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Assessing the Genetic Correlations Between Blood Plasma Proteins and Osteoporosis: A Polygenic Risk Score Analysis

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Abstract

Osteoporosis is a common metabolic bone disease. The impact of global blood plasma proteins on the risk of osteoporosis remains elusive now. We performed a large-scale polygenic risk score (PRS) analysis to evaluate the potential effects of blood plasma proteins on the development of osteoporosis in 2286 Caucasians, including 558 males and 1728 females. Bone mineral density (BMD) and bone areas at ulna & radius, hip, and spine were measured using Hologic 4500W DXA. BMD/bone areas values were adjusted for age, sex, height, and weight as covariates. Genome-wide SNP genotyping of 2286 Caucasian subjects was performed using Affymetrix Human SNP Array 6.0. The 267 blood plasma proteins-associated SNP loci and their genetic effects were obtained from recently published genome-wide association study (GWAS) using a highly multiplexed aptamer-based affinity proteomics platform. The polygenetic risk score (PRS) of study subjects for each blood plasma protein was calculated from the genotypes data of the 2286 Caucasian subjects by PLINK software. Pearson correlation analysis of individual PRS values and BMD/bone area value was performed using R. Additionally, gender-specific analysis also was performed by Pearson correlation analysis. 267 blood plasma proteins were analyzed in this study. For BMD, we observed association signals between 41 proteins and BMD, mainly

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Conflict of interest Xiao Liang, Yanan Du, Yan Wen, Li Liu, Ping Li, Yan Zhao, Miao Ding, Bolun Cheng, Shiqiang Cheng, Mei Ma, Lu Zhang, Hui Shen, Qing Tian, Xiong Guo, Feng Zhang, and Hong-Wen Deng declare that they have no conflicts of interest.

Compliance with Ethical Standards

Human and Animal Rights All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

Informed Consent "Informed consent was obtained from all individual participants included in the study."

including whole body total BMD versus Factor H (p value = 9.00×10^{-3}), whole body total BMD versus BGH3 (p value = 1.40×10^{-2}), spine total BMD versus IGF-I (p value = 2.15×10^{-2}), and spine total BMD versus SAP (p value = 3.90×10^{-2}). As for bone areas, association evidence was observed between 45 blood plasma proteins and bone areas, such as ferritin versus spine area (p value = 1.90×10^{-2}), C4 versus hip area (p value = 1.25×10^{-2}), and hemoglobin versus right ulna and radius area (p value = 2.70×10^{-2}). Our study results suggest the modest impact of blood plasma proteins on the variations of BMD/bone areas, and identify several candidate blood plasma proteins for osteoporosis.

Keywords

Genome-wide association study; Blood plasma proteins; Osteoporosis; Polygenic risk score analysis

Introduction

Osteoporosis (OP) is a common metabolic bone disease characterized by decreased bone mineral density (BMD) and increased risk of fragility fractures. OP is recognized as a major health problem worldwide. It affects more than 200 million individuals worldwide [1]. OP was reported to cause 2 million hip fractures and other debilitating bone fractures annually [1]. With the rapidly aging of populations, OP and its associated fragility fractures lead to heavy burden on the health care system and society. BMD has a strong heritable component with an estimated heritability of 50–85% [2]. BMD is widely used for diagnosing OP and evaluating the risk of osteoporotic fractures [2]. Extensive genetic studies of OP have been conducted and identified a group of susceptibility loci associated with the variation of BMD [2–4]. However, the genetic basis of OP remains largely unknown now.

Proteins play crucial roles for gene functions in biological organisms, and abnormal alterations of proteins could lead to changes of physiological conditions [5]. Notably, there are increasing interests in studying the relationships between blood plasma proteins and osteoporosis. Multiple studies found that protein was significantly associated with BMD [5–8]. For instance, it has been identified that lean elderly Thais with lower transthyretin levels had a higher risk of osteoporosis [6]. Another study found that ANXA2 protein expressed differentially between low BMD and high BMD subjects [5]. Recently, researchers have observed that the levels of autocrine motility factor receptor were lower in plasma of female osteoporosis patients [7]. In addition, Wu et al. suggested that plasmaadvanced oxidation protein products levels were negatively associated with BMD [8]. To the best of our knowledge, no large-scale study has been conducted to systematically evaluate the relationships between global blood plasma proteins and osteoporosis.

With the rapid development of DNA genotyping and sequencing technologies, a large amount of genetic susceptibility loci have been identified for human complex diseases or traits. Utilizing published susceptibility loci, polygenic risk score (PRS) [9] analysis is not only able to evaluate the effects of susceptible loci on disease risks, but also capable of exploring the genetic relationships between various complex diseases and traits. For instance, PRS analysis has been successfully applied to multiple complex diseases, such as

diabetes [10], obesity [11], and coronary heart disease [12]. Recently, Karsten Suhre et al. conducted a genome-wide association study using a highly multiplexed aptamer-based affinity proteomics platform (SOMAscan) in 1000 individuals, which identified a group of SNPs associated with multiple blood plasma protein levels [13]. Using the PRS of blood plasma protein levels as instrumental variables, we can explore the genetic relationships between global blood plasma proteins and osteoporosis.

In this study, we conducted a large-scale PRS analysis of 267 blood plasma proteins to explore the relationships between blood plasma proteins and BMD/bone area in 2286 Caucasians subjects. Our study results provide novel clues for understanding the mechanism of the observed effects of blood plasma protein on the variation of BMD and bone areas.

