other disease contexts.

The third study developed a novel metaPGS (meta polygenic score) to predict fracture risk by combining genetic information from multiple fracture-related traits. The metaPGS was evaluated alone and in combination with the clinical risk score recommended by guidelines. The results showed that the metaPGS demonstrated a significant association with incident fractures and was more potent than previously established individual PGSs for bone mineral density. Adding the metaPGS to the existing FRAX model improved the discrimination of fractures from non-fracture cases, suggesting that the metaPGS can help stratify fracture risk in the European population and for developing personalized prevention strategies. The study highlights the use of genomic information to stratify individuals for fracture risk and presents a powerful fracture genomic risk score to date. However, the predictive performance of the metaPGS is limited if

compared with some diseases, and future studies should validate the metaPGS in other populations and evaluate its clinical utility.

Appendix A. ICD10 Codes Used to Identify Fracture Cases

M80.8 Other osteoporosis with pathological fracture： M8080: Other osteoporosis with pathological fracture (Multiple sites) M8081: Other osteoporosis with pathological fracture (Shoulder region) M8082: Other osteoporosis with pathological fracture (Upper arm) M8085: Other osteoporosis with pathological fracture (Pelvic region and thigh) M8086: Other osteoporosis with pathological fracture (Lower leg) M8088: Other osteoporosis with pathological fracture (Other) M8089: Other osteoporosis with pathological fracture (Site unspecified)
M80.9 Unspecified osteoporosis with pathological fracture： M8090: Unspecified osteoporosis with pathological fracture (Multiple sites) M8091: Unspecified osteoporosis with pathological fracture (Shoulder region) M8092: Unspecified osteoporosis with pathological fracture (Upper arm) M8093: Unspecified osteoporosis with pathological fracture (Forearm) M8094: Unspecified osteoporosis with pathological fracture (Hand) M8095: Unspecified osteoporosis with pathological fracture (Pelvic region and thigh) M8096: Unspecified osteoporosis with pathological fracture (Lower leg) M8097: Unspecified osteoporosis with pathological fracture (Ankle and foot) M8098: Unspecified osteoporosis with pathological fracture (Other) M8099: Unspecified osteoporosis with pathological fracture (Site unspecified)
S22.0 Fracture of thoracic vertebra： S2200: Fracture of thoracic vertebra (closed) S2201: Fracture of thoracic vertebra (open)
S32.0 Fracture of lumbar vertebra： S3200: Fracture of lumbar vertebra (closed)
S42 Fracture of shoulder and upper arm： S4220: Fracture of upper end of humerus (closed) S4221: Fracture of upper end of humerus (open) S4230: Fracture of shaft of humerus (closed) S4231: Fracture of shaft of humerus (open) S4240: Fracture of lower end of humerus (closed) S4241: Fracture of lower end of humerus (open)
S52 Fracture of forearm： S5200: Fracture of upper end of ulna (closed) S5201: Fracture of upper end of ulna (open) S5210: Fracture of upper end of radius (closed) S5211: Fracture of upper end of radius (open) S5220: Fracture of shaft of ulna (closed) S5221: Fracture of shaft of ulna (open) S5230: Fracture of shaft of radius (closed) S5231: Fracture of shaft of radius (open) S5250: Fracture of lower end of radius (closed) S5251: Fracture of lower end of radius (open) S5260: Fracture of lower end of both ulna and radius (closed) S5261: Fracture of lower end of both ulna and radius (open) S5290: Fracture of forearm, part unspecified (closed)
S72 Fracture of femur： S7200: Fracture of neck of femur (closed) S7201: Fracture of neck of femur (open) S7210: Pertrochanteric fracture (closed) S7211: Pertrochanteric fracture (open)

S7220: Subtrochanteric fracture (closed)
S7221: Subtrochanteric fracture (open)
S7230: Fracture of shaft of femur (closed)
S7231: Fracture of shaft of femur (open)
S7240: Fracture of lower end of femur (closed)
S7241: Fracture of lower end of femur (open)
S7280: Fractures of other parts of femur (closed)
S7290: Fracture of femur, part unspecified (closed)
S7291: Fracture of femur, part unspecified (open)

Appendix B. UK Biobank Data Field IDs Used to Ascertain Conventional Risk Factors

