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Proteomics-driven discovery of intervention windows and risk subtypes in osteoporosis: A prospective cohort study

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ABSTRACT

Given the limited feasibility of population-wide bone mineral density screening and the infrequency of long-term monitoring in healthy individuals, identifying the window for early intervention and the populations to be prioritized for screening is critical. This study aimed to identify intervention windows for osteoporosis and to determine potential high-risk subtypes within the healthy population. Based on proteomic data from 41,408 healthy adults, we conducted the DE-SWAN method to identify change peaks in plasma protein during the pre-diagnostic osteoporosis phase, and employed finite Gaussian mixture model-based clustering to delineate high-risk subtypes of osteoporosis. We identified 122 protein biomarkers significantly associated with osteoporosis risk throughout the follow-up period. Importantly, we identified two critical peaks occurring approximately 10 and 6 years before diagnosis, with the former enriched in immune-related pathways and the latter prominently involving responses to retinoic acid and glucocorticoids. Furthermore, one high-risk subtype for osteoporosis was identified in both males and females, termed the Frailty and Obesity Subtype. This subtype is characterized by a high degree of frailty and obesity, accompanied by a significantly elevated risk of both osteoporosis and fractures. Finally, we developed a predictive model comprising 10 proteins for identifying high-risk subtypes of osteoporosis, which demonstrated better performance than the traditional risk factor model (AUC: 0.743 vs. 0.680). Our findings demonstrate that proteomic profiling can reveal early molecular changes and identify high-risk subtypes years before clinical onset, providing a foundation for screening and precision prevention of osteoporosis.

1. Introduction

Osteoporosis is a chronic skeletal disorder characterized by decreased bone mass and microarchitectural deterioration, accompanied by an increased risk of fractures [1,2]. Although dual-energy X-ray absorptiometry is an important tool for assessing bone mineral density (BMD), the feasibility of conducting population-wide screening with dual-energy X-ray absorptiometry is limited, and individuals rarely undergo regular monitoring of their bone health [3]. Moreover, previous studies have primarily focused on classification of individuals with osteoporosis, with limited attention given to identifying potential high-risk individuals within healthy populations [4,5]. Consequently, our

study focuses on two critical questions: when is the optimal time point for early intervention, and which populations should be prioritized for further BMD screening. From a preventive medicine perspective, by establishing these early biomarkers and identifying high-risk subgroups, we can better target screening efforts, increase individual awareness and proactive management, and optimize the allocation of healthcare resources.

Determining the optimal intervention window and identifying the target population require biomarkers that undergo significant alterations prior to clinical diagnosis. Advances in proteomics enable simultaneous quantification of thousands of proteins, creating unprecedented opportunities for early disease monitoring and risk stratification

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[6,7]. Recent evidence has identified several key plasma proteins undergo measurable changes before the clinical onset of disease, serving as effective biomarkers for predicting osteoporosis and fracture risk [8–10]. These findings underscore the significance of plasma proteomics in shifting osteoporosis management from a reactive to a preventive paradigm.

In this study, we extended the application of proteomics in osteoporosis research. Building upon the identification of protein biomarkers, our aim is to identify early changes in protein profiles and characterize high-risk osteoporosis subtypes that could serve as early-warning signals. Utilizing a large prospective cohort, our investigation focuses on three principal objectives: (i) evaluating proteins significantly associated with osteoporosis at different time points; (ii) mapping dynamic proteomic trajectories throughout the progression of osteoporosis; (iii) identifying distinct osteoporosis risk subtypes and elucidating underlying disease heterogeneity.

2. Materials and methods

2.1. Study design and participants

This study utilized data from the UK Biobank (UKB), a large-scale prospective cohort study that recruited over 500,000 participants between 2006 and 2010 (Fig. 1). A total of 41,408 individuals were included in the final analysis after applying the following exclusion criteria: (1) missing Olink proteomics data; (2) a baseline diagnosis of osteoporosis or fractures; (3) withdrawal of consent or lost to follow-up; (4) a missing rate of protein measurements exceeding 20%; or (5) incomplete covariate information (Supplementary Fig. 1). The study was approved by the NHS North West Multi-Centre Research Ethics Committee (11/NW/0382) under application number 92718.

2.2. Plasma proteomic measurement

Blood samples from participants were collected at baseline between 2007 and 2010. These samples were quantitatively analyzed using the antibody-based Olink Explore Proximity Extension Assay, measuring a total of 2923 proteins. The full names and abbreviations of these proteins are provided in Supplementary Table 1. Detailed protocols for the Olink proteomics measurements have been previously reported elsewhere [11]. Four proteins with missing values exceeding 20% were excluded from the analysis. For the remaining 2919 proteins, missing values were addressed through the K-nearest neighbors imputation method. Subsequently, protein values were standardized through the application of Z-scores.

2.3. Assessment of osteoporosis and fracture

The identification of osteoporosis and fractures was based on the International Classification of Diseases (ICD)-10: osteoporosis (M80, M81, M82) and fractures (M484, M485, M80, M843, M844, S12, S22, S32, S42, S52, S72, S82, T02, T08, T10, T12) [12]. Osteoporosis cases were defined through linked health records, including hospital admissions data, primary care records, death registers, and self-reported information. Follow-up duration was calculated from baseline assessment to the earliest occurrence of osteoporosis/fracture diagnosis, death, or the end of follow-up (November 30, 2022).

2.4. Statistical analysis

Baseline characteristics of the study population were shown as follows: continuous variables were presented as mean (standard deviation) and assessed for group differences using analysis of variance, while categorical variables were reported as frequencies (percentages) and compared across groups using the chi-square test. Pairwise comparisons between subtypes were tested using the *t*-test.

2.5. Association analysis

We employed Cox proportional hazards regression models to evaluate the associations between plasma proteins and the risk of osteoporosis at different time points (full follow-up time, within 10 years, and from 10 years onward). Covariates included age, sex, ethnicity, education, Townsend Deprivation Index, body mass index (BMI), smoking status, alcohol consumption, physical activity, and history of hypertension, diabetes, and cancer. Bonferroni correction was applied to regression analyses to assess significance associations ($P < 0.05$). Meanwhile, we provided regression results adjusted by the FDR method. Detailed descriptions of these covariates are provided in the Supplementary Methods. Given that many of the significant proteins may reflect broader systemic processes, such as inflammation or aging, rather than direct mechanisms of bone metabolism, we further adjusted for C-reactive protein in sensitivity analyses to ensure the robustness of our findings (both Bonferroni-adjusted and FDR-adjusted results were provided). Moreover, the pathway enrichment analysis was performed using the clusterProfiler package, with adjustment for multiple testing using the Benjamini–Hochberg method.

Furthermore, we utilized the DGIdb database to explore the druggability and therapeutic potential of the identified osteoporosis-related proteins. The DGIdb database is a public resource designed for drug prediction, enabling the identification of drugs that are either currently approved or have potential as therapeutic candidates targeting the corresponding proteins [13].

2.6. Protein dynamics during the pre-diagnostic phase of osteoporosis

We used the time from baseline to the diagnosis of osteoporosis as a proxy to simulate disease progression. This approach has been widely adopted and validated in studies of neurodegenerative diseases such as Alzheimer's disease [14–16]. These exploratory analyses have yielded valuable insights into early molecular changes that are difficult to capture through conventional cross-sectional association studies. Meanwhile, we acknowledge that this is an analytical approximation, as serial BMD measurements were not available to directly quantify progression at baseline. We used LOESS regression to model the association between baseline protein levels and the progression of osteoporosis [14,16]. Subsequently, the Euclidean distance was employed to calculate the pairwise differences between the LOESS predictions, and hierarchical clustering was carried out using the complete linkage method via the *hclust* function.

