New Horizons in Translational Medicine 3 (2017) 277–283

Contents lists available at ScienceDirect

New Horizons in Translational Medicine

journal homepage: www.elsevier.com/locate/nhtm

Research article

Analysis of osteoporosis gene interactome to identify heterogenic genes and pathways

Bharat Singh, Yasha Hasija*

Department of Biotechnology, Delhi Technological University, Shahbad Daulatpur, Main Bawana Road, Delhi 110042, India

ARTICLE INFO

Keywords:
GWAS
SNP
Osteoporosis
Interactome

ABSTRACT

Latest advances in genetics have prompted swift progress towards the efficient identification of genes tangled in complex diseases. Still, the comprehensive understanding of the relation between the physiological and molecular mechanism of genes and how they affect disease phenotypes remains a challenge for researchers and clinicians. Here, we wish to identify the osteoporosis disease module, i.e. the indigenous neighborhood of the interactome whose agitation is associated with osteoporosis, and endorse it for functional and pathophysiological application, using both computational and experimental methodologies. Recent studies in osteoporosis suggest that against certain genetic variations, the expression level of genes were different in both diseased and normal conditions. The osteoporosis disease module supplemented with uncertain GWAS p-values may also contain mechanisms that are collective with other disease modules. We, therefore, constructed the gene-gene and protein-protein interaction network for 104 genes with 173 reported SNPs accompanied by GO functional enrichment and KEGG pathway enrichment analysis and recognized the substantial genes of osteoporosis along with their molecular functions. Our analyses exposed polymorphism in SOST and LRP5 as significantly conservative SNPs.

Focal points:

- **Benchside:** Robust and concise curation of raw data for osteoporosis will help to make the presymptomatic data more valuable to perform wet lab studies.
- **Bedside:** Bioinformatics network studies are crucial in finding drug targets so it becomes necessary to process the huge data for osteoporosis to curate and produce significance targets. Further, unpredicted pathways and genes could be explored to support clinical studies.
- **Industry:** Data from disease network studies is essential for predicting clinical and non-clinical follow-ups for better drug development.
- **Community:** Ratification and standardization of redundant osteoporosis and gene polymorphism data helps to improvise clinical validation of drug targets. It is important that the data is upto the proper stringency level.
- **Government:** As for the ultimate purpose of drug development, refined gene expression data is the key in developing clinical products. Financial support from government to produce and validate such products is important as with time these will help both the patients and clinicians as well.

1. Introduction

There is collective evidence that disease genes in both complex and monogenic diseases are not scattered arbitrarily on the molecular interface network (interactome) [1], instead of that they tend to work together in analogous biological modules or pathways. Besides this, gene products i.e. proteins associated to the same phenotype have a robust propensity to interact with each other and to make clusters in the similar network vicinity [2]. This recommends the presence of disease modules and interlinked sub-networks that can be systematically allied to a particular disease phenotype. The exact identification of such disease modules could help in revealing the molecular mechanisms causing diseases and detect new genes associated with disease along with the pathways, which ultimately could help in coherent drug target identification. Presently, due to the lack of cellular network maps and listed genes to diseases the exact analysis about disease modules is incomplete. However, that recent advances in interactome mapping and disease gene identification have begun to

* Corresponding author.
E-mail address: yashahasija@gmail.com (Y. Hasija).

http://dx.doi.org/10.1016/j.nhtm.2017.04.001
Received 3 February 2017; Received in revised form 1 April 2017; Accepted 3 April 2017
Available online 06 April 2017

offer adequate network analysis and precision to enable identification of disease modules for some well-studied complex diseases.