Field ID	Description
21022	Age at recruitment
34	Sex
50	Standing height
12144	Height
21002	Weight
21001	Body mass index (BMI)
2463	Fractured/broken bones in last 5 years
20116	Smoking status
20003	Treatment/medication code (define glucocorticoid use)
131848	Date M05 first reported (seropositive rheumatoid arthritis)
131850	Date M06 first reported (other rheumatoid arthritis)
130706	Date E01 first reported (insulin-dependent diabetes mellitus)
3581	Age at menopause (last menstrual period)

Appendix C. Participant Characteristics of the UK Biobank Testing Set (N=455,663)

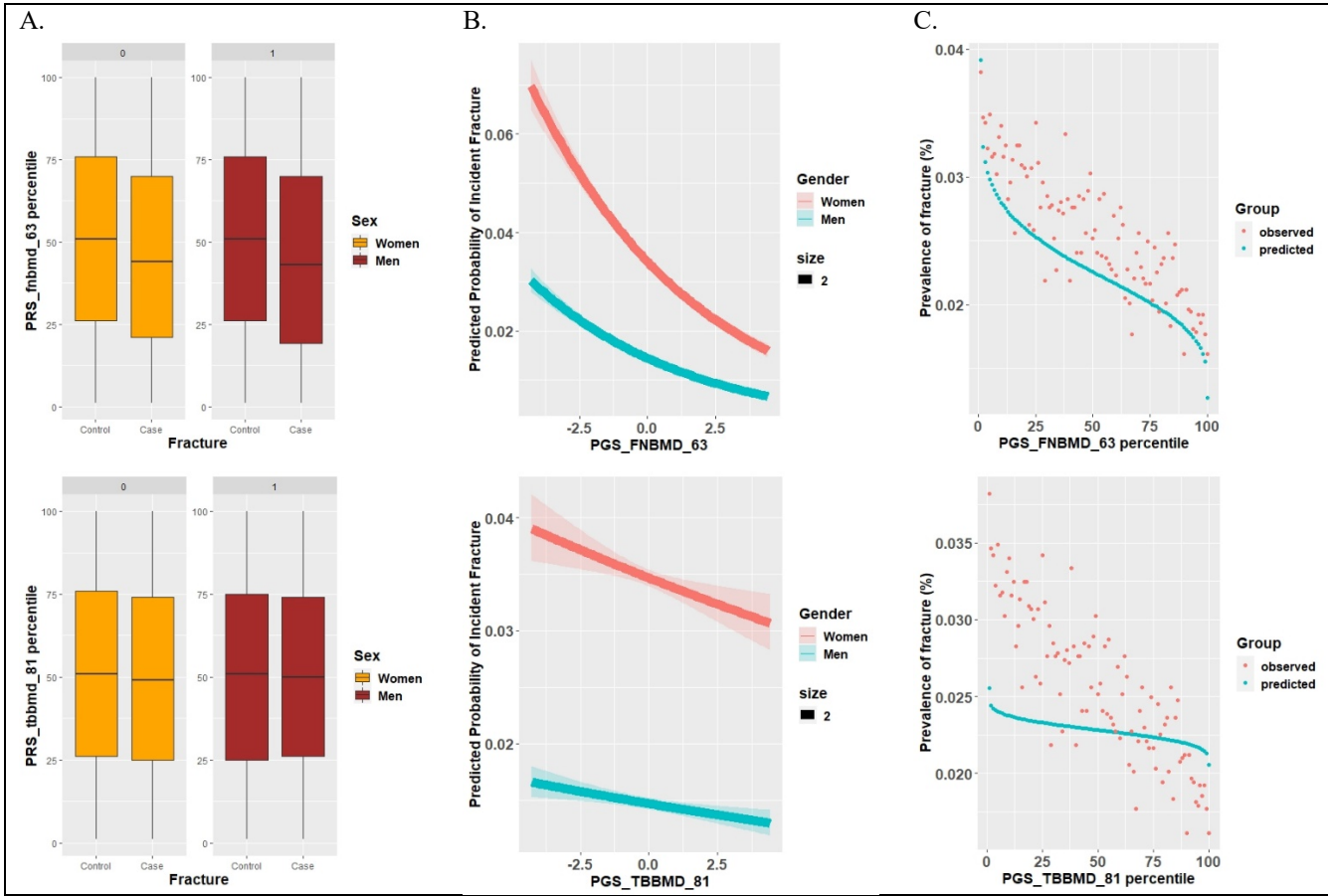
	UKB (N=455,663)	Male (n=208,271) (45.71%)	Female (n=247,392) (54.29%)
Age at assessment, yrs.	56.76 ± 8.02	56.99 ± 8.11	56.58 ± 7.95
Height (cm)	168.66 ± 9.25	175.84 ± 6.78	162.63 ± 6.25
Weight (kg)	78.16 ± 15.91	86.18 ± 14.30	71.40 ± 13.95
Current smoker	47,486 (10.42%)	25,392 (12.19%)	22,094 (8.93%)
Body mass index (BMI) ($\boldsymbol{kg/m^2}$)	27.39 ± 4.77	27.85 ± 4.24	27.01 ± 5.14
Fractures in the past 5 years	47,576 (10.44%)	19,262 (9.25%)	28,314 (11.44%)
Oral glucocorticoid user	2,426 (0.53%)	1,069 (0.51%)	1,354 (0.55%)
Rheumatoid arthritis	10,964 (2.41%)	3,612 (1.73%)	7,352 (2.97%)
Type 1 diabetes	4,336 (0.95%)	2,517 (1.21%)	1,819 (0.74%)
Menopause before age 45 years	35,657 (7.83%)	NA	35,657 (7.83%)