Materials and Methods

Study Subjects

Briefly, our study samples consisted of 2286 unrelated Caucasians subjects living at Kansas City and its surrounding areas. Anthropometric measures and a structured questionnaire including diet, lifestyle, medical history, family information, and others were acquired for all samples. The subjects with chronic diseases and conditions that might potentially affect BMD, bone areas, bone mass, or metabolism were excluded in this study. These diseases/conditions included chronic disorders involving skeletal diseases (osteogenesis imperfecta, rheumatoid arthritis, fractures, etc.), vital organs (heart, lung, liver, kidney, brain), serious metabolic diseases (diabetes, hyperthyroidism, etc.), chronic use of drugs affecting bone metabolism (hormone replacement therapy, corticosteroid therapy, anticonvulsant drugs), and malnutrition conditions (such as chronic diarrhea, chronic ulcerative colitis, etc.). In addition, the subjects taking antibone resorptive or bone anabolic agents/drugs, such as bisphosphonates were also excluded from this study. The BMD (including total body, hip, spine, ulna & radius, and femoral neck) and bone areas (including hip, spine, ulna and radius, and femoral neck) were measured using Hologic 4500W dual-energy X-ray absorptiometry (Hologic Inc., Bedford, MA, USA) that were calibrated daily. All phenotypic values of BMD/bone area were adjusted for age, sex, height, and weight using linear regression model. 5 mL peripheral blood specimens were drawn from each participant. Informed consent documents were signed by all participants before they entered the project.

Genome-Wide SNP Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN, USA). SNP genotyping was performed using Genome-Wide Human SNP Array 6.0 (Affymetrix, Santa Clara, CA, USA), according to the Affymetrix protocol. In brief, 250 ng of genomic DNA was digested with restriction enzyme NspI or StyI. Digested DNA specimens were adaptor-ligated and polymerase chain reaction (PCR)-amplified for each subject. Fragment PCR products were then labeled with biotin, denatured, and hybridized to the arrays. Arrays were then washed and stained using Phycoerythrin on Affymetrix Fluidics Station, and scanned using the GeneChip Scanner3000 7G. Data management and analyses were performed using the Genotyping Command Console. The SNPs with Hardy–Weinberg equilibrium (HWE) testing p values <

0.0001, minor allele frequencies (MAF) < 0.01, and genotyping call rate < 95% were excluded.

Blood Plasma Protein-Associated SNP Sets

Blood plasma protein-associated SNPs and their genetic effects were driven from a large-scale genome-wide study of blood plasma protein [13]. Briefly, this study comprised 1000 Caucasian subjects. Blood samples for omics analyses and DNA extraction were collected between 2006 and 2008 as part of the KORA F4 follow-up. Blood was drawn in the morning between 08:00 and 10:30 after a period of at least 10 h of overnight fasting. The SOMA scan platform was used to quantify protein levels. Genome-wide SNP genotyping was conducted using the Affymetrix Axiom Array. PLINK [14] was used to fit linear models to inverse-normalized probe levels, using age, sex, and body mass index as covariates. R version 3.1.3 (http://www.R-project.org/) was used for data organization, plotting, and additional statistical analyses outside of the actual GWAS, including computation of linear regression models based on raw and log-scaled data. After quality control, this study tested the associations between 509,946 common autosomal SNPs and 1124 blood plasma protein levels. 539 significant associations (p value < $8.72 \times 10^{-11} = 0.05/509{,}946/1124$) between 284 proteins and 451 independent SNPs were identified by this study. Detailed description of study subjects, experimental design, and statistical analysis can be found in the published study [13].

Statistical Analysis

For PRS calculation, the 284 proteins-associated SNPs were further aligned with our genome-wide genotype data of 2286 Caucasian subjects. After aligning, we obtained 267 blood plasma proteins, whose associated SNPs had genotype data in our 2286 Caucasian subjects. Using linear regression model, the raw BMD/bone area values were first adjusted for age, sex, height, and weight as covariates [15]. The residues from linear regression were then used as the phenotypic values of BMD/bone area for genetic correlation analysis [15]. All the SNPs in the GWAS were included in the genetic correlation analysis. The PRS of each subject for each blood plasma protein was calculated from the genotypes data of the 2286 Caucasians subjects by PLINK software [16]. Pearson correlation analysis was then conducted to evaluate the associations between each blood plasma protein and BMD/ bone areas by using calculated PRS as the instrumental variables of blood plasma protein. Pearson correlation analysis was conducted via R (https://www.r-project.org/). Similarly, Gender-specific analysis was also performed by Pearson correlation analysis. Genetic correlations between BMD/ bone areas and men/women were analyzed, respectively. A p value < 0.05 was considered statistically significant in this study.

Results

A total of 2286 men and women were included in this study, and the general characteristics of the subjects are presented in Table 1.

BMD-Associated Blood Plasma Proteins in Total Sample

We observed genetic correlation evidence between 41 proteins and BMD. For whole body total BMD, genetic correlations were detected for 7 blood plasma proteins, such as Factor H (p value = 9.00×10^{-3}), BGH3 (p value = 1.40×10^{-2}), and ferritin (p value = 3.00×10^{-2}). For spine total BMD, genetic correlations were also observed for IGF-I (p value = 2.15×10^{-2}), BGH3 (p value = 3.15×10^{-2}), and SAP (p value = 3.90×10^{-2}). For femoral neck BMD, genetic correlations were detected for 10 blood plasma proteins, mainly including ASAHL (p value = 1.20×10^{-2}) hemoglobin (p value = 4.45×10^{-2}) and BGH3 (p value = 4.35×10^{-2}). For right ulna and radius BMD, significant genetic associations were observed for ASAHL (p value = 1.00×10^{-3}), Factor H (p value = 2.00×10^{-3}), and C7 (p value = 1.15×10^{-2}). For hip total BMD, genetic correlations were detected for 18 blood plasma proteins, such as Kininogen, HMW (p value = 9.50×10^{-3}), sICAM-5 (p value = 9.50×10^{-3}), and HCC-4 (p value = 1.10×10^{-2}) (Fig. 1).

