A Genetic Correlation Scan Identifies Blood Proteins Associated With Bone Mineral Density

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Research Article

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Abstract

Introduction: Osteoporosis is a common metabolic bone disease characterized by low bone mass. However, limited efforts were paid to explore the functional relevance of blood proteome to bone mineral density across different life stages.

Methods: Using genome-wide association study summary data of blood proteome and two independent research of bone mineral density, we conducted a genetic correlation scan of bone mineral density and blood proteome. Linkage disequilibrium score regression analysis was conducted to assess the genetic correlations between each of the 3,283 plasma proteins and bone mineral density.

Results: Linkage disequilibrium score regression identified 18 plasma proteins showing genetic correlation signals with bone mineral density in TB-BMD cohort, such as MYOM2 (coefficient=0.3755, *P* value=0.0328) among subjects aged 0-15, POSTN (coefficient =-0.5694, *P* value=0.0192) among subjects aged 30-45 and PARK7 (coefficient =-0.3613, *P* value =0.0052) among subjects aged over 60.

Conclusions: Our results identified multiple plasma proteins associated with bone mineral density and provided novel clues for revealing the functional relevance of plasma proteins to bone mineral density.

Introduction

Osteoporosis (OP) is a kind of metabolic bone disease characterized by the low bone mass. It is thought to be induced by an imbalance between the process of bone resorption and that of bone formation [1]. The typical symptom of it is the bone fragility fracture which often occurs to the old, especially to women [2, 3]. In European Union, the number of osteoporosis patients was estimated to be 27.6 million early in 2010 [3]. The probability of suffering any major osteoporotic fracture is about 20% for men and 50% for women after 50s [4].

Bone mineral density (BMD) indicates the amount of bone mineral in bone tissue. Low BMD is the typical pathological change of osteoporosis. Due to the different status of bone resorption and formation in human life, BMD varies throughout the whole life course. The body mass keeps increasing during infancy and reaches the peak body mass (PBM) in the second or third decade of life [4, 5]. And it gradually declines due to the overwhelming bone resorbing which happens as people get older [4].

Human BMD and PBM can be affected by both environmental and genetic factors [6]. However, it seems that heredity exerts greater influence than environment [7]. The heritability of BMD is thought to range from 50–85% [8–11]. Recent genome-wide association studies (GWAS) identified a group of genes as new candidate determinant of BMD such as GPC6 and SPTBN1 [12, 13]. Our previous genetic studies also identified multiple proteins and blood metabolites being related to BMD, such as BGH3 and 1,5-anhydroglucitol [14, 15]. What's more, the physiology characteristics of bone varies in different growth stages, so does the BMD. However, the specific genetic characteristics underlying the BMD variation in all life stages still remains unclear until now.

Human plasma proteins, also known as blood proteins, include a substantial amount of proteins in circulatory system [16]. These proteins play important roles in various cell processes, such as mineral transportation and immunity defense. Since plasma proteins are critical to various physiology processes, some plasma proteins are recognized as good drug targets [16]. What's more, facilitated by the development of proteomics, numerous plasma proteins are found to be involved in a variety of pathological changes, which lead to the promotion of disease diagnose strategy [17]. For instance, Kim H et al. suggested that human plasma DPP4 level is related to osteoporotic fracture and may help to evaluate the risk of fracture [18].

It has been demonstrated that genetic factors contributed greatly to the regulation of gene expression [19]. Extensive efforts have been paid to explore the effect of DNA polymorphisms on gene expression level, and identified a lot of gene expression quantitative trait loci (eQTLs) [19]. However, as it is not enough to unveil the relationship between genetic variation and protein expression, the importance of identifying protein quantitative trait loci (pQTLs) is gradually highlighted [19, 20]. Benjamin et al. analyzed more than 3000 plasma proteins and identified a group of protein pQTLs loci in healthy subjects. This study is helpful for exploring the genetic relationships between plasma proteins and human complex diseases [16].

Genetic correlation is the part of variance that two or more traits share due to genetic factors. It is pretty common in complex diseases. Linkage disequilibrium score regression (LDSC) is a powerful tool and used frequently to estimate the genetic correlations among different diseases or traits accurately based on the summary data of GWAS [21]. For example, a previous study conducted LDSC analysis and identified almost 300 genetic correlations among 24 traits, including anorexia, obesity and educational attainment [22].

In this study, we conducted a large-scale scan of genetic correlation between BMD and human plasma proteins. LDSC analysis was conducted by integrating each GWAS summary dataset of 2 BMD cohorts and the protein pQTLs involving over 3000 human plasma. The first cohort is a GWAS database of life-course total body BMD (TB-BMD) and second cohort is a GWAS database of BMD estimated by quantitative ultrasound of the heel (eBMD) [23] [24]. The proteins identified by both two cohorts was regarded as candidate ones in this study. Our research provided novel clues for the etiology studies of BMD.