Furthermore, we employed the DEswan algorithm to model nonlinear temporal dynamics in the risk of osteoporosis using baseline proteomic profiles [17]. To standardize temporal analysis, follow-up durations for incident osteoporosis cases were rounded to the nearest integer year and analyzed across 15 centers with ± 1 -year windows, ensuring robust detection of phase-specific biomarker patterns.

2.7. Clustering-based identification of osteoporosis subtypes

We employed finite Gaussian mixture models, implemented using the R package *mclust*, to conduct gender-specific clustering and identify subtypes exhibiting similar protein characteristics [18,19]. The optimal model was selected based on the Bayesian Information Criterion (BIC) to ensure the best fit. Meanwhile, models with subtypes representing less than 5% of the total sample size were excluded from selection. For feature selection, the 122 proteins identified in the association analysis were incorporated into the LASSO model for variable selection, with ten-fold cross-validation used to determine the optimal penalization parameter.

2.8. Mendelian randomization analysis

To further explore the genetic causal relationship between the key

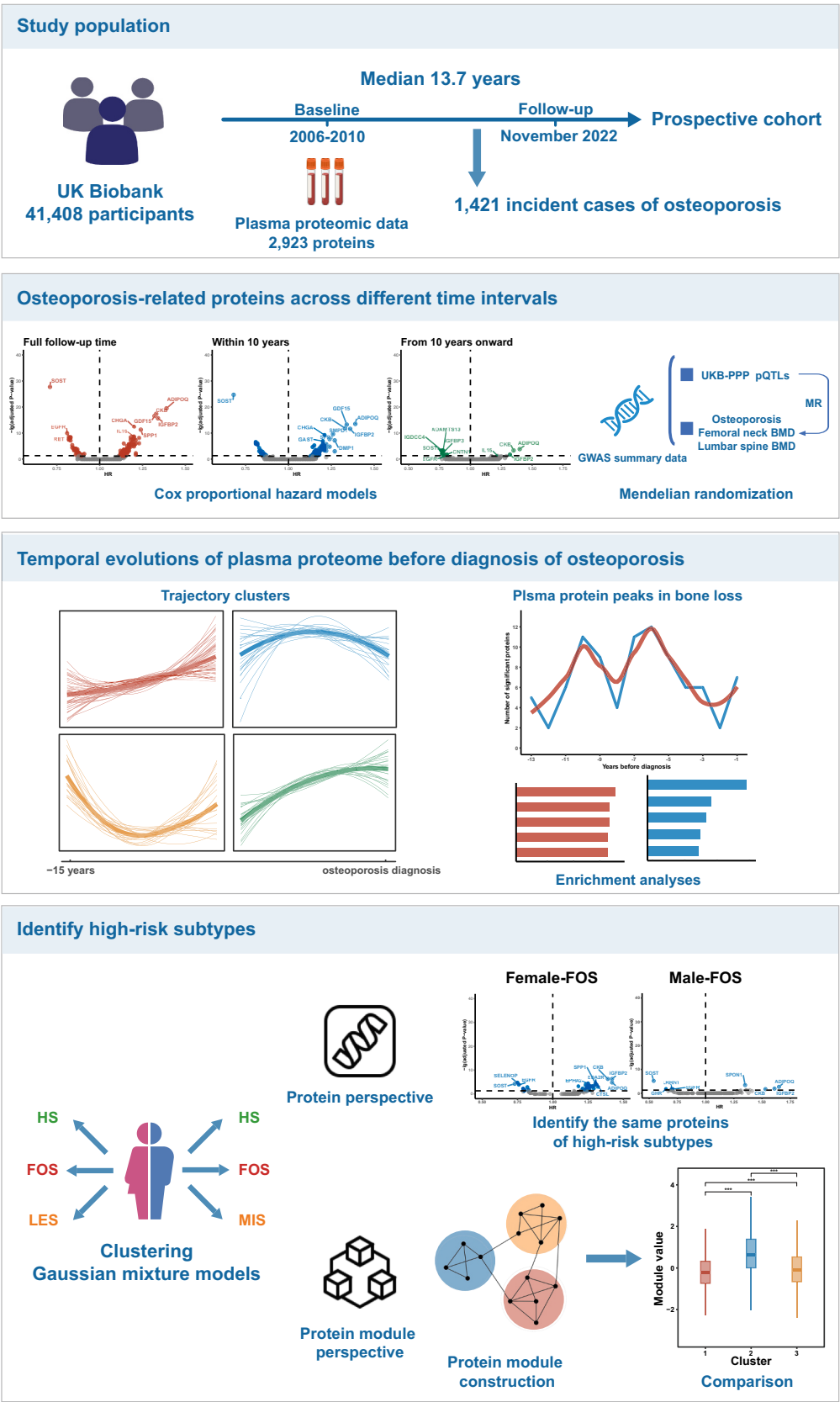


Fig. 1. Study overview.

proteins identified in the association analysis and bone loss (osteoporosis, lumbar spine BMD, femoral neck BMD), we conducted two-sample Mendelian randomization (MR) analysis. Genetic instruments associated with proteins were derived from the GWAS summary data provided by the UKB Pharma Proteomics Project [11]. The GWAS summary statistics for osteoporosis, lumbar spine BMD, and femoral neck BMD were sourced from the FinnGen Biobank (Release 12; 10,461 cases and 473,264 controls) and the GEFOS consortium (lumbar spine BMD: ieu-a-982, sample size: 28,498; femoral neck BMD: ieu-a-980, sample size: 32,735) [20,21]. Several key steps were performed prior to the MR analysis. In the two-sample MR framework, following instrumental variable selection ($P < 5 \times 10^{-8}$), clumping ($R^2 = 0.001$, window size = 10,000 kb), and data harmonization, MR Pleiotropy Residual Sum and Outlier was utilized for identification and removal of pleiotropic outliers ($P < 0.05$) before MR. To mitigate the risk of false positives, we employed six methods including inverse variance weighted, MR-Egger, weighted median, weighted model, Contamination mixture method (ConMix), and constrained maximum likelihood and model averaging (cML-MA), which are based on different assumptions. Significant causal associations were identified when more than half of the methods showed nominal significance, while less than half indicated a suggestive causal association. All F-statistics of genetic instruments exceeded 10, indicating no evidence of weak instrument bias. Furthermore, the MR Steiger approach was utilized to assess directionality.

2.9. Constructing protein module networks

Considering that proteins do not operate independently, we used the Weighted Correlation Network Analysis to construct protein module networks, aiming to reveal the differences between subtypes. Additionally, to better interpret the biological functions of each protein module, we conducted pathway enrichment analysis. Functional annotation was performed using Gene Ontology Biological Processes (GO: BP). To reduce redundancy, we applied semantic simplification with a similarity threshold of 0.7, retaining the most biologically significant terms. The P -values were adjusted for multiple testing using Benjamini-Hochberg method.

