Functional relevance for associations between osteoporosis and genetic variants

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Abstract

Osteoporosis is characterized by increased bone loss and deterioration of bone microarchitecture, which will lead to reduced bone strength and increased risk of fragility fractures. Previous studies have identified many genetic loci associated with osteoporosis, but functional mechanisms underlying the associations have rarely been explored. In order to explore the potential molecular functional mechanisms underlying the associations for osteoporosis, we performed integrative analyses by using the publicly available datasets and resources. We searched 128 identified osteoporosis associated SNPs ($P < 10^{-6}$), and 8 SNPs exert cis-regulation effects on 11 eQTL target genes. Among the 8 SNPs, 2 SNPs (RPL31 rs2278729 and LRP5 rs3736228) were confirmed to impact the expression of 3 genes (RPL31, CPT1A and MTL5) that were differentially expressed between human subjects of high BMD group and low BMD group. All of the functional evidence suggested the important functional mechanisms underlying the associations of the 2 SNPs (rs2278729 and rs3736228) and 3 genes (RPL31, CPT1A and MTL5) with osteoporosis. This study may provide novel insights into the functional mechanisms underlying the osteoporosis associated genetic variants, which will help us to comprehend the potential mechanisms underlying the genetic association for osteoporosis.

Introduction

Osteoporosis is a skeletal disease characterized by low bone mineral density (BMD) and microarchitectural deterioration of bone tissue, leading to decreased bone strength and increased the risk of fracture[1]. Osteoporosis not only endangers the health and life quality of patients, but also brings huge economic burden to the global health. More than 2 million Americans suffered osteoporotic fractures in 2005 with treatment costs more than $17 billion [2]. Due to the aging of the population in the United States, it is expected that osteoporotic fractures rates will reach more than 3 million patients and $25.3 billion treatment costs over the next 25 years [3]. More and more developing countries will experience the rapid increase in the elderly population,
which will lead to a greater number of individuals suffering from osteoporosis and fractures. It is expected that by 2050, up to half of the hip fracture patients will appear in Asia [4].

Genome-wide association studies (GWAS) have been successful at identifying a number of promising genetic variants that are associated with osteoporosis and related traits. However, the statistical association between OP and genetic variants were only established in the DNA level at the present stage, functional relevance has rarely been explored. Such established associations do not provide direct understanding into functions of significant candidate genes or regulation of genes expressions that functionally establish a connection between OP phenotype and gene code information.

Transcriptional regulation plays an important role in functional mechanisms underlying genetic association. The change of binding affinity between variants and regulatory factors altered by genetic variants may influence the transcription and/or translation of target proteins. Numerous public gene expression datasets are available, which are invaluable and may provide the functional data support for a better understanding of the association between variants and phenotype.

We combined the integrative analyses[5–8] (gene relationships among implicated loci, expression quantitative trait loci (eQTL) analysis, differential gene expression analysis and functional prediction analysis) results to research functional mechanisms for OP-associated genetic variants by utilizing the available data sources and analyzing available GWAS results. Performing the integrative analyses by using public data resources may strengthen our understandings in the molecular genetic mechanisms underlying complex diseases.

Materials and methods

Selection of OP-associated SNPs

Phenotype-Genotype Integrator (PheGenI) (www.ncbi.nlm.nih.gov/gap/PheGenI/) is a bioinformatics online tool that can provide robust view and download analytic data (including SNPs, genes and association results) for published studies[9]. Using phenotype “BMD” and “osteoporosis”, we identified 128 interesting SNPs (S1 Table) with \( P \) value \(<10^{-6}\) by searching PheGenI[10–15].