Materials And Methods

GWAS summary data of TB-BMD cohort

The GWAS summary data of life-course total body BMD (TB-BMD) was used here [23]. This study consists of 30 epidemiological cohorts of TB-BMD and includes 66,628 subjects from America, Europe, and Australia. About 90% of the individuals are European ancestry. SNP genotyping was conducted via Affymetrix UK Biobank Axiom array / UK BiLEVE Axiom array, Omniexpress array and Illumina arrays. The genotype imputation was performed using the cosmopolitan (all ethnicities combined) 1000 Genomes

phase 1 v.3 (March 2012) reference panel. In this study, all subjects were divided into five age groups, including 0–15 years, 15–30 years, 30–45 years, 45–60 years, and 60 or more years. Dual-energy X-ray absorptiometry (DXA) was used to measure the TB-BMD of population above 15 years old. Total body less head (TBLH) was used in individual younger than 15 years old. After adjusting the age, weight, height, genomic principal components (derived from GWAS data) and other study-specific covariates for BMD, linear regression models were built in each study among all of the 5 groups. Other details of the participants, genotyping, imputation, meta-analysis and quality control can be found in the published study [23].

GWAS summary data of eBMD cohort

GWAS summary data of eBMD were driven from the UK Biobank [24]. In this study, GWAS analysis were conducted in 426,824 UK Biobank full release White British individuals (55% female). A total of 1,103 conditionally independent signals were identified to be related to BMD at genome-wide significance ($p<6.6\times10^{-9}$). All of the participants in the UK Biobank were genotyped by the Affymetrix UK BiLEVE Axiom or Affymetrix UK Biobank Axiom array and imputed centrally by UK Biobank. For heel bone quality estimating, quantitative ultrasound speed of sound (SOS) and broadband ultrasound attenuation (BUA) Quantitative ultrasound assessment of calcanei was used via Sahara Clinical Bone Sonometer [Hologic Corporation (Bedford, Massachusetts, USA)]. More information of the subjects, genotyping, imputation and statistics could be found in the previous study [24].

Protein pQTLs data of human plasma proteome

The GWAS summary data of human plasma proteome were derived from a latest plasma proteome study [16]. In brief, 1,927 genetic associations (protein pQTLs) with 1,478 proteins was identified in this study. A total of 3622 plasma proteins in 3,301 subjects from the INTERVAL study were quantified through an expanded version of an aptamer-based multiplex protein assay (SOMAscan). A multiplexed, aptamer-based approach (SOMAscan assay) was utilized to measure the relative concentrations of the plasma proteins. The quality control was made both at the sample and SOMAmer levels via controlling aptamers and calibrator samples. The qualified samples were genotyped on the Affymetrix Axiom UK Biobank genotyping array at Affymetrix (Santa Clara, California, US). Variants were phased by SHAPEIT3 and successively imputed utilizing a combined 1000 Genomes Phase 3-UK10K reference panel via the Sanger Imputation Server (https://imputation.sanger.ac.uk). A total of 3283 plasma proteins were included in the GWAS summary data after quality control, which were utilized in following genetic correlation analysis with BMD. More information of the participants, sample preparation and statistics could be found in previous publication [16].

Genetic correlation scan

In this study, we conducted a large-scale LDSC scan for potential genetic correlations between BMD and human blood proteins. Following the document of LDSC tool (https://github.com/bulik/ldsc), the GWAS summary data of BMD and plasma proteins were prepared and input into LDSC for genetic correlation

scan using the default parameters recommended by the developers of LDSC. During LDSC analysis, the GWAS Z statistics of BMD and plasma proteins were driven from the GWAS and used as dependent variables for genetic correlation analysis [25]. In principle, LDSC analysis calculated each SNP's ability to tag adjacent variants, denoted as "LD score" [21]. High LD score of a SNP indicates that this SNP can tag more other genetic variants, including causal sites [21]. As an efficient tool for assessing the genetic relationships among various complex traits and diseases, LDSC utilizes GWAS summary data rather than individual level genotype data, which makes it more available and convenient [21].