2.10. Osteoporosis prediction

Using the Light Gradient Boosting Machine (LGBM) model, we focused on predicting osteoporosis within the high-risk subtype population. Given that a subtype with high risk of osteoporosis and fractures has been observed in both males and females, these two subtypes were combined to increase sample size and enhance the generalizability of the predictive model. Among the 63 proteins used for clustering, the LGBM model was employed to identify the top 10 proteins contributing most significantly to osteoporosis prediction within the high-risk subtype. These selected proteins were visualized using SHAP plots. To further evaluate the predictive performance of these proteins, we compared the model incorporating the 10 selected proteins with a model based on traditional risk factors, including age, sex, ethnicity, smoking status, alcohol consumption, Townsend Deprivation Index, and BMI. During the model development process, hyperparameter optimization was performed through grid search. The data was randomly split into training and testing sets, with 80% used for training and 20% for testing. To train the model, 10-fold cross-validation was utilized, and evaluation was conducted on the separate testing set.

3. Results

3.1. Identifying osteoporosis-associated proteins

The sample included 41,408 participants from the UKB, with a mean age of 56.7 years, comprising 53.6% females and 46.4% males (Supplementary Table 2). During a median follow-up period of 13.7 years,

1421 (3.43%) incident cases of osteoporosis were recorded. Out of 2919 plasma proteins, Cox regression identified 122 proteins significantly associated with osteoporosis risk in full follow-up time (Fig. 2A and Supplementary Table 1). Among these, SOST, ADIPOQ, and CKB, GDF15, IGFBP2 emerged as the five most strongly associated proteins. In the sensitivity analysis, after further adjusting for C-reactive protein, 81 plasma proteins remained significantly associated with osteoporosis (Bonferroni correction), while all 122 plasma proteins maintained a significant association under FDR correction, indicating the robustness of the results (Supplementary Table 1). The number of proteins associated with osteoporosis risk decreased to 85 within the 10-years, but SOST, ADIPOQ, GDF15, CKB, and IGFBP2 remained the most significant associations with osteoporosis risk. However, the number of significant proteins dropped sharply to nine from 10 years onward. Of the original five most strongly associated proteins, only ADIPOQ and CKB maintained robust associations, while IGDCC4, ADAMTS13 and IGFBP3 also emerged with the most significant correlations to osteoporosis risk.

Subsequently, we characterized the biological regulatory pathways associated with osteoporosis-related proteins across different follow-up periods (Fig. 2B and Supplementary Tables 3–5). Throughout the full follow-up duration, these proteins were predominantly enriched in immune-related processes, including positive regulation of cell adhesion, cytokine-mediated signaling pathway, and leukocyte proliferation. Within the 10 years, in addition to the aforementioned immune functions, the associated proteins were also enriched in pathways related to the response to glucocorticoid. In contrast, proteins associated with osteoporosis from 10 years onward were mainly enriched in pathways involving IGF and Wnt signaling pathway, peptidyl-tyrosine phosphorylation, as well as the regulation of smooth muscle cell migration and proliferation, and key processes involved in kidney development and function.

3.2. Mendelian randomization and assessment of druggability

Subsequently, we employed two-sample Mendelian randomization to assess the genetic causal relationships between plasma proteins and osteoporosis or BMD (Fig. 2C-D, and Supplementary Tables 6–9). We identified 20, 20, and 19 proteins that demonstrated significant ($P < 0.05$ in at least three MR methods) or suggestive ($P < 0.05$ in at least two MR methods) associations with osteoporosis, femoral neck BMD, and lumbar spine BMD, respectively (Fig. 2D). Importantly, among the five key proteins identified in the previous results, four proteins (ADIPOQ, IGFBP2, SOST, and EGFR) showed significant or suggestive associations with osteoporosis or BMD.

Using the DGIdb database, we identified a series of potential drugs directly or indirectly associated with osteoporosis-related proteins (Supplementary Table 10). Among the approved drugs, *alendronate sodium*, *romosozumab* and *denosumab* are already used in the clinical treatment of osteoporosis. Nonsteroidal anti-inflammatory drugs, such as *diclofenac sodium* and *celecoxib*, are used for pain relief and management of skeletal diseases. Additionally, glucocorticoids such as *prednisone* and *dexamethasone* are closely associated with bone loss. Additionally, *follicle stimulating hormone*, *testosterone*, and *fenofibrate micronized* have been approved for hormone or cholesterol regulation.

3.3. Waves of osteoporosis-associated proteins

We performed hierarchical clustering on the 122 plasma proteins that were significantly associated with osteoporosis risk over the full follow-up time and identified four distinct trajectories (Fig. 3A and Supplementary Table 11). Cluster 1 exhibited a linear increasing pattern and was primarily enriched in pathways related to the positive regulation of cell adhesion, leukocyte proliferation, and T cell activation. Cluster 2 peaked approximately 6–7 years before diagnosis and was mainly associated with protein processing, ossification, and muscle atrophy. Cluster 3 showed a trough between 6 and 10 years before