Appendix D. Association Between Polygenic Scores (PGSs) and measured BMD in the UKB Testing Set: Results of Multiple Linear Regression Analysis (N=38,204)

BMD sites	PGSs	Regression Coefficient (SE)	P-values	Standardized regression
Femoral neck BMD	$\boldsymbol{PGS\_FNBMD_{63}}$	0.026 (0.0006)	<0.0001	0.184
	$\boldsymbol{PGS\_FNBMD_{ldpred}}$	0.030 (0.0007)	<0.0001	0.199
Total body BMD	$\boldsymbol{PGS\_TBBMD_{81}}$	0.005 (0.0006)	<0.0001	0.036
	$\boldsymbol{PGS\_TBBMD_{ldpred}}$	0.041 (0.0006)	<0.0001	0.264

*Separated multiple linear regressions were conducted for each PGS; each estimate was adjusted for age, sex, and the first four principal components.

Appendix E. Risk for Incident Fracture According to Restricted PGSs.



(A) PGSs percentile among fracture cases versus controls in the UK Biobank testing set. Within each boxplot, the horizontal lines reflect the median, the top and bottom of each box reflect the interquartile range, and the whiskers reflect the maximum and minimum values within each group. (B) Predicted Probability of Incident Fracture by PGSs: Risk gradient for fractures according to the PGS percentiles. 100 groups of the testing dataset were derived according to the percentile of each of the two PGSs. (C) Predicted versus Observed prevalence of incident fracture according to PGS percentiles.

Appendix F. The cumulative incidence of fracture by PGS groups using the cumulative incidence function.