Bone Areas-Associated Blood Plasma Proteins in Total Sample

We observed genetic correlation evidence between 45 proteins and bone area. For hip area, genetic correlations were detected for 9 blood plasma proteins, such as C7 (p value = 8.50×10^{-3}), C4 (p value = 1.25×10^{-2}), and LAG-1 (p value = 3.35×10^{-2}). For spine area, genetic correlations were observed for Mn SOD (p value = 1.00×10^{-3}), C4 (p value = 1.30×10^{-2}), and ferritin (p value = 1.90×10^{-2}). For right ulna and radius area, 16 blood plasma proteins show genetic correlations evidence, mainly including BST1 (p value = 4.50×10^{-3}), CPNE1 (p value = 1.05×10^{-2}), and hemoglobin (p value = 2.70×10^{-2}). For femoral neck area, genetic correlations were detected for 22 blood plasma proteins, such as RANTES (p value = 1.00×10^{-3}), TAFI (p value = 2.00×10^{-3}), and ferritin (p value = 3.95×10^{-2}) (Fig. 2).

BMD/Bone Area-Associated Blood Plasma Proteins in Male

We detected 37 blood plasma proteins associated with male BMD (Fig. 3) and 45 blood plasma proteins associated with male bone area (Fig. 4), such as whole body total BMD versus MMP-8 (p value = 1.86×10^{-2}), hip total BMD versus Hemopexin (p value = 1.60×10^{-3}), spine total BMD versus ENTP5 (p value = 1.90×10^{-3}), and femoral neck BMD versus IL-12Rb1 (p value = 1.40×10^{-2}). In addition, we observed several genetic correlations between blood plasma proteins and bone area, mainly including PPAC versus hip area (p value = 7.80×10^{-3}), Protein C versus femoral neck area (p value = 4.00×10^{-4}), and Coagulation Factor VII versus right ulna and radius area (p value = 4.00×10^{-4}).

BMD/Bone Area-Associated Blood Plasma Proteins in Female

We observed that 31 blood plasma proteins were associated with female BMD (Fig. 5) and 42 blood plasma proteins were associated with female bone areas (Fig. 6), such as whole body total BMD versus Factor H (p value = 2.90×10^{-3}), hip total BMD versus Kininogen, HMW (p value = 2.76×10^{-2}), spine total BMD versus C7 (p value = 2.30×10^{-3}), and femoral neck BMD versus CYTF (p value = 1.51×10^{-2}). Additionally, we observed several genetic correlations between blood plasma proteins and bone area, mainly including C7

versus hip area (p value = 3.00×10^{-3}), TAFI versus femoral neck area (p value = 4.00×10^{-4}), and CNDP1 versus spine area (p value = 9.00×10^{-4}).

Discussion

To reveal the potential effects of blood plasma proteins on the development of osteoporosis, we conducted a large-scale PRS analysis of 267 blood plasma proteins in 2286 Caucasians. We observed multiples genetic correlations between BMD/bone areas and blood plasma proteins, and identified several candidate blood plasma proteins for osteoporosis. To the best of our knowledge, this is the first large-scale PRS analysis of blood plasma proteins for osteoporosis. Our study results suggest that some blood plasma proteins were associated with the variations of BMD and bone areas.

One important finding of this study is the transforming growth factor-beta-induced protein ig-h3 (beta ig-h3/BGH3, also known as RGD-CAP), which showed correlation with BMD at different skeletal sites, including whole body total BMD, hip total BMD, spine total BMD, right ulna and radius BMD, and femoral neck BMD. BGH3 is an extracellular matrix (ECM) protein expressed in a wide variety of tissues, including cartilage, skin, and bone [17]. It has been demonstrated that BGH3 is an intriguingly versatile molecule role in a wide range of physiological and pathological conditions. For instance, BGH3 is capable of negatively regulating the mineralization of hypertrophic chondrocytes at the terminal stage of chondrogenic differentiation [18] and in the end stage of endochondral ossification [19], indicating that BGH3 functions as an negative regulator of osteogenesis. Interestingly, BGH3 may also play an important role in the maintenance of periodontal ligament homeostasis by regulating mineralization [20]. In addition, it has been identified that BGH3 could regulate periosteal bone formation rate and thereby bone mass, bone size, and bone strength [21]. This correlation also was observed in women, whereas this correlation was not found in the men. Long-term follow-up studies of the mechanism of BGH3 will be helpful to clearly understand the association between BGH3 levels and BMD.

Ferritin is another interesting blood plasma protein identified by this study. Ferritin is an essential component of the body, showing genetic correlation evidence with both whole body total BMD, spine area, and femoral neck area in this study. The functional relevance of ferritin with BMD has been demonstrated by previous studies [22–26]. For instance, Ahn et al. found that serum ferritin levels were inversely associated with the BMD of lumbar spine and femur neck in Korea women [22]. Mona Sarrai et al. observed a significant relationship between high ferritin levels and abnormal BMD, suggesting that higher ferritin levels increased the risk of developing low BMD in the adults with sickle cell disease [23]. Furthermore, increased serum ferritin was significantly associated with a decrease in BMD on the lumbar spine, which would cause more cases of osteoporosis in postmenopausal women [24]. In contrast, some studies also suggested that serum ferritin levels were positively associated with BMD [25, 26]. For instance, Kyung Shik Lee et al. reported a positive correlation between serum ferritin levels and BMD in elderly Korean men [25]. In a study of postmenopausal women, it has been identified that high serum ferritin was associated with BMD positively, indicating that serum ferritin was negatively correlated with the risk of osteoporosis [26]. Despite the inconsistent results, all of above study results

support the impact of serum ferritin on the variation of BMD. However, ferritin shows no correlation with BMD/bone area in either men or women after gender-specific analysis. Further biological studies are warranted to clarify the potential roles of ferritin in the pathogenesis of OP.