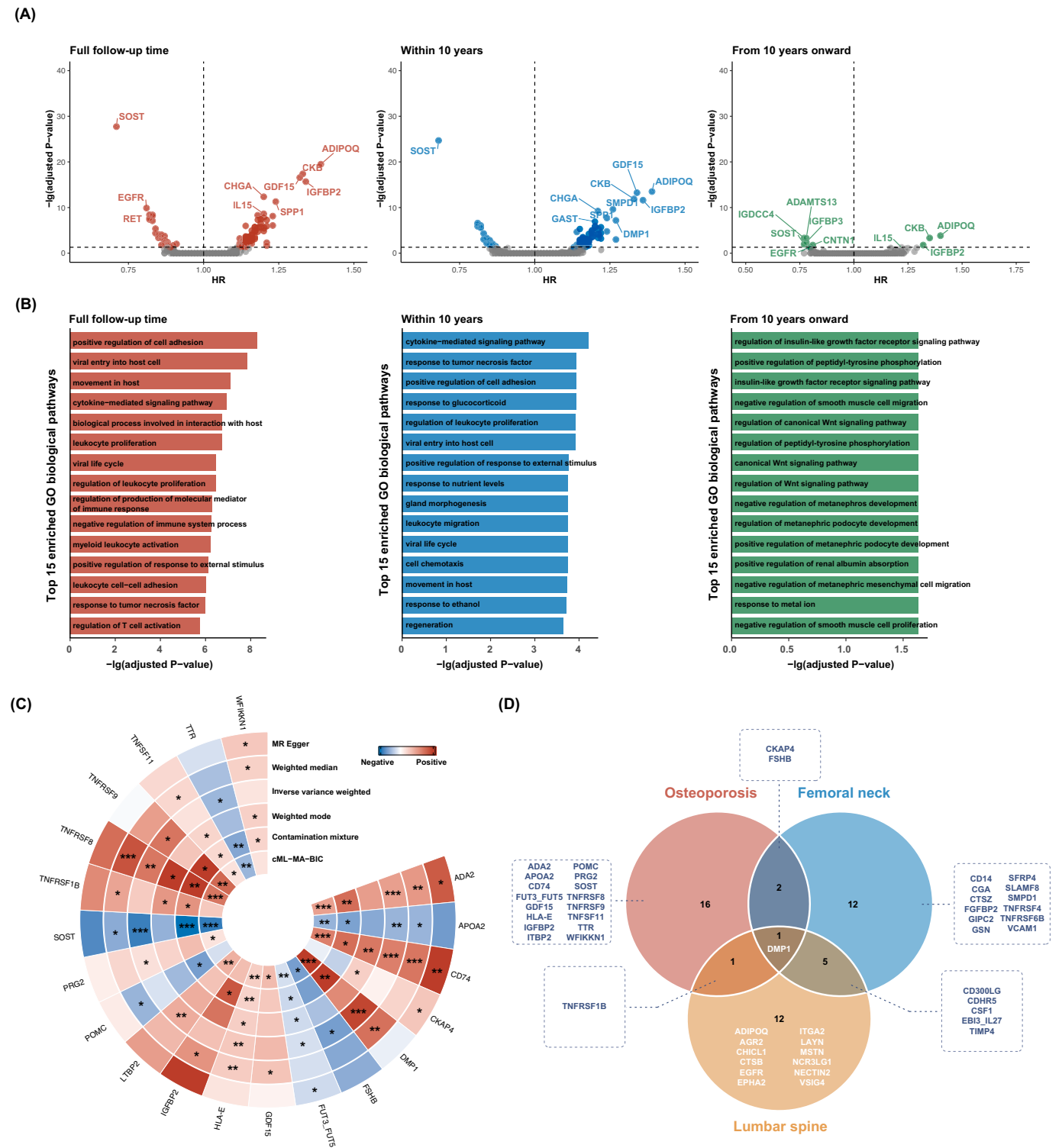


Fig. 2. Proteins associated with osteoporosis. (A) The associations of 2919 proteins with osteoporosis in different follow-up time. Model was adjusted for age, sex, ethnicity, education level, Townsend Deprivation Index, BMI, smoking status, alcohol consumption, physical activity, history of hypertension, history of diabetes, and history of cancer. Gray points represent non-significant proteins. Red, blue, and green points indicate proteins significantly associated with osteoporosis during the full follow-up time, within 10 years, and from 10 years onward, respectively. The top 10 proteins were labeled. The *P*-values were Bonferroni-corrected. (B) Top 15 enriched GO biological pathways. Enrichment analysis was performed using proteins significantly associated with osteoporosis during each follow-up period (full follow-up time, within 10 years, and from 10 years onward). The *P*-values were adjusted for multiple testing using Benjamini–Hochberg method. Causal proteins identified through Mendelian randomization in (C) osteoporosis, (D) femoral neck BMD, and lumbar spine BMD. Six different Mendelian randomization methods were utilized to mitigate false positive discoveries. Significant causal associations were identified when more than half of the methods showed nominal significance ($P < 0.05$), while two indicated a suggestive causal association. Abbreviations: BMD, bone mineral density; BMI, body mass index; HR, hazard ratio; 95% CI, 95% confidence interval. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

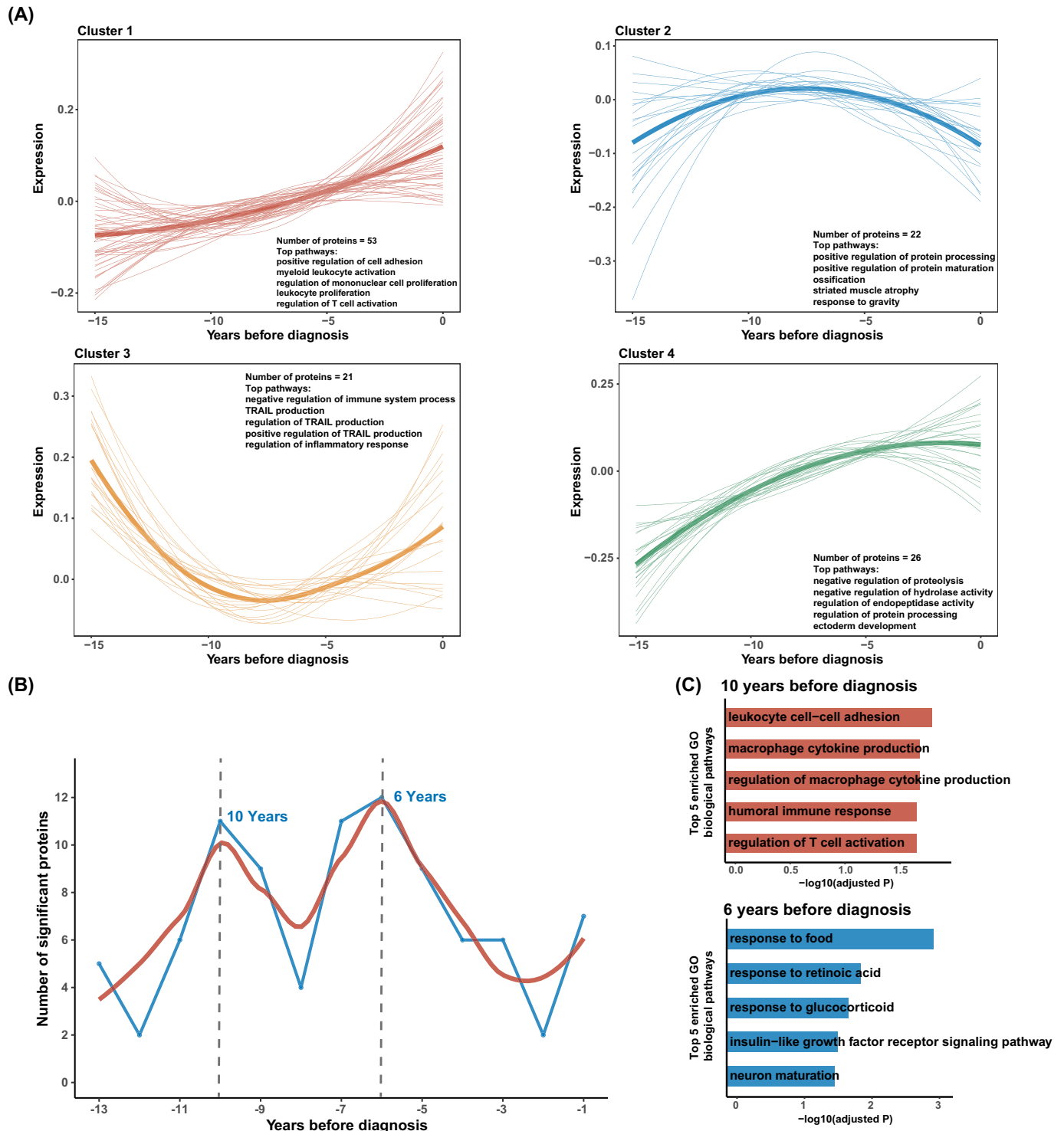


Fig. 3. Temporal evolutions of proteins before diagnosis of osteoporosis. (A) Clustering to identify protein trajectories. Bold lines indicate cluster-specific mean trajectories, while thin lines represent the individual trajectories of each protein within the cluster. Corresponding protein counts and significantly enriched pathways are annotated. (B) The number of differentially expressed plasma proteins during the pre-diagnostic phase of osteoporosis. Two peaks were observed at 10 and 6 years before diagnosis (indicated by dashed lines). The red curve was fitted using LOESS. (C) Top 5 enriched GO biological pathways at different peaks (10 and 6 years before diagnosis). Red bars represent enrichment results for differentially expressed proteins at the 10-year peak, and blue bars represent those at the 6-year peak. The *P*-values were adjusted for multiple testing using Benjamini–Hochberg method. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

diagnosis and was involved in the regulation of immune system processes. Cluster 4 displayed a logarithmic pattern and was primarily linked to the regulation of proteolysis.