	CIF											
	1 year	2 years	3 years	4 years	5 years	6 years	7 years	8 years	9 years	10 years	11 years	12 years
$\boldsymbol{PGS\_FNBMD_{63}}$												
<1%	2.50%	6.54%	8.27%	10.01%	12.14%	14.67%	19.94%	23.46%	27.97%	32.28%	36.01%	39.15%
1-5%	2.38%	4.88%	7.21%	9.56%	12.19%	15.07%	18.19%	21.21%	25.53%	28.90%	33.82%	37.13%
5-20%	1.89%	3.85%	5.91%	8.25%	10.53%	13.40%	16.29%	19.74%	23.20%	26.84%	30.79%	33.77%
20-40%	1.64%	3.89%	6.21%	8.32%	10.55%	13.01%	15.85%	18.87%	22.10%	25.28%	28.81%	31.43%
40-60%	1.63%	3.90%	6.20%	8.31%	10.55%	13.01%	15.82%	18.85%	22.10%	25.28%	28.81%	31.42%
60-80%	1.79%	3.90%	5.95%	8.16%	10.38%	12.89%	15.40%	18.36%	21.00%	24.43%	27.53%	29.64%
80-95%	1.44%	3.34%	5.28%	7.13%	9.10%	11.23%	13.63%	16.11%	18.51%	21.75%	24.76%	26.83%
95-99%	1.33%	3.03%	4.96%	6.85%	8.87%	10.86%	12.91%	15.35%	17.85%	20.43%	23.27%	25.29%
>99%	1.63%	2.57%	3.77%	5.73%	7.26%	8.93%	11.06%	13.00%	15.65%	18.75%	21.92%	23.53%
$\boldsymbol{PGS\_TBBMD_{81}}$												
<1%	1.67%	3.98%	7.64%	9.55%	12.19%	14.60%	18.28%	21.97%	24.92%	27.38%	30.58%	32.30%
1-5%	1.70%	3.98%	6.09%	8.91%	11.63%	14.38%	16.90%	20.09%	23.58%	26.16%	29.29%	31.45%
5-20%	1.99%	4.16%	6.02%	8.10%	10.18%	12.73%	15.43%	17.77%	20.56%	24.09%	27.07%	29.33%
20-40%	1.67%	3.66%	5.89%	7.89%	10.25%	12.69%	15.38%	18.27%	21.15%	24.41%	27.98%	30.40%
40-60%	1.55%	3.76%	5.77%	7.91%	9.90%	12.30%	14.91%	18.14%	21.23%	24.15%	27.37%	29.77%
60-80%	1.61%	3.29%	5.26%	7.25%	9.32%	11.73%	14.29%	17.27%	20.02%	23.38%	26.90%	29.54%
80-95%	1.61%	3.60%	5.45%	7.40%	9.31%	11.53%	13.97%	16.62%	19.53%	23.09%	26.54%	28.86%
95-99%	1.76%	3.34%	5.35%	7.79%	10.07%	12.20%	13.83%	16.67%	19.94%	23.09%	26.37%	27.63%
>99%	0.47%	2.60%	4.97%	6.16%	8.58%	10.78%	13.71%	15.42%	17.14%	20.56%	24.23%	27.17%
$\boldsymbol{PGS\_FNBMD_{ldpred}}$												
<1%	2.89%	5.36%	7.84%	9.92%	12.65%	16.27%	19.09%	24.76%	26.72%	29.34%	33.26%	36.09%
1-5%	2.51%	4.51%	6.97%	10.25%	12.27%	15.31%	18.73%	21.74%	25.53%	29.09%	34.00%	36.55%
5-20%	1.83%	3.96%	6.39%	8.84%	11.51%	14.21%	16.84%	19.92%	23.46%	26.98%	30.57%	33.63%
20-40%	1.60%	3.65%	5.91%	7.95%	10.05%	12.52%	15.35%	18.30%	21.43%	24.69%	28.04%	30.41%
40-60%	1.70%	3.93%	5.81%	7.82%	9.82%	12.04%	14.67%	17.65%	20.49%	23.74%	26.99%	29.47%
60-80%	1.60%	3.34%	5.28%	7.21%	9.22%	11.62%	13.97%	17.03%	19.62%	22.87%	26.20%	28.30%
80-95%	1.38%	3.15%	4.84%	6.55%	8.62%	10.75%	12.82%	14.92%	17.28%	20.37%	23.38%	25.28%
95-99%	1.64%	3.67%	5.00%	6.66%	8.47%	10.56%	13.32%	15.29%	18.26%	21.16%	23.80%	25.78%

>99%	0.78%	2.07%	3.37%	5.20%	6.78%	9.14%	10.98%	12.83%	14.41%	17.31%	19.41%	20.47%
$\boldsymbol{PGS\_TBBMD_{ldpred}}$												
<1%	2.42%	5.96%	9.69%	13.07%	16.47%	19.32%	23.14%	26.78%	32.15%	34.26%	38.09%	41.15%
1-5%	2.58%	4.95%	7.53%	10.75%	14.35%	17.91%	21.14%	24.21%	27.71%	31.48%	35.57%	39.07%
5-20%	2.05%	4.51%	6.93%	9.11%	11.54%	14.32%	17.11%	20.87%	24.27%	27.79%	31.91%	34.64%
20-40%	1.57%	3.53%	5.65%	7.95%	10.23%	13.04%	15.91%	18.94%	22.08%	25.56%	29.27%	31.72%
40-60%	1.66%	3.81%	5.67%	7.77%	9.76%	12.22%	14.77%	17.55%	20.17%	23.49%	26.74%	29.26%
60-80%	1.52%	3.27%	5.29%	7.09%	9.21%	11.18%	13.45%	15.83%	18.52%	21.68%	24.40%	26.34%
80-95%	1.34%	2.94%	4.47%	6.08%	7.49%	9.35%	11.53%	14.00%	16.67%	19.50%	22.50%	24.61%
95-99%	1.48%	2.69%	4.31%	6.01%	7.45%	8.64%	10.67%	12.93%	14.70%	17.38%	19.57%	21.13%
>99%	1.34%	2.95%	3.75%	4.57%	5.93%	8.39%	10.58%	12.52%	14.45%	16.93%	19.42%	20.52%

Appendix G. Odds Ratios (OR), Hazard Ratios (HR), and Their Corresponding 95% Confidence Intervals (CI) for Incident Fracture Per 1 SD Decrease in PGS. Results from Multiple Linear Regression and Cox Proportional Hazard Models in the UKB Testing Set.