Gene Relationship Across Implicated Loci (GRAIL)

Under normal circumstances, one disease-associated SNP has one or more predisposing genes or locus in the region near this SNP. In order to carry out the further functional studies, selecting genes located at the two sides of the associated SNP as potential candidate genes is a conventional method to identify the candidate genes[5]. With the purpose of discover more candidate genes, we performed GRAIL analysis (http://www.broadinstitute.org/mpg/grail/), which is an online statistical method that examining relationships automatically between seed regions and candidate genes were selected through PubMed abstracts to prioritizes the best candidate gene[16]. The seed regions are SNPs by searching PheGenI (250–500 kb flanking regions of the SNP). GRAIL analysis can select new potential candidate genes near OP-associated SNPs automatically[6].

eQTL analysis

Variation occurs at the level of DNA may lead to the changes of genes expression, which subsequently account for a significant proportion of the phenotypic variance[6] (e.g. BMD) and susceptibility to OP. Therefore, eQTL analysis in specific tissues or cells is a favorable tool to identify candidate SNPs[17,18] and it is very important to study the functional molecular
mechanism of the association by detecting whether the OP-associated SNP affects the levels of candidate gene expression. Some previous studies have found quite a lot of eQTLs in a variety of cells and tissues [19–23], we can search those databases quickly by eQTL Browser (http://eqtl.uchicago.edu/cgi-bin/gbrowse/eqtl/). We used this database to detect the eQTL effects of the identified OP-associated SNPs in monocytes and lymphoblastoid cell lines (LCLs). In humans vivo, the favorable working cell model for studying gene/protein expression patterns and their regulation mechanisms with reference to osteoporosis risk is monocyte [24,25]. Monocytes as the precursors of osteoclasts [26], related to osteoclast differentiation, activation, and apoptosis [27]. The expression level of genes are very similar in both human osteoblasts and LCLs and are enriched in pathways that are important in cellular growth and survival [28–31].

Differential expression analysis
Three previous in vivo genome wide gene expression studies in our group using Human Genome U133 Plus 2.0 or U133A Arrays were performed to identify genes differentially expressed in monocytes or B cells between low and high BMD women., and we uploaded those data to GEO (gene expression omnibus) Datasets. The experimental procedures and data analysis were detailed in the original studies, and the GSE numbers are: GSE7158, GSE13850, GSE2208 [32–34]. The differential expression analysis ($P<0.05$) of for the identified eQTL genes between low and high BMD were detected by using t-tests.

Functional prediction
For the identified eQTL SNPs, we used two online tools to predict the potential functions. F-SNP is a database to provide the latest information about already confirmed and presumed potential functional effect of SNPs at the multiple level, such as splicing, transcriptional, translational, and post-translational (http://compbio.cs.queensu.ca/F-SNP/). RegulomeDB [35] is an online public data resource, by integrating multiple data resources (such as high-throughput and experimental data) to perform predictions and annotations and then to identify potential functional variants (http://regulome.stanford.edu/). Different regulomeDB score (1–6) is represent the different degree for functional variant as transcription factor binding site. Score 1 indicates the strongest evidence for a SNP being located in a functional region. Phyre2 (http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index) is an online tool to predict and analyze the secondary and tertiary structure and function of proteins [36]. Users can submit a number of sequences of amino acid, Phyre2 will predict the secondary and tertiary structure of their models automatically.

Results
We found 128 OP-associated SNPs (S1 Table) and 157 associated genes by searching the PheGenI. We detected 88 genes for the 128 SNPs by GRAIL analysis: 31 genes can also be detected by PheGenI and 57 newly detected genes were. As shown in S1 Table, the columns “Gene 1” and “Gene 2” listed 157 unique genes, which were physically located at two sides of the corresponding 128 SNPs, and the “implicated genes” listed 88 unique genes from GRAIL analysis.

eQTL analysis [37–39] is an effective method to detect the functional mechanism underlying association by detecting the association between the genetic variants at DNA level and the variations in mRNA expression of genes near SNPs. Among the 128 unique SNPs, we found that 8 SNPs have potential eQTL effects on a total of eleven eQTL target genes (Table 1) in LCLS or monocytes [28–30] which are two type of cells closely related with bone metabolism and all of them act as cis-effect regulators.
Table 1. Expression quantitative trait locus (eQTL) analysis results between SNPs and the corresponding eQTL target genes.