To further characterize the dynamic changes of plasma proteins

during the progression of bone loss, we applied DE-SWAN analysis (Fig. 3B–C). Two peaks of significant proteins were identified approximately 10 and 6 years before osteoporosis diagnosis, as detailed in Supplementary Table 12. Notably, the proteins comprising these two

peaks exhibited distinct functional enrichment patterns. Proteins peaking around 10 years before diagnosis were predominantly involved in immune-related processes (Supplementary Table 13). In contrast, the peak observed approximately 6 years before diagnosis was enriched in pathways related to responses to food (particularly retinoic acid) and glucocorticoid, insulin-like growth factor receptor signaling pathway, and neuron maturation.

3.4. Plasma protein clustering identifies distinct osteoporosis risk subtypes

Nextly, LASSO regression was applied, further narrowing the selection to 63 plasma proteins for clustering analysis (Supplementary Table 14). Using the selected proteins, we performed clustering analyses separately for males and females. In both males and females, the BIC indicated that the optimal clustering model consisted of three distinct subtypes (Supplementary Table 15). Consequently, a total of six unique subtypes were identified, comprising three subtypes for males and three for females (Fig. 4A). The largest subtype in both males and females was designated as the Healthy Subtype (HS), as their rates of osteoporosis

and fracture incidence were comparable to or lower than the overall population (Supplementary Table 16). Detailed characteristics of the clustered subgroups are presented in Supplementary Table 16. The frailty index in cluster 2 was significantly higher in both females and males, accompanied by the highest BMI (Fig. 4 and Supplementary Table 16). Therefore, this subtype was designated as the Frailty and Obesity Subtype (FOS). In females, cluster 3 exhibited significantly lower estrogen levels compared to both the HS and the FOS, and was thus named the Low Estrogen Subtype (LES). In males, cluster 3 showed slightly elevated CRP levels compared to the HS, leading to its designation as the Mild Inflammation Subtype (MIS). Furthermore, differences observed in C-reactive protein, testosterone levels, and body impedance in the FOSs indicate elevated inflammation, reduced sex hormone levels, and decreased muscle mass.

3.5. Differences in osteoporosis and fracture risk across identified subtypes

In females, Kaplan-Meier curves revealed significant differences

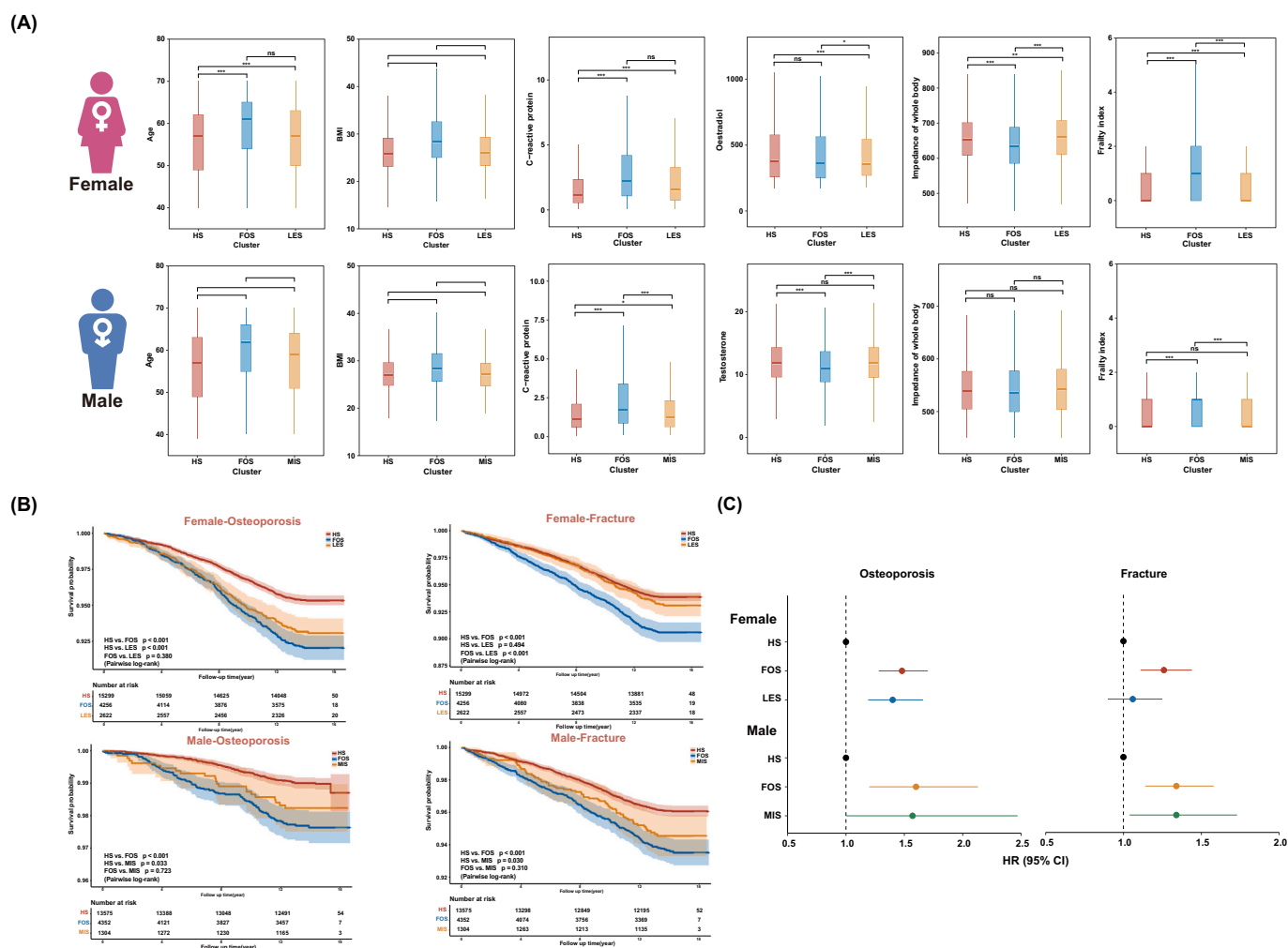


Fig. 4. Identification of high-risk subtypes of osteoporosis. (A) Phenotypic characteristics of different subtypes. Phenotypic features include age, BMI, C-reactive protein, oestradiol, impedance of the whole body, and frailty index. Red boxplots represent the HS, blue boxplots represent the FOS, and yellow boxplots represent the LES (female) or the MIS (male). Pairwise comparisons between subtypes were performed using the *t*-test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. (B) Unadjusted Kaplan-Meier curves present the clinical progression to osteoporosis and fracture over time. In each panel, the lines represent the different clusters (HS in red, FOS in blue and LES and MIS in yellow). Survival curves were compared using the pairwise log-rank test. (C) HRs and 95% CIs for osteoporosis and fracture, according to different subtypes. Model was adjusted for age, sex, ethnicity, education level, Townsend Deprivation Index, BMI, smoking status, alcohol consumption, physical activity, history of hypertension, history of diabetes, and history of cancer. Abbreviations: BMI, body mass index; FOS, Frailty and Obesity Subtype; HS, Healthy Subtype; LES, Low Estrogen Subtype; MIS, Mild Inflammation Subtype; 95% CI, 95% confidence interval. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

between FOS (P -value <0.001) and LES (P -value <0.001) compared to the HS in terms of osteoporosis progression (Fig. 4B). However, for fracture progression, significant differences were observed only between FOS and the HS (P -value <0.001). Further Cox regression indicated that both FOS (HR = 1.48; 95% CI: 1.28 to 1.70) and LES (HR = 1.40; 95% CI: 1.19 to 1.66) exhibited a higher risk of osteoporosis compared to the HS (Fig. 4B). However, for fracture risk, FOS demonstrated a significantly higher risk than the HS (HR = 1.26; 95% CI: 1.11 to 1.44), whereas no significant difference was observed for LES (HR = 1.06; 95% CI: 0.90 to 1.25) (Fig. 4C). While both FOS and LES display characteristics of high osteoporosis risk, they differ in fracture risk, highlighting the heterogeneity of osteoporosis risk.