Results

Analysis results of TB-BMD cohort

For TB-BMD cohort, LDSC observed genetic correlations between 18 plasma proteins and life-course BMD (Table 1). At the group aged 0 ~ 15 years, MYOM2 was detected (coefficient = 0.3755, *P* value = 0.0328). At the group of 30 ~ 45 years, 7 plasma proteins were identified, such a Periostin (coefficient=-0.5694, *P* value = 0.0192) and G3PT (coefficient = 0.6272, *P* value = 0.0315). At the group of 45 ~ 60 years, 8 plasma proteins were identified, such as GPNMB (coefficient = 0.4921, *P* value = 0.008) and CHST15 (coefficient = 0.4835, *P* value = 0.0151). At age more than 60 years, 3 plasma proteins were identified, such as PARK7 (coefficient=-0.3613, *P* value = 0.0052) and F10 (coefficient=-0.4772, *P* value = 0.0271). Additionally, we found that PLXB2 appeared to be correlated with the BMD of both 30 ~ 45 years group (coefficient = 0.5184, *P* value = 0.0488) and 45 ~ 60 years group (coefficient = 0.3927, *P* value = 0.0348) (Table 1, Fig. 1 to Fig. 4).

Table 1

List of human plasma proteins identified by LDSC[a] for Life course BMD[b]

Age group (years)	Plasma protein	Gene	Coefficients	*P* value
15 less	Myomesin-2	MYOM2	0.3755	0.0328
30 ~ 45	Periostin	POSTN	-0.5694	0.0192
	Glyceraldehyde-3-phosphate Dehydrogenase, testis-specific	G3PT	0.6272	0.0315
	Fas apoptotic inhibitory molecule 3	FAIM3	-0.3988	0.0341
	RING finger protein 148	RN148	0.4332	0.0424
	Interferon regulatory factor 1	IRF1	-0.4638	0.0459
	Dynein light chain 1, cytoplasmic	DLC8	0.6325	0.0481
	Plexin-B2	PLXB2	0.5184	0.0488
45 ~ 60	Transmembrane glycoprotein NMB	GPNMB	0.4921	0.008
	Carbohydrate sulfotransferase 15	CHST15	0.4835	0.0151
	Testican-2	SPOCK2	0.3097	0.0332
	Plexin-B2	PLXB2	0.3927	0.0348
	Carbohydrate sulfotransferase 15	CHST15	0.4848	0.0409
	ERO1-like protein beta	ERO1B	-0.3512	0.0419
	Myosin-binding protein C, slow-type	MYBPC1	0.2941	0.0464
	Stem Cell Growth Factor-beta	SCGF-beta	-0.4148	0.0468
60 more	Protein deglycase DJ-1	PARK7	-0.3613	0.0052
	Coagulation Factor X	F10	-0.4772	0.0271
	Metalloproteinase inhibitor 4	TIMP-4	-0.4097	0.0483

[a]: Linkage disequilibrium score regression;

[b]: Bone mineral density

Analysis results of eBMD cohort

For eBMD cohort, LDSC identified 21 plasma proteins for eBMD, such as RIPK2 (coefficient=-0.2357, *P* value = 0.0072), RANTES (coefficient=-0.2108, *P* value = 0.0145) and CRHBP (coefficient = 0.1533, *P* value = 0.0297) (Table 2).

Table 2

List of human plasma proteins identified by LDSC [c] for eBMD[d] (*P* value < 0.05)

Plasma protein	GENE	Coefficients	*P*-value
Receptor-interacting serine/threonine-protein kinase 2	RIPK2	-0.2357	0.0072
C-X-C motif chemokine 16	CXCL16, soluble	0.2008	0.0119
C-C motif chemokine 5	RANTES	-0.2108	0.0145
Cathepsin Z	CATZ	0.1998	0.0178
Interleukin-7	IL-7	-0.2414	0.0212
Cysteine-rich hydrophobic domain-containing protein 2	CHIC2	0.1121	0.0256
Phenylalanine–tRNA ligase, mitochondrial	SYFM	0.2221	0.0256
Corticotropin-releasing factor-binding protein	CRHBP	0.1533	0.0297
O-acetyl-ADP-ribose deacetylase MACROD1	MACD1	0.1633	0.0319
Guanylate cyclase activator 2B	GUC2B	0.2065	0.033
SPARC-like protein 1	SPARCL1	0.1513	0.0362
Dickkopf-related protein 4	Dkk-4	-0.2058	0.0373
E3 ubiquitin-protein ligase ZNRF3	ZNRF3	0.1993	0.0419
Cellular retinoic acid-binding protein 1	RABP1	0.1496	0.0428
Cell growth regulator with EF hand domain protein 1	CGRE1	-0.1792	0.0436
Serine/threonine-protein kinase pim-1	PIM1	0.2707	0.0446
Creatine kinase M-type	CK-MM	0.2035	0.0471
Alpha-2-antiplasmin	a2-Antiplasmin	0.2072	0.0478
Tenascin-X	Tenascin-X	0.3227	0.0483
Thrombospondin-3	TSP3	0.2816	0.0488
Dickkopf-related protein 1	DKK1	-0.177	0.0495

[c]: Linkage disequilibrium score regression;

[d]: Bone mineral density estimated by quantitative ultrasound of the heel.