| SNP      | Chr | position | Context | Allele | Association P Value | Gene1 | Gene2 | Implicated Gene | eQTL Gene | Effect | Score | Target | Regulome DB Score |
|----------|-----|----------|---------|--------|---------------------|-------|-------|----------------|------------|--------|-------|--------|------------------|
| rs2278729 | 2   | 101668856 | intron  | A/G    | 1.00E-07            | TBC1D8 | TBC1D8 | RPL31          | RPL31      | exon-QTL | 7.87  | monocyes | 1f                |
| rs13182402| 5   | 125918148 | intron  | A/G    | 2.00E-09            | ALDH7A1 | ALDH7A1 | ALDH7A1        | ALDH7A1    | eQTL    | 45.14 | LCLS  | 5                |
| rs227584  | 17  | 42225547  | missense| G/T    | 9.00E-07            | C17orf53 | C17orf53 | SLC4A1         | SLC4A1     | eQTL    | 35.55 | LCLS  | 6                |
| rs228769  | 17  | 42193185  | intron  | G/C    | 2.00E-08            | HDAC5  | HDAC5  | SLC4A1         | SLC4A1     | eQTL    | 32.60 | monocyes | 4                |
| rs1007738 | 11  | 46849360  | intron  | G/A    | 7.00E-07            | CKAP5  | CKAP5  | CREB3L1        | MTCH2      | exon-QTL | 3.59  | LCLS  | 1f                |
| rs3762288 | 11  | 68201295  | missense| C/T    | 6.00E-12            | LRP5   | LRP5   | LRP5           | CPT1A      | eQTL    | 20.78 | monocyes | 1f                |
| rs1038304 | 6   | 15193175  | intron  | A/G    | 4.00E-11            | C6orf97 | C6orf97 | C6orf97        | C6orf97    | eQTL    | 13.26 | monocyes | 6                |
| rs884205  | 18  | 60054857  | intergenic | T/G  | 9.00E-09            | TNFRSF11A | RPL17P44 | TNFRSF11A     | TNFRSF11A  | eQTL    | 1.00  | monocyes | 5                |

Note
1°Association P-value” are the results of PheGene1.
2°Gene 1” and “Gene 2” are the nearby genes physically located at two sides of the SNP.
3°Implicated genes” are the results from Gene Relationships Across Implicated Loci (GRAIL) analysis.
4°eQTL gene”, “Effect”, “Score”, “Target” are the result of eQTL analysis. Score = −log10(P-value). The P-values represented the differences of gene expression of the different genotypes.
5°”exon-QTL”: quantified reads for known exons by RNA sequencing against SNPs. All the eQTL genes have cis-effect.
6°Regulome DB Score” is a scoring scheme of RegulomeDB and represents the source of supporting evidence. Among those, “1f” refers to the evidence from “eQTL + TF binding/ DNase peak”, “4” refers to TF binding + DNase peak, “5” refers to TF binding or DNase peak, “6” refers to the evidence from “other”LCLs: lymphoblastoid cell lines.

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Then, the differential expression analyses for the 11 identified eQTL target genes from the above 8 SNPs with potential eQTL effects in monocytes and B Lymphocytes was performed (Table 2). B cells are known regulators of bone metabolism, particularly for osteoclastogenesis [40,41]. We used the t-tests to compare the mRNA expression levels of the 11 eQTL target genes between high-BMD subjects and low-BMD subjects. The gene ribosomal protein L31 (RPL31) showed significantly differential expressions in GSE7158 and GSE13850 cell samples, with p value < 0.05 (GSE7158: \( P = 4.73E-02 \), GSE13850: \( P = 5.42E-03 \)). The genes carnitine palmitoyltransferase 1A (CPT1A) and metallothionein-like 5 (MTL5) showed significantly differential expressions in GSE13850 cell sample (CPT1A: \( P = 1.56E-02 \), MTL5: \( P = 4.83E-02 \)) (Table 2). The three genes were upregulated in the high bone mass group. We used the Regulome DB to investigate whether the 8 eQTL SNPs are functional variants with putative regulation effects. As shown in Table 1, three SNPs (RPL31 rs2278729, CREB3L1 rs1007738 and LRP5 rs3736228), scored 1f, were annotated as the most credible functional variants with potential regulation effects and likely accounted for allelic-specific expressions of the 5 eQTL target genes (RPL31, CPT1A, MTL5, MTCH2, PHF21A). Using the F-SNP program, we found that SNP rs3736228 located in nonsynonymous region and alteration of allele C to T caused a missense mutation of LRP5 protein with amino acid residue alteration from Alanine (A) to Valine (V). We used 101 amino acids (50 amino acids before and 50 after the missense mutation of rs3736228) to predict the secondary structure of protein. We found that the amino acid residue alteration from Alanine (A) to Valine (V) caused loss of alpha helix and gain of beta strand before the missense mutation (Fig 1). We speculate that this SNP may influence osteoporosis risk through changing the amino acid sequence and secondary structure of the LRP5 protein.