In males, Fig. 4B indicated significant differences in both osteoporosis and fracture risk between FOS (both P -value <0.001), MIS (P -value = 0.033 for osteoporosis, P -value = 0.030 for fracture), and the HS. However, after further adjustment for covariates, FOS was associated with significantly increased risks of both osteoporosis (HR = 1.60; 95% CI: 1.20 to 2.13) and fracture (HR = 1.34; 95% CI: 1.14 to 1.58) compared to the HS (Fig. 4C). In contrast, MIS demonstrated a significantly elevated fracture risk (HR = 1.34; 95% CI: 1.04 to 1.73) but did not differ significantly from the HS in terms of osteoporosis risk (Fig. 4C).

3.6. Subtype-specific characteristics at the protein and network modules level

Considering the similarities in disease manifestation observed in both male and female FOSs, which showed high risks for osteoporosis and fractures, we identified FOSs as the primary high-risk subtype deserving focused attention. At the individual protein level, notable commonalities were observed among these high-risk groups. All eight proteins significantly associated with osteoporosis in male FOS were also significantly associated in female FOS, with five of them (ADIPOQ, IGFBP2, SOST, CKB, and EGFR) ranking among the top ten most significant proteins in female FOS (Fig. 5A and Supplementary Table 17). Notably, these five proteins were also among the top ten most significant osteoporosis-associated proteins identified in the overall population (Fig. 2A, Fig. 5A). In contrast, substantial differences were observed in the proteins most significantly associated with osteoporosis risk between FOS, LES (SOST, KLKB1, ITGAV, CCL7, and MSTN) and MIS (CHGA, GDF15, SPON2, and GAST), highlighting the heterogeneity of osteoporosis risk across distinct subtypes (Fig. 5A).

Through Weighted Correlation Network Analysis, we identified four distinct protein modules among the 122 proteins, with the number of proteins in each module ranging from 15 to 63 (Fig. 5B). Pathway enrichment analysis revealed functional associations of these modules with diverse biological processes, including immune regulation, lipid metabolism, follicle-stimulating hormone (FSH) signaling pathways, and skin-muscle aging (Fig. 5C and Supplementary Table 18). The immune, lipid metabolism, and FSH modules exhibited significant positive associations with osteoporosis risk, whereas the skin-muscle module demonstrated a significant inverse association with osteoporosis risk (Fig. 5D). Importantly, we also observed significant dysregulation at the protein module level across the different subtypes (Fig. 5E). Both FOSs, LES, and MIS demonstrated significantly elevated expression levels in the immune, lipid metabolism (except for female FOS), and FSH modules compared to the HS. In contrast, the expression levels of the skin-muscle module were markedly reduced in these clusters.

3.7. Development of machine learning models to predict osteoporosis risk in the high-risk subtype

We ranked 63 osteoporosis-associated proteins based on their importance in the LGBM model within the high-risk subtypes (FOSs). From these, the top 10 proteins (FSHB, SOST, LTBP2, ADIPOQ, EDA2R, OXT, SRPX, CTSL, HPGDS, CGA) with the strongest predictive capability

for osteoporosis risk in the high-risk subtypes were selected for further analysis (Fig. 6A and Supplementary Table 19). The SHAP plot revealed that FSHB exhibited the widest range of contributions, underscoring its robust predictive power in the high-risk subtype (Fig. 6B). Subsequently, we developed two predictive models: Model 1 was based on 10 selected plasma proteins, while Model 2 incorporated traditional risk factors, including age, sex, ethnicity, smoking status, alcohol consumption, Townsend Deprivation Index, and BMI (Fig. 6C). In the FOS population, the protein-based model demonstrated superior performance in predicting the risk of osteoporosis compared with model 2 (AUC: 0.743 versus 0.680, DeLong test $P = 0.014$).

4. Discussion

In this study, we investigated the dynamic changes of plasma proteins during the pre-diagnostic phase of osteoporosis and identified distinct high-risk subtypes for osteoporosis from a proteomic perspective. Our findings revealed that plasma proteins associated with osteoporosis exhibit temporal heterogeneity. We further delineated four distinct protein trajectory patterns and captured two critical intervention windows, approximately 10 and 6 years before diagnosis. Moreover, with a focus on identifying high-risk populations for prioritized screening, we discovered a high-risk subtype (FOS) in both male and female, characterized by a significantly elevated risk of osteoporosis and fractures.

One of the key findings of our study is the observation of fluctuating patterns in plasma proteins throughout the pre-diagnostic phase of osteoporosis. We identified two distinct protein peaks occurring approximately 10 and 6 years before osteoporosis diagnosis, which may represent critical windows for early intervention. These two peaks exhibited minimal overlap in both protein composition and functional enrichment, a pattern that has also been reported in previous studies on aging [16]. The earlier peak was predominantly enriched for immune-related pathways, suggesting that inflammation may play an important role as an early mechanism in bone loss [22]. In addition, the peak occurring 6 years before diagnosis are mainly enriched in response to food (especially retinoic acid) and glucocorticoids, which is important for osteoporosis prevention. Retinoic acid, a metabolite of vitamin A, is involved in osteoblast regulation. A previous study demonstrated that inhibition of retinoic acid signaling in osteoblasts led to increased BMD, alterations in bone microarchitecture, and significantly reduced bone strength in mice, resulting in osteoporosis-like disorders [23]. Consistently, studies in zebrafish have shown that retinoic acid can alleviate symptoms of osteoporosis [24]. Moreover, long-term or high-dose use of glucocorticoids leads to rapid bone loss and an increased risk of fractures, potentially through mechanisms involving the suppression of bone formation, promotion of bone resorption, and inhibition of gonadal function [25]. These findings underscore the potential value of monitoring vitamin A intake and glucocorticoid use as preventive strategies against osteoporosis; however, further research is needed to establish optimal intake and dosing guidelines.