Discussion

In this study, to explore the potential correlation between human plasma proteins and BMD across different life courses, we conducted a large-scale LDSC analysis using two independent cohorts. We

identified multiple plasma proteins correlated with BMD, such as PARK7, SCGF-beta, POSTN, GAPDS and RANTES.

PARK7 (Parkinson disease protein 7, also known as Protein deglycase DJ-1) belongs to the peptidase C56 family. According to the view of a previous study, PARK7/DJ-1 protein level was increased up to 3 times in MLO-Y4 osteocytic cells, which were treated by N-BPs (Nitrogen-containing bisphosphonates), a kind of osteoporosis drug [26]. And this change was demonstrated to be involved with a pathway which plays a role in the effect of N-BPs on osteocytes [26]. As a result of a study, short stature and brachydactyly are two characteristics observed in the parkinsonism patients without PARK7 region in the DJ-1 gene [26, 27]. The authors indicated that the PARK7 region may contain a modifier gene for bone growth [27].

SCGF-beta (Stem Cell Growth Factor-beta), also named as Osteolectin or CLEC11A, was recognized as an osteogenic growth factor [28, 29]. Researchers have found that this protein promotes Leptin Receptor+ (LepR+) skeletal stem cells and other osteogenic progenitors in bone marrow to differentiate into osteoblasts and to maintain the adult skeletal bone mass [29]. Andriani GA et al. indicated that CLEC11A is a component of SASP (senescence-associated secretory phenotype) [30]. They also suggested that aneuploid cells which accumulate during aging in some mammalian tissues potentially play key roles in age-related pathologies via SASP secretion [30].

Periostin is a secreted extracellular matrix protein in human, which is encoded by POSTN gene. It was expressed in many tissues including skeleton and originally identified in periosteum and bone [31]. A previous study indicated that periostin participates in the early stages of osteoblast differentiation and bone formation [32]. It stimulates osteoblast functions and bone formation via integrin receptors and Wnt-beta-catenin pathways [33]. Experimental mice without periostin proportionately suffered from severe periodontal disease and bone density reduction [31]. Pepe J et al. suggested that Serum periostin levels were associated with radial cortical porosity, even after adjusted by age [34]. Moreover, periostin expression declines with the skeletal growth, but it could re-express in the process of fracture healing and bone repair [32]. It also has been demonstrated that it plays a key role in postmenopausal osteoporosis for that serum levels could be measured to predict BMD and the risk of fracture [33].

GAPDS (Glyceraldehyde-3-phosphate dehydrogenase, testis-specific) is a member of GAPDH (glyceraldehyde-3-phosphate dehydrogenase) family that play an important role in carbohydrate metabolism. GAP was recognized as an osteoblast marker enzyme with high mRNA expression in periosteal cells from young rats [35]. Vitamin K2, a homologue to GAPDH, confirmed by the observations of Biochem Pharmacol et al, play a crucial role in bone metabolism [36]. It is able to post-translationally modify the osteocalcin, induce osteoclast apoptosis and imped the osteoclast formation and resorption activity [36].

RANTES, also known as CCL5 (C-C Motif Chemokine Ligand 5), is a member of chemokine genes clustered on the q-arm of chromosome 17, which are involved in immunoregulatory and inflammatory processes. By sharing common signaling pathways and regulatory mechanisms, the bone systems are closely related to the immune systems [37]. According to the previous studies, CCL5 is directly associated

with disturbed bone metabolism in nonpainful rheumatoid arthritis [37]. Additionally, the chemokine CCL5 is overexpressed in the FDOJ (fatty oxide osteoporosis/osteolysis in the jawbone) cases [38]. More, in the knowledge that hyperhomocysteinemia is a risk factor for osteoporotic fractures, another previous study indicated that protein CCL5 could be generated in the osteobalsts after homocysteine induces serum amyloid A3 [38]. In summary, CCL5 may play an important role in the bone metabolism, which needs more confirmative evidence.

It is well known that bone remodeling is a dynamic process and BMD varies at different human life stages [23]. Because of bone reformation, BMD increases dramatically during childhood and adolescent periods, peaking at the third decade of life approximately. Around the age of over 50, the process of bone resorption gradually overwhelms the process of bone reformation, which results in a decrease of BMD, particularly for postmenopausal women. In this study, we identified several plasma proteins showing age specific effects on BMD. For instance, coagulation Factor X showed negative genetic correlation with BMD in the subjects aged more than 60 years. Coagulation Factor X is a vitamin K-dependent enzyme of blood coagulation cascade and plays a critical role in blood coagulation [39]. Gigi R et al. demonstrated that Rivaroxaban, an anticoagulant against factor Xa, could significantly induce the reduction in osteoblastic cell growth and energy metabolism, and the inhibition of alkaline phosphatase, which was a kind of osteoblastic marker [40]. Coagulation Factor X may contribute to the decrease of BMD of the subjects aged more than 60 years through activating osteoblast. Additionally, MYBPC1 appeared to be correlated with BMD in the subjects aged 45–60 years in this study. MYBPC1 encodes myosin binding protein c (slow type), which plays an important role in muscle contraction. MYBPC1 mutation has been linked to skeletal muscle atrophy related disorder [41]. Furthermore, previous studies have demonstrated the positive association between body lean mass and BMD [42]. It is well known that adult body lean mass trend to decrease with age, especially after 40 years. Based on previous studies and our study results, we may infer that MYBPC1 contributed to the variation of BMD through affecting skeletal muscle loss in the subjects aged 45–60 years.