### Discussion

In this study, we performed integrative analyses to explore functional mechanisms underlying the associations for OP by using publically available datasets. A total of 8 SNPs (rs2278729, rs13182402, rs227584, rs228769, rs1007738, rs3736228, rs1038304, rs884205) acting as cis-effect regulators on the 11 corresponding eQTL genes (RPL31, ALDH7A1, C17orf53, TMUB2,
C17orf65, MTCH2, PHF21A, CPT1A, MTL5, C6orf97, ENSG0000014) were identified. Among these SNPs, two eQTL SNPs (RPL31 rs2278729 and LRP5 rs3736228) likely accounted for allelic-specific expressions of the 3 eQTL target genes (CPT1A, MTL5 and RPL31) which showed significantly differential expression in monocytes and LCLs.

Integrative analyses by utilizing the public data resources can provide new understandings into the molecular genetic mechanisms of human diseases. In genetics, the genetic information carried on DNA is transferred to RNA through transcription and then translated into protein molecules[5]. The functional mechanisms for OP-associated genetic variants at the DNA level may be that genetic variants lead to variation of gene expression, and then cause the variation of susceptibility to OP[5]. Thus, integrating abundant evidences from multiple levels can help us to understand potential functional mechanism of genes and their contribution to variation in susceptibility to OP. We draw out the robust genetic associations between 2 SNPs of the corresponding genes (CPT1A, MTL5, CREB3L1 and RPL31) and OP.

The previous study showed that rs3736228 in the LRP5 gene was strongly associated with the BMD[42]. Through the eQTL analysis, the SNP rs3736228 may serve as a cis-effect regulator of genes CPT1A and MTL5[19]. CPT1A is a key regulator to facilitate the transfer of long-chain fatty acids across the mitochondrial membrane for β-oxidation in mammals[43]. MTL5

Fig 1. Predicted secondary structure of protein carrying either (A) rs3736228-C allele or (B) rs3736228-T allele. A: before missense mutation; B: after missense mutation. NOTE: The arrows point the position of the missense mutation. The 50 amino acids before and after missense mutations, which are: cdgfpemcdqdeg cpvcsaaqpcargqcvdrlrccdgsaqcdqರdadciclpqfrcaagqcvlickqcdsfpdcidgsdelmceitkpp sdds and cdgfpemcdqdeg cpvcsaaqpcargqcvdrlrccdgsaqcdqರdadciclpqfrcaagqcvlickqcdsfpdcidgsdelmceitkpp sdds, respectively.
may play a central role in the regulation of cell growth and differentiation [44]. Previous studies showed CPT1A and MTL5 [45] genes were associated with lumbar spine BMD and CPT1A located in BIOCARTA_LEPTIN_PATHWAY which is important in the biology and etiology of osteoporosis [12]. In this study, we found the gene CPT1A and MTL5 in B cells were significantly differentially expressed between high and low BMD group (P<0.05). SNP rs3736228 acts as potential eQTL cis-effect on CPT1A and MTL5. Therefore, we speculate that rs3736228 plays important roles in the pathological mechanism of osteoporosis by regulating differential mRNA transcriptions of CPT1A and MTL5. On the other hand, since rs3736228 caused a missense mutation for LRP5 protein, LRP5 rs3736228 may influence and even induce osteoporosis through changing the amino acid sequence and secondary structure of LRP5 protein. Our finding strongly suggested that rs3736228 was a causal variant functioning either by regulating expression of CPT1A and MTL5 and/or by influencing function of LRP5 protein. Further functional study on the molecular mechanism of CPT1A, MTL5 gene and LRP5 rs3736228 may help us comprehend the association between osteoporosis and candidate gene, understand the pathogenesis of osteoporosis and the genetic mechanism.