Furthermore, our findings demonstrate that plasma proteins can effectively identify high-risk subtypes of osteoporosis within a healthy population, providing new insights into the heterogeneity of the disease. Recently, a study conducted in a Chinese population clustered individuals with osteoporosis into two distinct subtypes [4]. These subtypes exhibited varying fracture risks, further validating the concept of heterogeneity observed in our results. However, there are notable distinctions between our study and theirs. Our research emphasizes early intervention and monitoring in healthy individuals to prevent disease onset, while their study focuses more on treatment strategies for clinical patients. This distinction highlights the complementary nature of the two studies: understanding disease heterogeneity in clinical populations can inform therapeutic strategies, whereas identifying high-risk subtypes in asymptomatic individuals can facilitate preventive measures. Together, these perspectives underscore the importance of tailoring

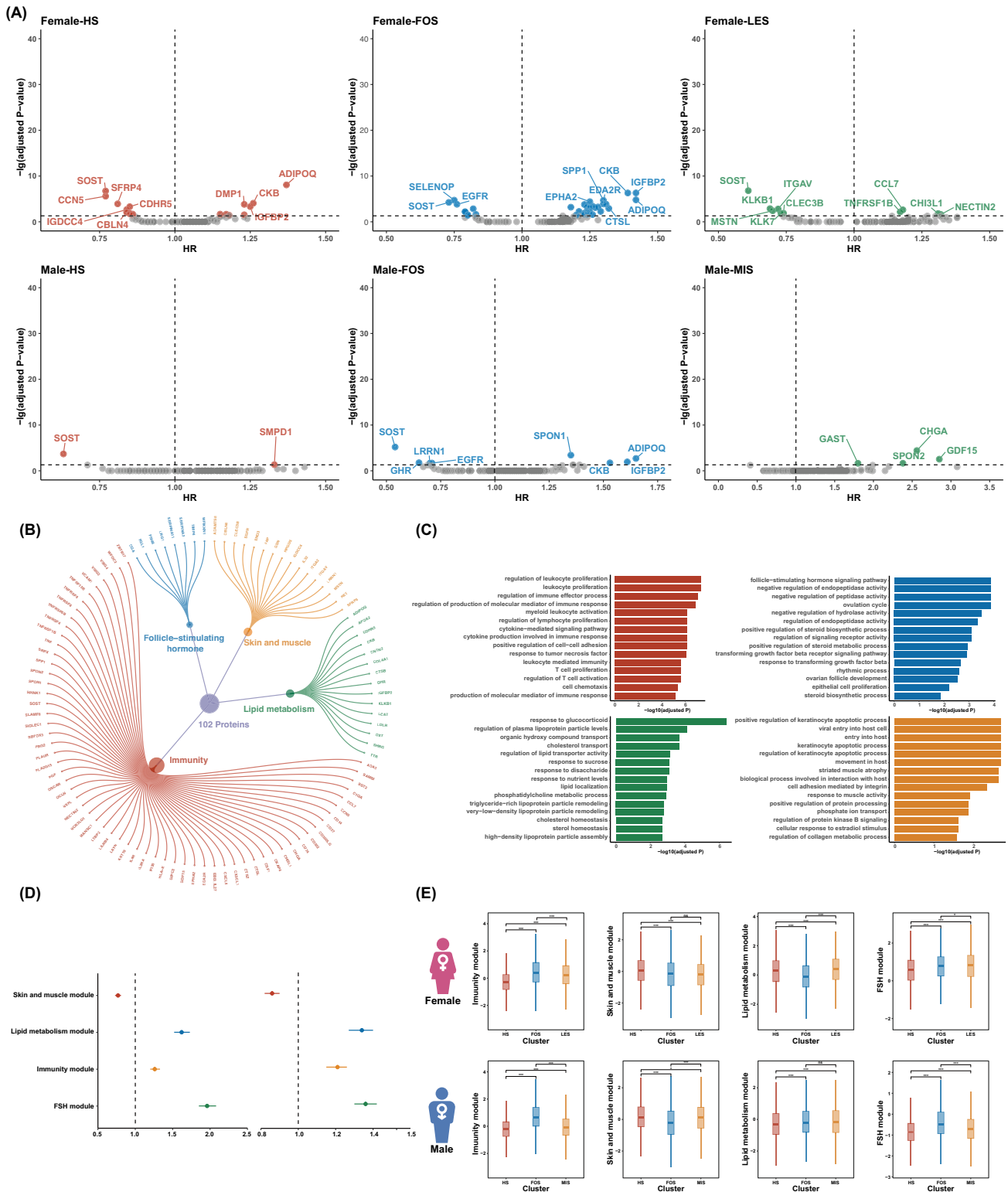


Fig. 5. The characteristics of different subtypes. (A) HRs and 95% CIs between plasma proteins and osteoporosis, stratified by cluster. All models were multivariate adjusted for age, sex, ethnicity, education level, Townsend Deprivation Index, BMI, smoking status, alcohol consumption, physical activity, history of hypertension, history of diabetes, and history of cancer. All the P -values were Bonferroni-corrected. (B) Specific proteins included in each module. The principal biology for each module was defined by enrichment analysis. (C) Top 15 enriched GO biological pathways in different modules. The P -values were adjusted for multiple testing using Benjamini–Hochberg method. (D) HRs and 95% CIs for osteoporosis and fracture, according to different protein modules. Model was adjusted for age, sex, ethnicity, education level, Townsend Deprivation Index, BMI, smoking status, alcohol consumption, physical activity, history of hypertension, history of diabetes, and history of cancer. (E) The comparison of protein module expression values among different subtypes. Abbreviations: BMI, body mass index; FOS, Frailty and Obesity Subtype; HR, hazard ratio; HS, Healthy Subtype; LES, Low Estrogen Subtype; MIS, Mild Inflammation Subtype; 95% CI, 95% confidence interval.

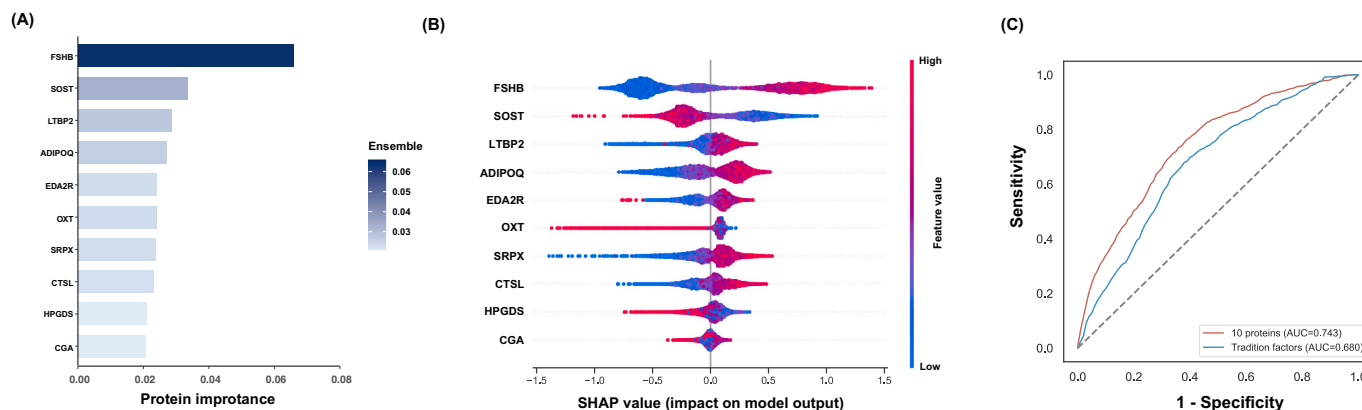


Fig. 6. Evaluating the predictive performance of proteins in high-risk subtypes, alone or in combination with other factors. (A) Importance of protein based on predictive contribution in LGBM model. (B) SHAP visualization plot of selected proteins. (C) ROC curves for the prediction of future osteoporosis over the entire full follow-up period.