There are some limitations that should be noted in this study. Firstly, we used the GWAS data driven from European, American, Australian and multiethnic populations, in which most of the subjects were European. Because of the different genetic background of different populations, it should be careful to apply our study results to other populations. Especially for the group [0–15], there's more subjects of not-european ancestry in the group [0–15] comparing to other groups. Secondly, the stability of LDSC result could be influenced by the small sample size of the study, which is worth noted. Thirdly, among the two datasets of BMD in our study, different body parts and different method was used to measure BMD, which may have some effect on our results. Further efforts are still need to confirm our results and clarify the potential biological mechanism underlying the observed genetic correlations between plasma proteins and BMD.

Conclusion

To evaluate the genetic correlation between human plasma proteins and life-course BMD, we performed LDSC analysis in 2 cohorts. A number of human proteins were detected. Our results may provide new clues for the physiological process of human BMD and help improve the treatment of OP.

Abbreviations

OP: Osteoporosis

BMD: Bone mineral density

PBM: peak body mass

GWAS: genome-wide association studies

eQTLs: expression quantitative trait loci

pQTLs: protein quantitative trait loci

LDSC: Linkage disequilibrium score regression

TB-BMD: total body BMD

PARK7: Parkinson disease protein 7

SCGF-beta: Stem Cell Growth Factor-beta

GAPDS: Glyceraldehyde-3-phosphate dehydrogenase

CCL5: C-C Motif Chemokine Ligand 5

Declarations

Conflicts of interest

Jiawen Xu, Shaoyun Zhang, Haibo Si, Yi Zeng, Yuangang Wu, Yuan Liu, Mingyang Li, Limin Wu, Bin Shen declare that they have no conflict of interest.

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Availability of data and material

The large-scale LDSC scan for potential genetic correlations between BMD and human blood proteins was performed following the document of LDSC tool (https://github.com/bulik/ldsc).

Ethics approval and consent to participate

The source of the data was a publicly available data base and no human participants were involved, hence ethical parameters are not applicable

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Not applicable

Author Contributions

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(VII) Final approval of manuscript: All authors