In addition, we found that insulin-like growth factor-I (IGF-I) was correlated with spine total BMD. IGF-I, one of the most abundant growth factors expressed in bone, has been shown to be an important regulator of bone remodeling and growth [27, 28]. Janssen et al. observed that fasting serum-free IGF-I was positively associated with BMD at the lumbar spine in men [27]. Johansson et al. detected positive relationship between low plasma levels of IGF-I and BMD in young men with osteoporosis, suggesting that IGF-I had endocrine effects on bone mass [29]. Similarly, it has also been demonstrated that serum IGF-I levels were significantly reduced in osteoporotic patients, and positively associated with BMD at lumbar spine in postmenopausal women [30]. Interestingly, serum IGF-I levels were positively associated with BMD at lumbar spine in women, who participated in the Framingham Osteoporosis Study [28]. Moreover, another study observed that plasma IGF-I levels were positively associated with BMD in pre- and postmenopausal women [31].

Notably, genetic correlation of hemoglobin was also observed for femoral neck BMD and right ulna and radius area in this study. Previously, several studies have directly or indirectly suggested the association between hemoglobin levels and BMD [32–36]. For instance, it has been shown that hemoglobin levels were positively associated with femoral neck BMD in Korean men [32]. Korkmaz et al. observed a significant association between hemoglobin values and femur BMD [33]. Additionally, anemia has been suggested to be a risk factor for low BMD in postmenopausal Turkish women [33]. In a population-based study, it has been suggested that hemoglobin levels < 140 g/L in men and < 130 g/L in women could predict the development of osteoporosis [34]. Besides, hemoglobin can serve as a BMD predictor in peritoneal dialysis patients [35]. A study by Cesari et al. investigated the association between hemoglobin levels and BMD in 950 older individuals. They found a positive significant association between hemoglobin levels and BMD [36], suggesting that hemoglobin levels could contribute to the development of OP [36].

Our study also observed that total serum alkaline phosphatase (SAP) was correlated with spine total BMD. Alkaline phosphatase, a ubiquitous enzyme that clears phosphate from nucleotides and proteins [37], is implicated in the abnormal skeletal mineralization [38]. In a recent study, researchers demonstrated that total alkaline phosphatase and bone-specific alkaline phosphatase were inversely associated with total body BMD at baseline in end-stage renal disease patients starting dialysis [38]. In addition, researchers have shown that bone alkaline phosphatase was significantly associated with BMD in diabetes patients, suggesting that bone alkaline phosphatase may interact with other factors affecting bone metabolism in diabetes patients [39].

Besides confirming the potential relevance of previously reported proteins with osteoporosis, our study also identified several novel blood plasma proteins for osteoporosis, such as ASAHL, component C7, and Factor H. To the best of our knowledge, few efforts have been paid to investigate the potential impacts of these blood plasma proteins on the development

of osteoporosis. Further biological studies are warranted to confirm our finding and clarify the potential roles of novel candidate proteins in the pathogenesis of OP.

Previous studies have observed skeletal site-specific effects of genetic loci on the variations of BMD [40–42]. For instance, Hsu et al. suggested that genes regulated BMD differently at different skeletal sites [41]. Our previous study observed genetic heterogeneity of peak BMD across different skeletal sites, suggesting that different genes were responsible for the regulation of peak BMD at different skeletal sites [40]. Additionally, several studies investigated the associations between blood plasma proteins and BMD at different skeletal sites [27, 28, 30, 32, 33]. For instance, previous studies have detected the associations between hemoglobin and femoral neck BMD [27, 28, 30], IGF-I and spine total BMD [32, 33], which were consistent with our study results. Based on previous and our study results, it is reasonable to infer that blood plasma proteins have site-specific effects on the variations of BMD. However, the mechanism underlying the potential site-specific effects of blood plasma proteins on the variations of BMD remains unclear. Further biological studies are needed to confirm our findings.

There are two limitations of this study that should be noted. First, blood plasma proteins-associated SNP sets and their genetic effects were driven from a previous GWAS of blood plasma proteins [13]. This study comprised 1000 unrelated Caucasian subjects, which is a relatively small GWAS sample and may affect the power of this study. Second, all subjects of this study were Caucasians. Due to the difference in genetic background, our study results should be interpreted with caution when applied to other populations. Further studies with large samples and biological studies are needed to confirm our findings.

In conclusion, we conducted a large PRS analysis to investigate the impact of blood plasma proteins on the variations of BMD and bone areas in 2286 Caucasians subjects. We observed genetic correlations between the blood plasma proteins and the variations of BMD. We hope that our study results can provide novel insights into the pathogenic studies of osteoporosis.