RPL31, belongs to the L31E family of ribosomal proteins. The encoded protein is one of the members of the ribosomal 60S subunit. Ribosomal proteins have a lot of functions, such as participation in regulation of gene transcription and translation, involved in DNA repair, regulation of cell proliferation, differentiation, apoptosis and so on. In addition, ribosomal proteins may play an important role in tumor occurrence, development, metastasis and tumor suppression [46,47]. Allele A of rs2278729 was previously reported to be associated with smaller femoral neck-shaft angle in men and lower RPL31 expression in lymphocytes and osteoblasts. In this integrative study, we confirmed that SNP rs2278729 acts as cis-effect regulators for RPL31. We speculate that rs2278729 can regulate differential expression of protein by regulating differential mRNA transcriptions of gene RPL31, therefore, rs2278729 plays important roles in the variation of susceptibility to OP.

We identified two SNPs having combined OP-associated functional evidence among the searched 128 OP-associated SNPs. Failure to find functional evidence for some promising functional SNPs in BMD hot loci, like RANKL-OPG, ESR and LRP5 in this study does not exclude the importance of the associations of the BMD hot loci with OP. Instead, those associated SNPs may be involved in pathogenesis of OP by other mechanisms (such as epigenetic regulation or protein translation) instead of directly regulating target gene expression. Small effect size of the SNPs and small sample size will lead to limited statistical power, which is a reason for the lack of association between OP-associated SNPs and mRNA expression. Further functional studies are required to understand the functional association between genetic variants and OP, which may provide new insights into mechanisms underlying the association detected at DNA level.

There are two limitations to this study. First, although we identified some functional evidence for the associations between 2 SNPs and OP, it is still needed to further study the causal variants with potential functional effects. Second, although we used two methods to select the candidate genes, there is still another possibilities that other genes near the associated-SNPs are also directly or indirectly involved in the association around the SNPs [5]. In short, this is not a perfect method by public data sources to research the functional mechanisms of diseases, but to some degree, this is a simple, complementary, and effective way to reveal the functional link underlying the association between SNPs and diseases.

In summary, all of the functional analysis results we found in this study demonstrated the significance of the 2 candidate OP-associated SNPs (rs2278729 and rs3736228). We confirmed that the 2 SNPs have cis-regulation effects on the expression of 3 genes (RPL31, CPT1A and MTL5), at the same time, these 3 genes were differentially expressed between high BMD group
and low BMD group. The above evidence provides some important clues for us to perform further study on the functional molecular mechanism between OP and OP-associated genetic variants. Performing the integrative analyses by utilizing the public data resources may provide a new perspective into the functional molecular genetic mechanisms underlying human complex diseases.

Supporting information

S1 Table. Information for the 128 genetic associations. N/A: not available. “Associated P-value”, “Source”, “PMID”, “Gene 1” and “Gene 2” are the results of Phenotype-Genotype Integrator (PheGenI) and the “Gene 1” and “Gene 2” represent the nearest genes physically located at two sides of the SNP. “Implicated gene” are the results from Gene Relationships Across Implicated Loci (GRAIL) analysis. PMID: Pubmed ID for association results. “RegulomeDB Score” is a scoring scheme of RegulomeDB and represents the source of supporting evidence. Among those, “1f” refers to the evidence from “eQTL + TF binding/DNase peak”, “2b” refers to TF binding + any motif + DNase Footprint + DNase peak”, “3a” refers to TF binding + any motif + DNase peak, ”4” refers to TF binding + DNase peak, ”5” refers to TF binding or DNase peak, ”6” refers to the evidence from ”other” LCLs: lymphoblastoid cell lines.

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References

1. El Hage R. Geometric indices of hip bone strength in obese, overweight, and normal-weight adolescent boys. Osteoporosis international: a journal established as result of cooperation between the European
2. Burge R, Dawson-Hughes B, Solomon DH, Wong JB, King A, Tosteson A. Incidence and economic burden of osteoporosis-related fractures in the United States, 2005–2025. Journal of bone and mineral research: the official journal of the American Society for Bone and Mineral Research. 2007, 22(3):465–75.