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Figures

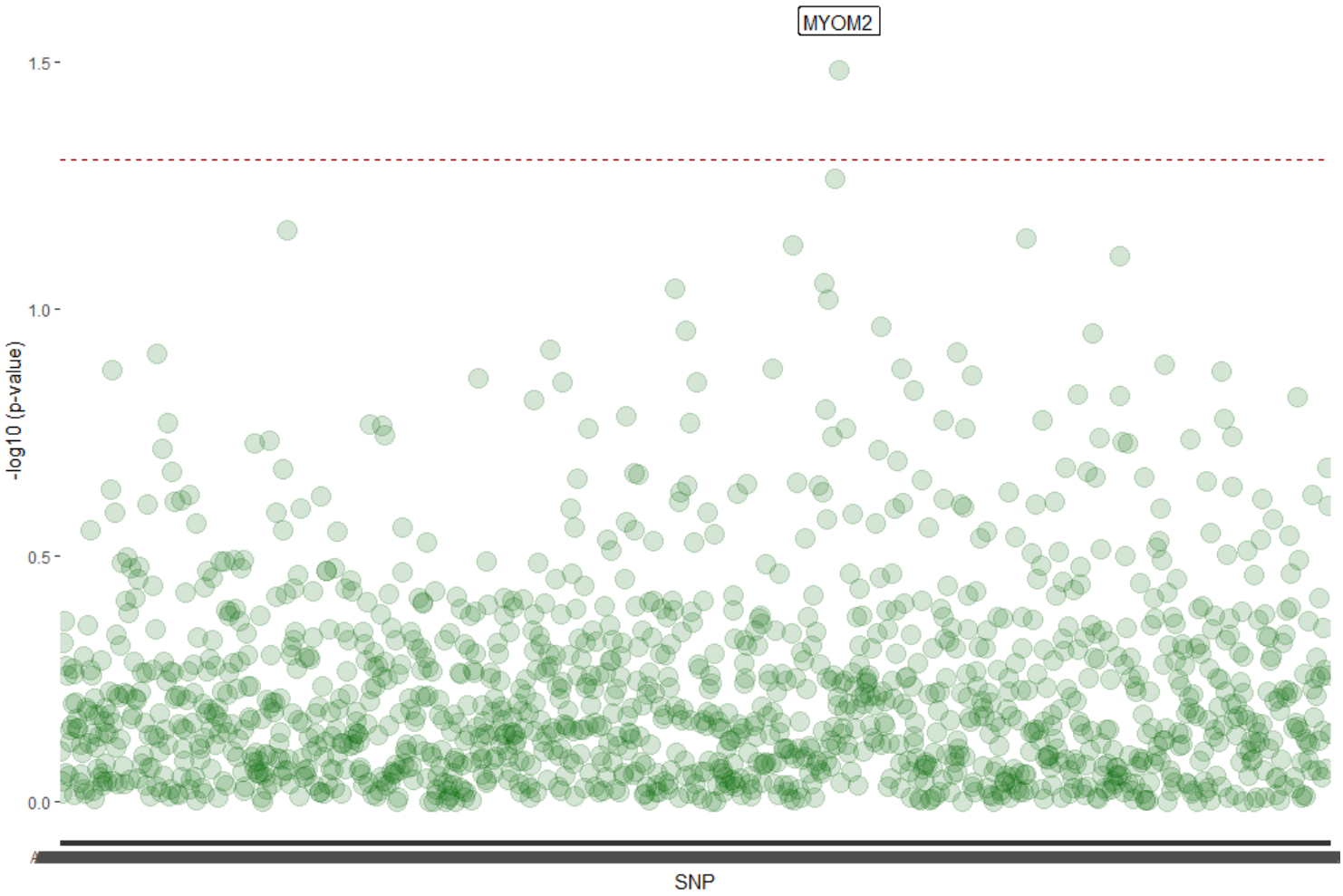

Figure 1

Human plasma proteins identified by linkage disequilibrium score regression for life course bone mineral density in subjects aged 0-15