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Blood plasma proteins	P value				
	Whole body total BMD	Hip total BMD	Spine total BMD	Right ulna and radius BMD	Femoral neck BMD
ASAHL	3.90E-02	2.55E-02	1.61E-01	1.00E-03	1.20E-02
Haptoglobin	1.25E-02	5.40E-02	1.95E-02	1.50E-01	1.40E-02
CYTF	3.00E-01	1.66E-01	4.25E-02	1.79E-01	1.50E-02
Kallikrein12	3.16E-01	4.17E-01	3.90E-02	1.90E-01	2.25E-02
MED-1	5.23E-01	4.05E-02	9.31E-01	7.13E-01	2.55E-02
Calcineurin	8.20E-02	2.64E-01	1.14E-01	2.80E-02	3.75E-02
BGH3	1.40E-02	1.80E-02	3.15E-02	3.35E-02	4.35E-02
Hemoglobin	2.46E-01	1.51E-01	5.45E-02	1.40E-01	4.45E-02
I-TAC	3.29E-01	1.56E-01	5.34E-01	1.88E-01	4.70E-02
Kininogen	5.80E-02	9.50E-03	1.25E-02	1.15E-01	4.95E-02
MSP	2.37E-01	3.90E-02	3.81E-01	3.22E-01	5.15E-02
Angiostatin	1.33E-01	6.66E-01	4.41E-01	2.70E-02	7.55E-02
IL-17E	1.31E-01	3.00E-02	2.46E-01	1.03E-01	1.20E-01
IL-18Ra	8.60E-02	3.50E-02	8.00E-02	8.95E-02	1.22E-01
PDGFRb	5.35E-02	5.55E-02	1.10E-02	1.55E-02	1.23E-01
LCMT1	1.25E-01	1.16E-01	4.10E-02	1.22E-01	1.24E-01
BST1	1.19E-01	9.73E-01	8.10E-02	4.90E-02	1.96E-01
SAP	3.75E-01	8.69E-01	3.90E-02	8.67E-01	2.15E-01
Complement C7	1.90E-02	6.95E-02	3.50E-03	1.15E-02	2.31E-01
ABL2	4.08E-01	4.70E-02	9.94E-01	5.83E-01	2.39E-01
BCAR3	4.08E-01	4.70E-02	9.94E-01	5.83E-01	2.39E-01
DRAK2	4.08E-01	4.70E-02	9.94E-01	5.83E-01	2.39E-01
MSR	4.08E-01	4.70E-02	9.94E-01	5.83E-01	2.39E-01
OCAD1	4.08E-01	4.70E-02	9.94E-01	5.83E-01	2.39E-01
TYK2	4.08E-01	4.70E-02	9.94E-01	5.83E-01	2.39E-01
FXI	4.61E-01	5.67E-01	1.38E-01	3.00E-02	2.47E-01
BCAM	8.40E-02	1.69E-01	9.85E-02	2.45E-02	2.55E-01
Notch1	8.40E-02	1.69E-01	9.85E-02	2.45E-02	2.55E-01
TCCR	8.40E-02	1.69E-01	9.85E-02	2.45E-02	2.55E-01
Factor H	9.00E-03	4.46E-01	8.35E-02	2.00E-03	2.59E-01
sICAM-5	7.20E-01	9.50E-03	6.04E-01	3.83E-01	2.62E-01
Ferritin	3.00E-02	6.67E-01	8.40E-02	3.44E-01	3.10E-01
AMPM2	2.62E-01	6.30E-02	1.70E-02	1.45E-01	3.12E-01
ART	4.06E-01	3.55E-02	2.50E-02	1.86E-01	3.13E-01
Hemopexin	7.25E-01	1.80E-02	3.46E-01	1.15E-01	3.78E-01
HCC-4	1.16E-01	1.10E-02	9.20E-01	4.74E-01	4.19E-01
GPVI	4.85E-02	-	6.23E-01	5.55E-02	4.82E-01
b-Endorphin	8.61E-01	4.93E-01	7.19E-01	4.30E-02	5.45E-01
IGFBP-3	4.17E-01	8.16E-01	2.15E-02	6.46E-01	6.86E-01
IGF-I	4.17E-01	8.16E-01	2.15E-02	6.46E-01	6.86E-01
sTie-2	2.17E-01	2.40E-02	2.25E-01	5.87E-01	7.09E-01

Fig. 1.

Heat map of the genetic correlation between 41 proteins and BMD. The color changed from white to red. A darker color represented a more significant association between a blood plasma protein and BMD

Blood plasma proteins	P value			
	Hip area	Spine area	Right ulna and radius area	Femoral neck area
ProteinC	8.50E-03	3.80E-02	4.90E-02	8.00E-03
Complement C7	8.50E-03	1.25E-01	3.50E-02	4.98E-01
Elafin	1.15E-02	1.30E-01	1.45E-02	4.25E-02
Complement C4	1.25E-02	1.30E-02	4.75E-02	2.95E-02
Contactin-5	1.85E-02	2.55E-01	5.42E-01	7.40E-02
FCRL3	3.30E-02	6.39E-01	9.40E-02	2.73E-01
LAG-1	3.35E-02	2.38E-01	5.29E-01	6.67E-01
Heparin cofactor II	3.55E-02	8.60E-02	2.60E-02	2.59E-01
IL-17E	5.00E-02	1.40E-01	7.61E-01	9.50E-02
BST1	5.30E-02	1.10E-02	4.50E-03	1.87E-01
CPNE1	7.20E-02	1.00E-03	1.05E-02	4.50E-03
Ferritin	7.20E-02	1.90E-02	1.31E-01	3.95E-02
MMP-12	7.60E-02	1.12E-01	1.35E-01	1.85E-02
Nidogen	8.70E-02	1.52E-01	3.20E-01	2.35E-02
IL-18Rb	8.90E-02	1.30E-01	1.20E-02	1.69E-01
SPARCL1	9.30E-02	2.44E-01	2.60E-01	2.80E-02
CYTN	9.55E-02	1.02E-01	1.60E-02	7.95E-02
sTie-2	1.20E-01	1.39E-01	2.49E-01	9.00E-03
Haptoglobin Mixed Type	1.40E-01	3.40E-02	1.49E-01	1.20E-01
IGF-IIreceptor	1.41E-01	7.47E-01	9.23E-01	1.90E-02
Cadherin-5	1.71E-01	6.27E-01	4.38E-01	3.45E-02
HAI-1	1.87E-01	2.60E-01	1.57E-01	1.05E-02
CYTT	1.90E-01	3.60E-02	2.60E-02	1.07E-01
SREC-I	2.06E-01	5.38E-01	5.05E-02	3.90E-02
TAFI	2.27E-01	3.70E-02	3.00E-02	2.00E-03
RGM-C	2.39E-01	9.65E-02	1.02E-01	2.30E-02
Hemoglobin	2.43E-01	2.45E-01	2.70E-02	7.68E-01
MED-1	2.44E-01	5.28E-01	2.85E-01	4.35E-02
MnSOD	2.52E-01	1.00E-03	1.86E-01	2.97E-01
CD109	2.53E-01	6.12E-01	3.50E-02	3.06E-01
CDON	2.71E-01	1.25E-02	4.70E-02	1.83E-01
BCAM	2.81E-01	2.49E-01	2.80E-01	3.80E-02
Notch1	2.81E-01	2.49E-01	2.80E-01	3.80E-02
TCCR	2.81E-01	2.49E-01	2.80E-01	3.80E-02
FCN1	2.95E-01	2.30E-02	8.90E-01	3.98E-01
PPase	3.15E-01	3.45E-02	3.07E-01	9.27E-01
PCSK7	3.18E-01	2.75E-02	4.20E-01	4.88E-01
RANTES	3.21E-01	3.40E-02	1.97E-01	1.00E-03
HCC-1	3.43E-01	3.85E-02	2.35E-01	2.89E-01
Endothelin-convertingenzyme1	4.22E-01	1.45E-01	3.30E-02	7.51E-01
CNDP1	5.95E-01	3.05E-02	2.30E-01	3.56E-01
Carbonicanhydrase6	6.20E-01	3.45E-01	2.35E-02	8.86E-01
bFGF	8.42E-01	4.40E-02	8.02E-01	8.00E-03
sTie-1	8.85E-01	4.73E-01	9.83E-01	4.55E-02
b-Endorphin	9.03E-01	1.50E-02	3.64E-01	6.55E-02