3. Cauley JA. Public health impact of osteoporosis. The journals of gerontology Series A, Biological sciences and medical sciences. 2013, 68(10):1243–51. https://doi.org/10.1093/gerona/glt093 PMID: 23902935

4. Cooper C, Cole ZA, Holroyd CR, Earl SC, Harvey NC, Dennison EM, et al. Secular trends in the incidence of hip and other osteoporotic fractures. Osteoporosis international: a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2011, 22(5):1277–88.

5. Deng FY, Lei SF, Zhang YH, Zhang ZL, Guo YF. Functional relevance for associations between genetic variants and systemic lupus erythematosus. PloS one. 2013, 8(1):e53037. https://doi.org/10.1371/journal.pone.0053037 PMID: 2341919

6. Lin X, Deng FY, Mo XB, Wu LF, Lei SF. Functional relevance for multiple sclerosis-associated genetic variants. Immunogenetics. 2015, 67(1):7–14. https://doi.org/10.1007/s00251-014-0803-4 PMID: 25308866

7. Zhu XW, Deng FY, Wu LF, Tang ZX, Lei SF. Functional mechanisms for type 2 diabetes-associated genetic variants. Journal of diabetes and its complications. 2013, 28(4):497–501. https://doi.org/10.1016/j.jdiacomp.2012.05.007 PMID: 23754502

8. Deng FY, Lei SF, Zhu H, Zhang YH, Zhang ZL. Integrative analyses for functional mechanisms underlying associations for rheumatoid arthritis[J]. The Journal of rheumatology. 2013, 40(7):1063–8. https://doi.org/10.3899/jrheum.121119 PMID: 23678157

9. Yu W, Yesupriya A, Wulf A, Hindorff LA, Dowling N, Khoury MJ, et al. GWAS Integrator: a bioinformatics tool to explore human genetic associations reported in published genome-wide association studies. European journal of human genetics: EJHG. 2011, 19(10):1095–9. https://doi.org/10.1038/ejhg.2011.91 PMID: 21610748

10. Rivadeneira F, Styrkarsdottir U, Estrada K, Halldorsson BV, Hsu YH, Richards JB, et al. Twenty bone-mineral-density loci identified by large-scale meta-analysis of genome-wide association studies. Nature genetics. 2009, 41(11):1199–206. https://doi.org/10.1038/ng.446 PMID: 19801982

11. Styrkarsdottir U, Halldorsson BV, Gretarsdottir S, Gudbjartsson DF, Walters GB, Ingvarsson T, et al. Multiple genetic loci for bone mineral density and fractures. The New England journal of medicine. 2008, 358(22):2355–65. https://doi.org/10.1056/NEJMoa0801197 PMID: 18445777

12. Hsu YH, Zillikens MC, Wilson SG, Farber CR, Demissie S, Soranzo N, et al. An integration of genome-wide association study and gene expression profiling to prioritize the discovery of novel susceptibility loci for osteoporosis-related traits. PLoS genetics. 2010, 6(6):e1000977. https://doi.org/10.1371/journal.pgen.1000977 PMID: 20548944

13. Kiel DP, Demissie S, Dupuis J, Lunetta KL, Murabito JM, Karasik D. Genome-wide association with bone mass and geometry in the Framingham Heart Study. BMC medical genetics. 2007, 8 Suppl 1:S14.

14. Richards JB, Rivadeneira F, Inouye M, Pastinen TM, Soranzo N, Wilson SG, et al. Bone mineral density, osteoporosis, and osteoporotic fractures: a genome-wide association study. Lancet. 2008, 371(9623):1505–12. https://doi.org/10.1016/S0140-6736(08)60599-1 PMID: 18453228

15. Lunetta KL, D’Agostino RB Sr., Karasik D, Benjamin EJ, Guo CY, Govindaraju R, et al. Genetic correlates of longevity and selected age-related phenotypes: a genome-wide association study. Framingham Study. BMC medical genetics. 2007, 8 Suppl 1:S13.