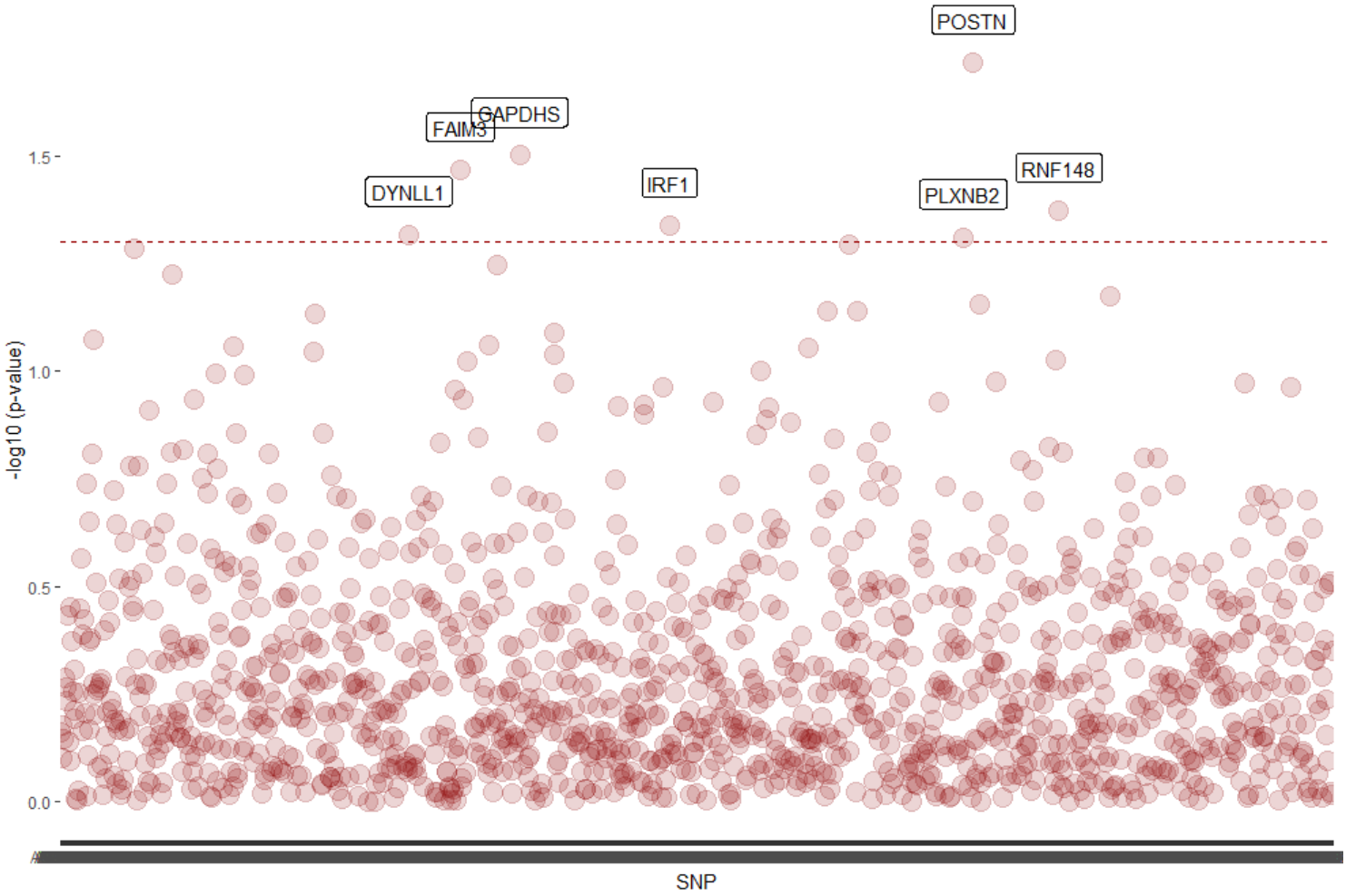

Figure 2

Human plasma proteins identified by linkage disequilibrium score regression for life course bone mineral density in subjects aged 30-45.