Fig. 2.

Heat map of the genetic correlation between 45 proteins and Bone area. The color changed from white to red. A darker color represented a more significant association between a blood plasma protein and bone area

Blood plasma proteins	P value				
	Whole body total BMD	Hip total BMD	Spine total BMD	Right ulna and radius BMD	Femoral neck BMD
AK1A1	9.85E-02	4.40E-03	3.99E-01	8.99E-01	3.95E-02
AMPM2	7.06E-01	NA	2.68E-02	9.55E-01	4.75E-01
ATS13	1.37E-01	1.01E-01	2.69E-02	5.73E-02	2.58E-01
CD109	6.30E-01	9.76E-01	4.64E-02	2.29E-01	7.08E-01
CollectinKidney1	1.58E-01	3.18E-02	5.70E-01	9.57E-01	3.51E-02
CYTD	5.24E-02	3.93E-02	3.37E-01	4.20E-01	2.54E-01
CYTN	4.68E-01	9.74E-01	7.29E-01	2.47E-02	3.66E-01
DKK3	2.47E-02	8.39E-02	2.03E-02	2.59E-01	4.80E-01
ENTP5	1.61E-01	3.97E-01	1.90E-03	3.59E-01	3.32E-02
EphA1	2.48E-01	2.47E-01	7.18E-02	4.62E-02	3.62E-01
EPHB2	1.00E-01	8.59E-01	1.89E-02	1.78E-01	1.56E-01
FCN1	4.25E-02	8.04E-02	7.23E-01	2.31E-01	6.51E-02
GOT1	3.98E-02	1.15E-01	4.25E-01	1.66E-01	1.54E-02
HCC-4	9.65E-02	2.24E-02	1.81E-01	9.56E-02	6.11E-02
Hemoglobin	1.21E-01	3.50E-02	2.65E-02	1.97E-01	7.37E-02
Hemopexin	9.69E-01	1.60E-03	8.36E-01	6.03E-01	1.98E-01
IGFBP-7	4.50E-01	5.12E-01	2.78E-01	2.91E-02	4.13E-01
IL-12Rb1	4.45E-02	2.79E-01	4.20E-02	1.21E-02	1.40E-02
IL-18Ra	2.78E-02	7.60E-03	3.72E-01	6.42E-02	1.42E-01
IL-19	1.58E-01	3.18E-02	5.70E-01	9.57E-01	3.51E-02
IL-1b	1.58E-01	3.18E-02	5.70E-01	9.57E-01	3.51E-02
IMDH1	2.57E-01	6.51E-01	7.80E-03	7.61E-01	4.72E-01
Kallistatin	8.68E-01	2.23E-02	8.46E-02	4.01E-01	3.17E-01
LD78-beta	5.41E-02	3.70E-01	1.29E-01	9.86E-02	3.52E-02
MAPK2	2.13E-02	9.25E-01	4.37E-01	5.50E-03	1.71E-02
MMP-1	4.97E-02	7.96E-02	4.06E-01	4.01E-01	4.90E-01
MMP-8	1.86E-02	6.71E-02	6.90E-03	2.61E-01	9.47E-02
NPS-PLA2	5.29E-01	4.13E-01	5.89E-01	2.36E-02	2.90E-01
PARC	5.63E-01	3.29E-01	7.39E-01	8.85E-01	3.15E-02
PDE5A	8.63E-01	2.42E-02	9.59E-01	5.42E-01	3.26E-01
PDK1	1.64E-01	1.92E-01	2.24E-01	5.70E-03	1.40E-01
PPAC	8.60E-01	4.22E-02	5.64E-01	5.03E-01	8.22E-01
PPID	2.04E-01	5.84E-01	4.58E-02	1.52E-01	1.32E-01
PPIE	5.14E-01	3.22E-02	6.97E-01	5.43E-01	7.05E-01
Siglec-9	4.44E-02	9.36E-01	1.84E-01	4.53E-02	4.13E-02
sTie-2	1.18E-01	4.70E-03	8.94E-02	7.46E-01	1.51E-01
Trypsin2	4.51E-02	5.92E-01	3.06E-01	1.28E-01	2.83E-02

Fig. 3.