16. Raychaudhuri S, Plenge RM, Rossin EJ, Ng AC, Purcell SM, Sklar P, et al. Identifying relationships among genomic disease regions: predicting genes at pathogenic SNP associations and rare deletions. PLoS genetics. 2009, 5(6):e1000534. https://doi.org/10.1371/journal.pgen.1000534 PMID: 19557189

17. Cookson W, Liang L, Abecasis G, Moffatt M, Lathrop M. Mapping complex disease traits with global gene expression. Nature reviews Genetics. 2009, 10(3):184–94. https://doi.org/10.1038/nrg2537 PMID: 19223927

18. Dixon AL, Liang L, Moffatt MF, Chen W, Heath S, Wong KC, et al. A genome-wide association study of global gene expression. Nature genetics. 2007, 39(10):1202–7. https://doi.org/10.1038/ng2109 PMID: 17873877

19. Zeller T, Wild P, Szymczak S, Rotival M, Schillert A, Castagne R, et al. Genetics and beyond—the transcriptome of human monocytes and disease susceptibility. PloS one. 2010, 5(5):e10693. https://doi.org/10.1371/journal.pone.0010693 PMID: 20502693
20. Dimas AS, Deutsch S, Stranger BE, Montgomery SB, Borel C, Attar-Cohen H, et al. Common regulatory variation impacts gene expression in a cell type-dependent manner. Science. 2009, 325(5945):1246–50. https://doi.org/10.1126/science.1174148 PMID: 19644074

21. Veyrieras JB, Kudaravalli S, Kim SY, Dermitzakis ET, Gilad Y, Stephens M, et al. High-resolution mapping of expression-QTLs yields insight into human gene regulation. PLoS genetics. 2008, 4(10):e1000214. https://doi.org/10.1371/journal.pgen.1000214 PMID: 1884210

22. Schadt EE, Molony C, Chudin E, Hao K, Yang X, Lum PY, et al. Mapping the genetic architecture of gene expression in human liver. PLoS biology. 2008, 6(5):e107. https://doi.org/10.1371/journal.pbio.0060107 PMID: 18462017

23. Pickrell JK, Marioni JC, Pai AA, Degnner JF, Engelhardt BE, Nkadori E, et al. Understanding mechanisms underlying human gene expression variation with RNA sequencing. Nature. 2010, 464(7289):768–72. https://doi.org/10.1038/nature08872 PMID: 20220758

24. Leung R, Cuddy K, Wang Y, Rommens J, Glogauer M. Sbds is required for Rac2-mediated monocyte migration and signaling downstream of RANK during osteoclastogenesis. Blood. 2011, 117(6):2044–53. https://doi.org/10.1182/blood-2010-05-282574 PMID: 21084708

25. Zeng Y, Zhang L, Zhu W, Xu C, He H, Zhou Y, et al. Quantitative proteomics and integrative network analysis identified novel genes and pathways related to osteoporosis. Journal of proteomics. 2016, 142:45–52. https://doi.org/10.1016/j.jprot.2016.04.044 PMID: 27153759

26. Higuchi S, Tabata N, Tajima M, Ito M, Tsurudome M, Sudo A, et al. Induction of human osteoclast-like cells by treatment of blood monocytes with anti-fusion regulatory protein-1/CD98 monoclonal antibodies. Journal of bone and mineral research: the official journal of the American Society for Bone and Mineral Research. 1998, 13(1):44–9.

27. Cohen-Solal ME, Graulet AM, Denne MA, Gueris J, Baylink D, de Vernejoul MC. Peripheral monocyte culture supernatants of menopausal women can induce bone resorption: involvement of cytokines. The Journal of clinical endocrinology and metabolism. 1993, 77(6):1648–53. https://doi.org/10.1210/jcem.77.6.8263153 PMID: 8263153

28. Tripuwahbrut P, Mustafa M, Gjerde CG, Brudvik P, Mustafa K. Effect of compressive force on human osteoblast-like cells and bone remodelling: an in vitro study. Archives of oral biology. 2013, 58(7):826–36. https://doi.org/10.1016/j.archoralbio.2013.01.004 PMID: 2353016

29. Lei SF, Wu S, Li LM, Deng FY, Xiao SM, Jiang C, et al. An in vivo genome wide gene expression study of circulating monocytes suggested GBP1, STAT1 and CXCL10 as novel risk genes for the differentiation of peak bone mass. Bone. 2009, 44(5):1010–4. https://doi.org/10.1016/j.bone.2008.05.016 PMID: 19223260