High PGS definition	Reference group	Odds ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
$\boldsymbol{PGS\_FNBMD_{63}}$					
Bottom 20% of distribution	Remaining 80%	1.35 (1.28 – 1.41)	<0.0001	1.21 (1.16 – 1.27)	<0.0001
Bottom 10% of distribution	Remaining 90%	1.39 (1.32 – 1.47)	<0.0001	1.27 (1.20 – 1.33)	<0.0001
Bottom 5% of distribution	Remaining 95%	1.41 (1.32 – 1.52)	<0.0001	1.30 (1.22 – 1.39)	<0.0001
Bottom 1% of distribution	Remaining 99%	1.59 (1.39 – 1.85)	<0.0001	1.33 (1.16 – 1.54)	<0.0001
$\boldsymbol{PGS\_FNBMD_{ldpred}}$					
Bottom 20% of distribution	Remaining 80%	1.32 (1.25 – 1.37)	<0.0001	1.20 (1.15 – 1.25)	<0.0001
Bottom 10% of distribution	Remaining 90%	1.35 (1.28 – 1.43)	<0.0001	1.20 (1.14 – 1.27)	<0.0001
Bottom 5% of distribution	Remaining 95%	1.41 (1.32 – 1.54)	<0.0001	1.25 (1.16 – 1.35)	0.002
Bottom 1% of distribution	Remaining 99%	1.59 (1.35 – 1.85)	<0.0001	1.25 (1.06 – 1.47)	<0.0001
$\boldsymbol{PGS\_TBBMD_{81}}$					
Bottom 20% of distribution	Remaining 80%	1.02 (0.98 – 1.08)	0.27	1.02 (0.98 – 1.07)	0.26
Bottom 10% of distribution	Remaining 90%	1.02 (0.96 – 1.09)	0.46	1.06 (1.01 – 1.12)	0.05
Bottom 5% of distribution	Remaining 95%	1.08 (0.99 – 1.16)	0.09	1.13 (1.04 – 1.22)	0.002
Bottom 1% of distribution	Remaining 99%	1.08 (0.90 – 1.27)	0.41	1.14 (0.96 – 1.35)	0.14
$\boldsymbol{PGS\_TBBMD_{ldpred}}$					
Bottom 20% of distribution	Remaining 80%	1.47 (1.43 – 1.54)	<0.0001	1.28 (1.23 – 1.33)	<0.0001
Bottom 10% of distribution	Remaining 90%	1.59 (1.51 – 1.69)	<0.0001	1.31 (1.25 – 1.37)	<0.0001
Bottom 5% of distribution	Remaining 95%	1.69 (1.59 – 1.82)	<0.0001	1.36 (1.28 – 1.45)	<0.0001
Bottom 1% of distribution	Remaining 99%	1.89 (1.64 – 2.17)	<0.0001	1.47 (1.30 – 1.67)	<0.0001

Appendix H. Concordance Index and the Corresponding 95% Confidence Intervals of Predicted and Observed Fracture Risk for the Model with and without PGS.

	C-index	95% CI	P-value
Model 1 (Base model)	0.663	0.658 – 0.668	NA
Model 2 (Base model + $\boldsymbol{PGS\_FNBMD_{63}}$)	0.668	0.662 – 0.672	<0.001
Model 3 (Base model + $\boldsymbol{PGS\_TBBMD_{81}}$)	0.663	0.660 – 0.672	<0.001
Model 4 (Base model + $\boldsymbol{PGS\_FNBMD_{ldpred}}$)	0.665	0.661 – 0.672	0.007
Model 5 (Base model + $\boldsymbol{PGS\_TBBMD_{ldpred}}$)	0.668	0.661 – 0.673	<0.001

Appendix I. Sources of Genome-Wide Association Studies (GWAS) Summary Statistics used for the metaPGS construction.