Heat map of the genetic correlation between 31 proteins and BMD in men. The color changed from white to red. A darker color represented a more significant association between a blood plasma protein and BMD

Blood plasma proteins	P value			
	Hip area	Spine area	Right ulna and radius area	Femoral neck area
a1-Antichymotrypsin	4.99E-01	1.65E-01	7.45E-02	2.86E-02
ADAMTS-5	8.60E-03	4.02E-01	6.69E-01	2.07E-01
AK1A1	2.12E-02	5.37E-01	3.75E-01	1.21E-01
alpha-1-antichymotrypsincomplex	4.99E-01	1.65E-01	7.45E-02	2.86E-02
ApoE2	6.42E-01	4.71E-01	7.22E-01	2.77E-02
ASAH2	1.52E-02	3.70E-02	2.63E-01	2.54E-01
ATS13	6.30E-01	6.69E-01	8.62E-01	4.52E-02
b-Endorphin	6.27E-01	4.50E-02	4.05E-01	2.48E-01
bFGF	9.56E-01	2.40E-02	8.83E-01	2.55E-01
C8	5.67E-01	6.96E-02	2.39E-02	2.48E-02
CD23	8.91E-01	4.04E-01	2.48E-02	8.47E-01
CDON	5.94E-01	1.01E-01	3.73E-02	1.52E-01
CLM6	3.99E-02	8.12E-01	5.36E-01	9.77E-01
CNTN2	7.88E-02	7.05E-01	8.90E-02	2.77E-02
CoagulationFactorVII	5.60E-01	8.61E-02	4.00E-04	8.48E-02
DERM	1.87E-02	8.87E-01	1.15E-01	7.38E-01
EPHB2	5.83E-01	4.45E-02	9.16E-01	4.75E-02
EsteraseD	1.57E-01	2.54E-02	1.99E-01	6.17E-01
gp130,soluble	1.57E-02	3.61E-02	1.43E-01	1.32E-01
GPNMB	1.39E-02	5.01E-01	3.95E-01	3.93E-01
Granulysin	6.82E-01	3.77E-01	2.14E-02	4.26E-01
Gro-a	4.81E-01	5.50E-01	9.58E-01	2.52E-02
HCC-1	4.21E-02	1.10E-02	3.10E-03	9.47E-02
IGFBP-7	6.70E-01	3.34E-01	4.99E-02	5.44E-01
IGF-IIreceptor	2.12E-01	9.83E-01	1.33E-01	1.80E-03
IL-1RAcP	2.28E-01	2.89E-01	4.14E-02	9.43E-01
IMDH1	3.76E-01	8.01E-01	6.50E-03	7.92E-01
IMDH2	8.93E-01	7.07E-01	2.37E-02	4.98E-01
IP-10	6.03E-01	5.28E-01	2.06E-01	2.13E-02
MASP3	9.93E-01	5.84E-01	4.96E-02	7.58E-01
MFGM	9.39E-02	2.38E-01	6.44E-02	1.64E-02
MMP-12	3.51E-02	4.84E-01	9.36E-01	6.14E-01
MnSOD	4.98E-01	3.26E-02	1.97E-01	3.56E-02
MSP	6.16E-01	9.51E-01	7.51E-02	4.65E-02
NPS-PLA2	1.83E-02	2.95E-01	1.95E-01	8.41E-01
NXPH1	1.86E-01	3.26E-02	7.60E-03	5.44E-02
PLXC1	2.60E-01	4.57E-01	1.83E-01	3.27E-02
PPAC	7.80E-03	5.04E-01	5.64E-01	4.76E-01
ProteinC	1.09E-01	7.51E-02	8.91E-01	4.00E-04
RGM-C	2.08E-02	8.58E-01	5.93E-01	6.63E-01
RUXF	6.42E-01	4.71E-01	7.22E-01	2.77E-02
Semaphorin3E	4.06E-02	6.82E-01	7.51E-02	7.67E-01
SPINT2	5.09E-02	4.62E-01	2.72E-01	7.00E-03
TLR4:MD-2complex	7.64E-01	9.69E-01	1.22E-01	4.03E-02
TSG-6	6.14E-01	1.12E-01	2.81E-02	1.87E-01

Fig. 4.

Heat map of the genetic correlation between 45 proteins and Bone area in men. The color changed from white to red. A darker color represented a more significant association between a blood plasma protein and bone area

Blood plasma proteins	P value				
	Whole body total BMD	Hip total BMD	Spine total BMD	Right ulna and radius BMD	Femoral neck BMD
Afamin	2.86E-02	7.67E-02	7.34E-02	8.25E-02	2.40E-01
AMPM2	1.95E-01	1.24E-01	9.35E-02	3.49E-02	3.80E-01
Angiostatin	2.35E-02	6.34E-01	1.35E-01	6.00E-04	3.09E-02
ART	3.37E-01	6.01E-02	2.95E-02	4.77E-02	4.37E-01
ARTS1	2.82E-01	8.60E-01	6.80E-01	3.27E-02	5.73E-02
ASAHL	4.63E-02	5.48E-02	1.19E-01	3.00E-04	4.90E-02
BGH3	1.71E-02	9.47E-02	1.03E-01	2.20E-03	1.52E-01
C7	1.40E-02	5.12E-02	2.30E-03	4.50E-05	2.47E-01
Calcineurin	4.05E-02	2.28E-01	3.40E-02	5.40E-03	2.00E-02
CathepsinA	1.34E-02	5.56E-01	8.99E-02	1.87E-02	2.89E-01
CATZ	1.34E-02	5.56E-01	8.99E-02	1.87E-02	2.89E-01
Chitotriosidase-1	1.93E-01	9.17E-01	2.66E-01	1.16E-02	5.07E-01
CYTF	3.52E-01	3.94E-02	4.10E-02	2.97E-01	1.51E-02
ENTP5	2.18E-02	5.49E-01	5.99E-02	5.56E-01	2.40E-01
FactorH	2.90E-03	2.95E-01	4.45E-02	1.60E-03	1.49E-01
GOT1	5.59E-02	5.21E-01	8.75E-02	2.78E-02	1.32E-01
GPVI	3.67E-02	NA	6.20E-01	1.85E-02	4.70E-01
IGFBP-3	3.41E-01	9.31E-01	3.90E-02	9.90E-01	7.83E-01
IGF-I	3.41E-01	9.31E-01	3.90E-02	9.90E-01	7.83E-01
IL-17E	1.87E-01	6.91E-02	6.46E-01	3.55E-02	7.47E-02
IMDH2	9.58E-02	6.69E-01	4.18E-02	9.88E-01	4.88E-01
I-TAC	2.04E-01	1.73E-01	1.49E-01	7.69E-02	4.95E-02
kallikrein12	2.41E-01	2.21E-01	1.33E-01	1.36E-01	1.97E-02
Kininogen,HMW	1.27E-02	2.76E-02	6.10E-03	1.44E-02	6.36E-02
LCMT1	1.98E-02	1.13E-01	1.38E-02	2.03E-02	1.49E-01
MED-1	1.46E-01	1.27E-01	8.23E-01	3.25E-01	1.59E-02
NPS-PLA2	4.54E-02	7.94E-01	1.28E-01	9.02E-02	3.20E-01
PDGFRb	5.42E-02	3.74E-02	1.46E-02	1.68E-02	6.68E-02
Properdin	4.86E-02	2.32E-01	1.28E-01	3.15E-01	2.81E-01
SAP	5.75E-01	9.42E-01	3.87E-02	8.10E-01	2.39E-01
sL-Selectin	1.23E-01	4.15E-01	1.54E-01	1.05E-02	1.43E-01