30. Liu YZ, Dvornyk V, Lu Y, Shen H, Lappe JM, Recker RR, et al. A novel pathophysiologica mechanisms for osteoporosis suggested by an in vivo gene expression study of circulating monocytes. The Journal of biological chemistry. 2005, 280(32):29011–6. https://doi.org/10.1074/jbc.M501164200 PMID: 15962235

31. Xiao P, Chen Y, Jiang H, Liu YZ, Pan F, Yang TL, et al. In vivo genome-wide expression study on human circulating B cells suggests a novel ESR1 and MAPK3 network for postmenopausal osteoporosis. Journal of bone and mineral research: the official journal of the American Society for Bone and Mineral Research. 2008, 23(5):644–54.

32. Boyle AP, Hong EL, Harirahan M, Cheng Y, Schaub MA, Kasowski M, et al. Annotation of functional variation in personal genomes using RegulomeDB. Genome research. 2012, 22(9):1790–7. https://doi.org/10.1101/gr.137323.112 PMID: 22955989

33. Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJ. The Phyre2 web portal for protein modeling, prediction and analysis. 2015, 10(6):485–58. https://doi.org/10.1038/nprot.2015.053 PMID: 25950237

34. Stranger BE, Nica AC, Forrest MS, Dimas A, Bird CP, Beazley C, et al. Population genomics of human gene expression. Nature genetics. 2007, 39(10):1217–24. https://doi.org/10.1038/ng1472 PMID: 17873874

35. Sun W, Bennett VC, Eggins SM, Kamenetsky VS, Arculus RJ. Enhanced mantle-to-crust rhenium transfer in undegassed arc magmas. Nature. 2003, 422(6929):294–7. https://doi.org/10.1038/nature01482 PMID: 12646918
39. Stranger BE, Dermitzakis ET. The genetics of regulatory variation in the human genome. Human genomics. 2005, 2(2):126–31. https://doi.org/10.1186/1479-7364-2-2-126 PMID: 16004727

40. Horowitz MC, Bothwell AL, Hesslein DG, Pflugh DL, Schatz DG. B cells and osteoblast and osteoclast development. Immunological reviews. 2005, 208:141–53. https://doi.org/10.1111/j.0105-2896.2005.00328.x PMID: 16313346

41. Weitzmann MN, Cenci S, Haug J, Brown C, DiPersio J, Pacifici R. B lymphocytes inhibit human osteoclastogenesis by secretion of TGFbeta. Journal of cellular biochemistry. 2000, 78(2):318–24. PMID: 10842325

42. Funakoshi Y, Omori H, Yada H, Katoh T. A1330V polymorphism of the low-density lipoprotein receptor-related protein 5 gene and bone mineral density in Japanese male workers. Environmental health and preventive medicine. 2011, 16(2):106–12. https://doi.org/10.1007/s12199-010-0172-z PMID: 21432225

43. Wakil SJ, Abu-Elheiga LA. Fatty acid metabolism: target for metabolic syndrome. Journal of lipid research. 2009, 50 Suppl:S138–43. https://doi.org/10.1194/jlr.R800079-JLR200 PMID: 19047759

44. Sugihara T, Wadwha R, Kaul SC, Mitsui Y. A novel testis-specific metallothionein-like protein, tesmin, is an early marker of male germ cell differentiation. Genomics. 1999, 57(1):130–6. https://doi.org/10.1006/geno.1999.5756 PMID: 10191092

45. Mo XB, Lu X, Zhang YH, Zhang ZL, Deng FY, Lei SF. Gene-based association analysis identified novel genes associated with bone mineral density. PloS one. 2015, 10(3):e0121811. https://doi.org/10.1371/journal.pone.0121811 PMID: 25811989

46. Warner JR, McIntosh KB. How common are extraribosomal functions of ribosomal proteins?. Molecular cell. 2009, 34(1):3–11. https://doi.org/10.1016/j.molcel.2009.03.006 PMID: 19362532

47. Stoneley M, Willis AE. Cellular internal ribosome entry segments: structures, trans-acting factors and regulation of gene expression. Oncogene. 2004, 23(18):3200–7. https://doi.org/10.1038/sj.onc.1207551 PMID: 15094769