Phenotype/Trait	Individual PGS	Reference
Femoral neck BMD	PGS_FNBMD	Estrada et al., (Estrada, Karol et al., 2012)
Total body BMD	PGS_TBBMD	Medina-Gomez et al., (Medina-Gomez et al., 2018a)
Hand grip strength	PGS_HGS	Matteini et al., (Matteini et al., 2016)
Appendicular Lean Mass	PGS_ALM	Zillikens et al., (Zillikens et al., 2017)
Whole Body Lean Mass	PGS_WBLM	Zillikens et al., (Zillikens et al., 2017)
25-Hydroxy Vitamin D	PGS_VD	Jiang et al., (Jiang et al., 2018)
Serum Calcium Concentration	PGS_SCC	O'Seaghdha et al., al., (O'Seaghdha et al., 2013)
Homocysteine	PGS_HC	Van Meurs et al., (van Meurs et al., 2013)
Thyroid Stimulating Hormone Level	PGS_TSH	Teumer et al., (Teumer et al., 2018)
Fasting Glucose	PGS_FG	Lagou et al., (Lagou et al., 2021)
Fasting Insulin	PGS_FI	Lagou et al., (Lagou et al., 2021)
Type 1 Diabetes	PGS_T1D	Robertson et al., (Robertson et al., 2021)
Type 2 Diabetes	PGS_T2D	Vujkovic et al., (Vujkovic et al., 2020)
Rheumatoid Arthritis	PGS_RA	Ha et al., (Ha et al., 2021)
Inflammatory Bowel Disease	PGS_IBD	Liu et al., (Liu et al., 2015b)
Hip Bone Size	PGS_HBS	Styrkarsdottir et al., (Styrkarsdottir et al., 2019)
Coronary Artery Disease	PGS_CAD	Nikpay et al., (Nikpay et al., 2015)

Appendix J. UNLV IRB approval for study 1 and 2

UNLV Biomedical IRB - Administrative Review
Notice of Excluded Activity

DATE:	September 9, 2020
TO:	Qing Wu
FROM:	UNLV Biomedical IRB
PROTOCOL TITLE:	[1649226-1] Development of Genomic-enhanced Risk Prediction of Fragility Fracture
SUBMISSION TYPE:	New Project
ACTION:	EXCLUDED - NOT HUMAN SUBJECTS RESEARCH
REVIEW DATE:	September 9, 2020
REVIEW TYPE:	Administrative Review

Thank you for your submission of New Project materials for this protocol. This memorandum is notification that the protocol referenced above has been reviewed as indicated in Federal regulatory statutes 45CFR46.

The UNLV Biomedical IRB has determined this protocol does not meet the definition of human subjects research under the purview of the IRB according to federal regulations. It is not in need of further review or approval by the IRB.

We will retain a copy of this correspondence with our records.

Any changes to the excluded activity may cause this protocol to require a different level of IRB review. Should any changes need to be made, please submit a Modification Form.

If you have questions, please contact the Office of Research Integrity - Human Subjects at IRB@unlv.edu or call 702-895-2794. Please include your protocol title and IRBNet ID in all correspondence.

Office of Research Integrity - Human Subjects

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Appendix K. UNLV IRB approval for study 3

UNLV Biomedical IRB - Expedited Review
Modification Approved

DATE:	February 25, 2020
TO:	Qing Wu, M.D, Sc.D
FROM:	UNLV Biomedical IRB
PROTOCOL TITLE:	[829476-9] Validating the genomics-integrated model for osteoporosis prediction in Women's Health Initiative Study
SUBMISSION TYPE:	Revision
ACTION:	APPROVED
APPROVAL DATE:	February 11, 2020
REVIEW TYPE:	Expedited Review

Thank you for submission of Revision materials for this protocol. The UNLV Biomedical IRB has APPROVED your submission. This approval is based on an appropriate risk/benefit ratio and a protocol design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

Modifications reviewed for this action include:

1. Addition of developing personalized WHO FRAX to the research study.

Should there be *any* change to the protocol, it will be necessary to submit a Modification Form through ORI - Human Subjects. No changes may be made to the existing protocol until modifications have been approved.

ALL UNANTICIPATED PROBLEMS involving risk to subjects or others and SERIOUS and UNEXPECTED adverse events must be reported promptly to this office. Please use the appropriate reporting forms for this procedure. All FDA and sponsor reporting requirements should also be followed.

All NONCOMPLIANCE issues or COMPLAINTS regarding this protocol must be reported promptly to this office.

If you have questions, please contact the Office of Research Integrity - Human Subjects at IRB@unlv.edu or call 702-895-2794. Please include your protocol title and IRBNet ID in all correspondence.

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Appendix L. Copyright Approval for Study 1

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