interventions to specific population needs and stages of disease progression. Moreover, although the identified FOS phenotypically resembles a well-recognized clinical profile, it was derived in a data-driven manner based on proteomic signatures rather than predefined clinical characteristics. The proteomic features of this subtype, including coordinated alterations in immune, lipid metabolism, muscle-related, and endocrine pathways, provide mechanistic insights linking these clinical manifestations to their underlying biological processes. Notably, such molecular information is not fully captured by conventional clinical measures alone, highlighting the added value of proteomics in refining subtype characterization. Additionally, the improved predictive performance of the protein-based model further supports the utility of proteomic profiling in enhancing risk stratification beyond traditional approaches. However, the reliance on advanced Olink proteomic platforms may limit the immediate clinical applicability of our model due to considerations of cost, accessibility, and technical complexity. From a translational perspective, simplifying the model by reducing the number of proteins while maintaining predictive performance is warranted. Such simplification could facilitate the use of more accessible detection methods, thereby enhancing feasibility in routine clinical practice. For example, these proteins could be measured using targeted immunoassays or ELISA-based platforms. Notably, the relatively small number of proteins included in our model may facilitate its translation into simplified biomarker panels for risk detection and screening in high-risk populations. Future studies are needed to develop and validate simplified models in independent cohorts and to evaluate their performance in real-world clinical settings.

To further elucidate differences between subtypes, we performed integrated analyses at both the protein and network module levels. At the protein level, ADIPOQ, IGFBP2, SOST, CKB, and EGFR were consistently associated with osteoporosis risk in high-risk subtypes, suggesting their central roles in disease-related metabolic and signaling alterations. These proteins are functionally linked to lipid metabolism, bone formation, and musculoskeletal homeostasis [26–30]. At the network module level, high-risk subtypes exhibited coordinated dysregulation in immune, lipid metabolism, muscle-related, and FSH-associated modules. This suggests that osteoporosis development in high-risk subtypes is not driven by a single factor but rather represents the cumulative effects of multiple metabolic and signaling pathways. These alterations collectively reflect a systemic phenotype characterized by frailty- and obesity-related features, including inflammation, reduced sex hormone levels, and impaired muscle function. From an osteoimmunological perspective, chronic inflammation is a common driver of bone loss. Key pro-inflammatory cytokines, such as TNF- α , IL-1, and IL-6, promote osteoclast activity while simultaneously inhibiting osteoblast function through multiple pathways [31]. For instance, TNF- α impairs

osteoblast activity by upregulating inhibitory proteins such as SOST and Dickkopf-1 [32]. Meanwhile, it indirectly enhances osteoclastogenesis by increasing RANKL expression in osteoblasts [33]. In addition, dysregulation of lipid metabolism may influence the bone marrow micro-environment by altering bone marrow adipocytes [34]. These adipocytes secrete endocrine factors, including ADIPOQ and IGFBP2, which play important roles in the regulation of BMD [27]. For example, ADIPOQ can modulate the RANKL/OPG ratio, thereby promoting the proliferation and differentiation of osteoclast precursor cells and ultimately affecting bone resorption [35]. Moreover, growing evidence highlights the critical role of muscle–bone interactions in the pathogenesis of osteoporosis [36–38]. Impaired muscle function may affect bone homeostasis not only through reduced mechanical loading but also via endocrine and metabolic pathways [39]. Skeletal muscle releases myokines such as irisin, which can regulate bone remodeling by promoting osteoblast differentiation while also influencing osteoclast activity [40]. Beyond myokines, muscle-derived factors, such as kynurenic acid, have also been implicated in the regulation of bone resorption [41].

The strengths of our study include the use of a large-scale prospective cohort, high-quality plasma proteomic profiling, and a comprehensive analytical framework that integrates association analysis, causal inference, clustering, and network-based module identification. In addition, we characterized the dynamic fluctuations of plasma proteins during the pre-diagnostic phase of osteoporosis, an aspect that has received limited attention in previous studies. However, several limitations should be acknowledged. Firstly, the proteomics analysis did not encompass the entire human proteome, and some proteins that may play a crucial role in revealing the heterogeneity of osteoporosis might not have been included in our analysis. Secondly, the reliance on ICD codes and self-reported data may lead to underdiagnosis and misclassification of disease outcomes, thereby potentially affecting the accuracy of the results. For instance, dependence on ICD coding limits the ability to distinguish fragility fractures from traumatic fractures. In addition, clinically silent fractures, such as vertebral fractures, may be underreported or overlooked, further contributing to potential misclassification. Thirdly, due to the lack of longitudinal measurements of both BMD and plasma proteins, using time-to-diagnosis as a proxy to simulate bone loss progression may not accurately reflect the true progression. Therefore, our findings should be interpreted with caution, and further longitudinal studies are needed to validate these observations. Additionally, the analysis based on data from a predominantly European population in the UK Biobank may impact the generalizability of the findings to other ethnic groups, and further external validation is needed. Additionally, compared with the general population in the UK, the UKB participants tend to be healthier, which may introduce a “healthy volunteer” bias

[42]. Meanwhile, as the study population consists predominantly of individuals of European ancestry, the generalizability of the findings to other populations may be limited, and further validation in diverse populations is warranted. Furthermore, the predictive model has not been validated in an independent external cohort, which may reduce confidence in its robustness and broader applicability. Therefore, further validation in independent cohorts is warranted.

In conclusion, our study provides valuable insights into potential intervention windows and target populations for the prevention of osteoporosis from a proteomic perspective. Notably, we identified two critical windows (approximately 10 and 6 years before diagnosis), characterized by distinct plasma proteomic shifts, which may serve as early indicators for timely preventive strategies. Furthermore, we identified a high-risk subtype with significantly elevated risks of both osteoporosis and fracture. These findings highlight the potential of integrating proteomics into public health practice, providing scientific evidence to guide early monitoring and personalized interventions for high-risk populations.

Abbreviations

BMD	bone mineral density
BMI	body mass index
CIs	confidence intervals
cML-MA	constrained maximum likelihood and model averaging
ConMix	Contamination mixture method
FOS	Frailty and Obesity Subtype
FSH	follicle-stimulating hormone
GO:BP	Gene Ontology Biological Processes
HR	hazard ratio
HS	Healthy Subtype
ICD	International Classification of Diseases
LES	Low Estrogen Subtype
MIS	Mild Inflammation Subtype
MR	Mendelian randomization
UKB	UK Biobank

CRediT authorship contribution statement

Yi Zheng: Writing – original draft, Software, Investigation, Formal analysis, Data curation, Conceptualization. **Jincheng Li:** Software, Methodology, Investigation, Data curation. **Yucan Li:** Software, Investigation, Data curation. **Jiacheng Wang:** Software, Investigation, Data curation. **Chen Suo:** Writing – review & editing, Supervision, Resources. **Yanfeng Jiang:** Writing – review & editing, Supervision, Resources. **Li Jin:** Writing – review & editing, Supervision, Funding acquisition. **Kelin Xu:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Xingdong Chen:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

Ethics approval and consent to participate

This study received approval by the the NHS North West Multicentre Research Ethics Committee (11/NW/0382). All participants provided written informed consent.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bone.2026.117969>.

Data availability

UK Biobank data are available through application to the database <https://www.ukbiobank.ac.uk/>.

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