Fig. 5.

Heat map of the genetic correlation between 31 proteins and BMD in women. The color changed from white to red. A darker color represented a more significant association between a blood plasma protein and BMD

Blood plasma proteins	P value			
	Hip area	Spine area	Right ulna and radius area	hip_neck_area
Alkalinephosphatase,bone	7.74E-01	7.55E-01	7.76E-01	1.82E-02
ApoE2	9.05E-01	1.42E-01	3.69E-01	8.90E-03
ASAH2	7.44E-01	4.68E-02	3.71E-01	7.56E-01
ATS13	2.50E-01	1.96E-02	7.89E-01	1.86E-01
bFGF	8.54E-02	9.39E-01	2.19E-01	4.57E-02
BGH3	4.86E-01	1.22E-02	5.88E-01	9.73E-01
BST1	7.38E-01	9.72E-02	4.03E-02	5.47E-01
C7	3.00E-03	2.12E-01	2.83E-01	6.66E-01
C8	7.65E-01	2.76E-02	2.20E-02	4.38E-01
Carbonicanhydrase6	9.91E-01	9.86E-01	3.55E-02	8.12E-01
CarbonicanhydraseXIII	9.41E-01	3.53E-01	9.07E-01	1.68E-02
Chitotriosidase-1	6.15E-02	6.96E-01	2.27E-02	1.09E-01
CNDP1	3.64E-01	9.00E-04	4.70E-01	6.80E-01
CPNE1	9.53E-01	1.70E-03	9.34E-02	7.55E-02
CYTD	3.46E-01	3.81E-02	8.43E-02	7.29E-01
Elafin	9.50E-03	3.05E-01	1.73E-01	3.97E-02
EMR2	4.00E-01	2.25E-01	1.26E-02	7.69E-02
EPHB2	2.22E-01	7.75E-01	9.65E-01	4.86E-02
EsteraseD	4.17E-02	7.33E-01	9.04E-01	4.87E-02
FCG2A/B	3.37E-02	4.00E-01	6.87E-01	9.04E-01
Haptoglobin,MixedType	8.25E-01	3.23E-02	6.90E-01	3.21E-01
HCC-1	4.93E-01	4.87E-02	7.94E-01	3.48E-01
IMDH1	3.26E-02	7.90E-01	2.03E-01	7.57E-01
Integrina1b1	8.47E-01	2.32E-01	4.56E-02	8.54E-01
kallikrein12	3.79E-01	3.75E-02	1.97E-01	7.60E-01
LY9	4.53E-02	6.46E-01	5.94E-01	9.22E-01
MIF	7.48E-01	7.14E-02	1.85E-02	1.83E-01
MMP-7	7.40E-01	2.98E-02	6.14E-01	4.26E-01
MnSOD	7.99E-01	2.90E-03	8.20E-01	6.01E-01
Nidogen	2.40E-01	7.34E-01	4.35E-01	4.36E-02
PCSK7	3.82E-01	3.83E-01	3.19E-02	2.13E-01
PPase	2.77E-01	9.40E-03	1.97E-01	8.50E-01
PPID	1.19E-02	6.62E-01	1.57E-01	4.32E-01
ProteinC	2.89E-02	1.86E-01	1.57E-02	3.05E-01
RANTES	4.13E-01	9.17E-02	1.32E-01	3.60E-03
RELT	7.61E-01	2.32E-02	8.86E-01	9.72E-02
RUXF	9.05E-01	1.42E-01	3.69E-01	8.90E-03
sFRP-3	9.11E-01	3.63E-01	4.91E-02	9.37E-01
sLeptinR	2.46E-01	3.59E-01	3.58E-02	4.99E-01
SPARCL1	1.71E-01	2.12E-01	2.98E-01	2.70E-03
sTie-2	7.38E-01	9.57E-01	8.94E-01	1.77E-02
TAFI	1.22E-01	1.96E-02	2.70E-03	4.00E-04

Fig. 6.

Heat map of the genetic correlation between 42 proteins and Bone area in women. The color changed from white to red. A darker color represented a more significant association between a blood plasma protein and bone area

Table 1

Basic characteristics of 2286 Caucasian study subjects

Covariates	Total	Men	Women
Age (years)	51.37 ± 13.75	50.72 ± 16.18	51.59 ± 12.98
Height (cm)	166.35 ± 8.47	175.87 ± 6.27	163.28 ± 7.24
Weight (kg)	75.27 ± 17.54	87.12 ± 16.03	71.45 ± 16.74

Results are showed as mean ± SD