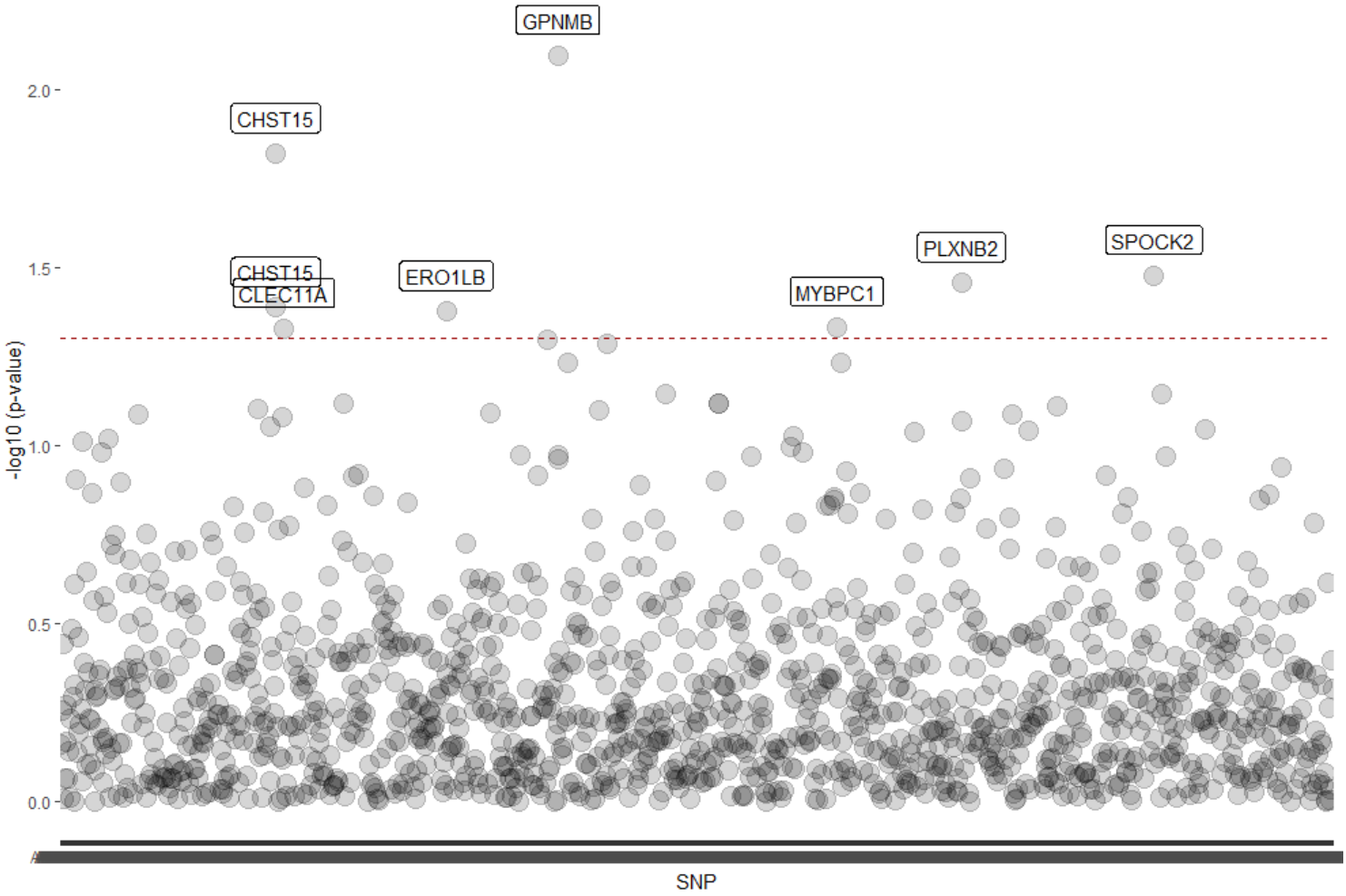

Figure 3

Human plasma proteins identified by linkage disequilibrium score regression for life course bone mineral density in subjects aged 45-60.

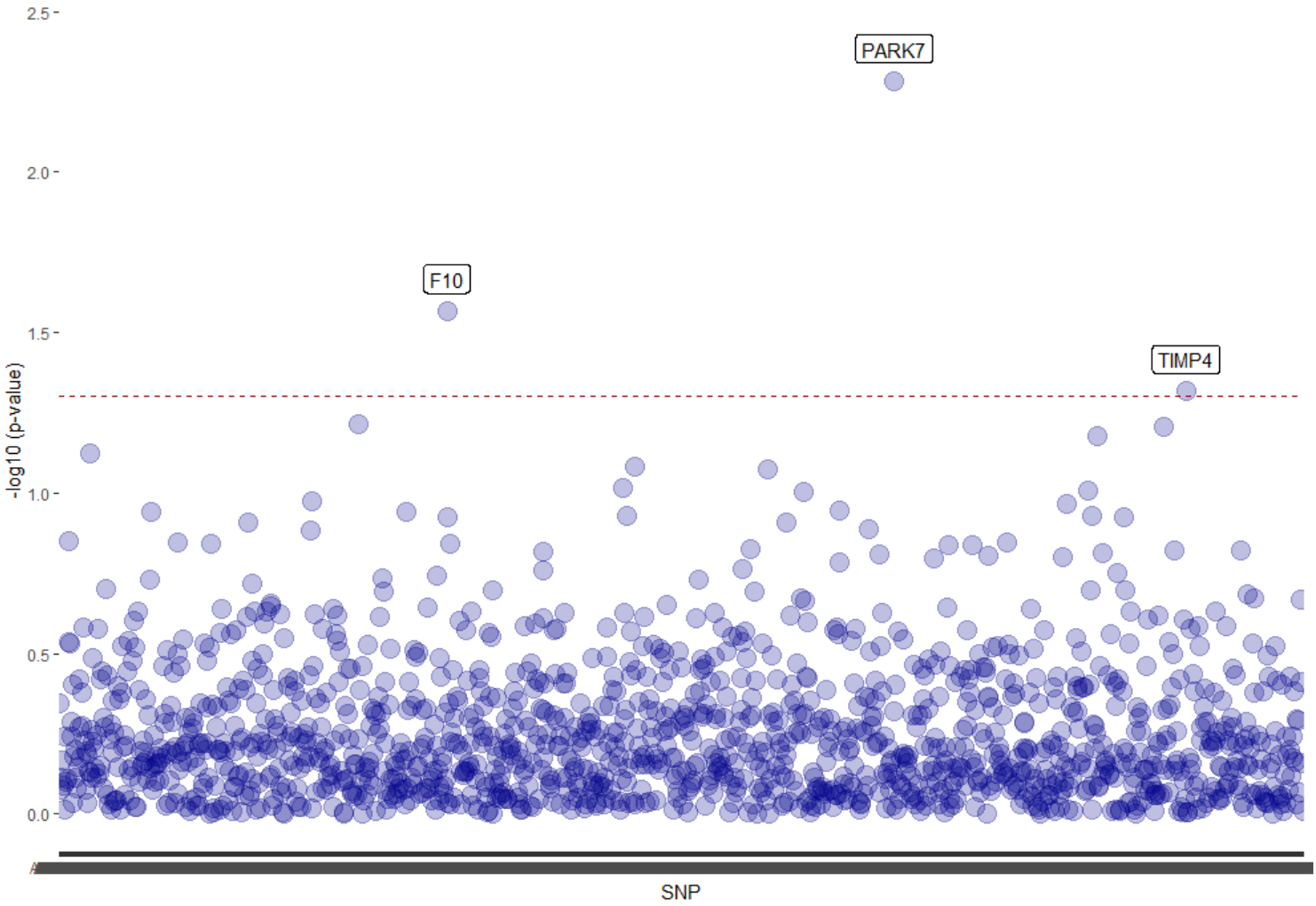

Figure 4

Human plasma proteins identified by linkage disequilibrium score regression for life course bone mineral density in subjects aged 60 or more years