Osteoporosis, being one of the most widely studied complex diseases is described by the loss of bone mass and strength, and the advancement of microarchitecture damage leading to fragility fractures [3]. As such, it has become a substantial clinical problem in health care services primarily associated with aging populations [4,5]. The vulnerability to osteoporosis is controlled by a variety of factors, such as genetic variants, age, sex steroid production, lifestyle and environment [5–7]. In context to age studies with subjects including wide age range have proven the fact that in case of postmenopausal osteoporosis change in BMD can be observed well before the age of 70/80 [8–10]. In case of sex steroids previous studies have suggested that the lack of androgen due to castration in prostate cancer is highly associated with bone loss and altered BMD [11–13]. A number of studies have explored the pathogenesis of osteoporosis at the molecular levels. Genome-wide association studies (GWASs) have also been conducted to identify the genes that regulate bone mineral density [14,15]. Traditional single gene pathway based approaches [16,17] have been proved to have limited utility due to environmental and genetic factors. Despite the identification of several susceptibility alleles and genes by GWAS and other tools, our awareness about the fundamental etiologic mechanisms accountable for osteoporosis remains limited. Hence, the fundamental etiology of osteoporosis is yet far from complete and as such the identification of new therapeutic targets for osteoporosis is required.

In recent years, various approaches have been formulated to find the association between characteristic properties of proteins and protein network to different type of 'omics' data to determine novel genes and pathways [18,19]. These approaches are based on the local impact hypothesis, assuming that if we can identify certain specific disease components, we could be able to identify other disease-related components supposed to exist in their network vicinity. Consequently, each disease can be associated to a definite indigenous neighborhood of the interactome, called the disease module. The goal of our work is to clearly implement such approaches to identify drug modules by developing disease module for osteoporosis which counts for approximately 40 million patients in India. In order to identify differentially expressed genes we further wish to analyze different osteoporosis module using bioinformatics methods to reveal osteoporosis-specific gene expression patterns and to determine its association to indigenous neighborhood with an aim to provide novel targets for the diagnosis and treatment of osteoporosis. This study attempts to employ a whole network based approach which might boost our understanding of the indigenous network neighborhood of a disease using osteoporosis as an example.

1.1. Disease heterogeneity and need to develop interactome

The first step to molecular dissection of genetic factors in osteoporosis comprises of determining the chromosomal location and the identification and characterization of the set of genes, variants of which are responsible for different subphenotypes of osteoporosis. However, the complex character of osteoporosis has made it quite resistant to the methods of analysis that in the past few decades worked well for the monogenic diseases. Therefore, different and often more cumbersome approaches have to be applied.

An accurate model of gene-gene interaction and PPI networks will allow better estimation of all types of network statistics along with generating synthetic networks of species for which protein-protein interactions have not been experimentally dogged. This might help understand cellular processes and lead to future biological experiments.

2. Materials and method

2.1. Data collection

To analyze the association of gene polymorphism and osteoporosis SNP information was obtained from the central PUBMED server. We searched the GWAS article relating to osteoporosis using search term as "OSTEOPOROSIS GENES", "OSTEOPOROTIC GENE POLYMORPHISM", "BONE FRACTURE AND GENE POLYMORPHISM", "BMD AND GENE POLYMORPHISM" and extracted osteoporosis associated SNPs with significant P-value and genes (updated to 1 March 2016).

From the central GENE database provided by NCBI, total 104 genes were reported to be associated with osteoporosis. Information regarding these polymorphisms was collected and used to develop a data sheet consisting information about following attributes: PMID, Gene name, RS-ID, Type of polymorphism, P-value, Odds ratio, Ethnicity of the population, Chromosome number, Location, Variant DNA, Variant RNA, Variant protein, Type of mutation.

2.2. Gene ontology

The GO delineates concepts/classes used to define gene function, and relationships between these concepts. It categorizes functions along three aspects: molecular function (molecular activities of gene associated products), cellular component (functional site of the gene products), and biological process (pathways and greater routes made up of the activities of numerous gene products).

Central gene ontology consortium (http://geneontology.org/) server was used; a widely embraced source of gene functional annotation describing cellular component, molecular function and biological process for the selected gene set. Gene ontology consortium central server was used for GO analysis with default settings with selecting Homo sapiens as the species background. In addition, PANTHER (Protein Analysis Through Evolutionary Relationship) classification system, which can offer spontaneous visualization of images of GO analysis was used to categorize proteins and their genes in order to simplify high through output analysis.

2.3. Developing Interactome (protein-protein interaction and gene-gene interaction)

A structured network layout explaining network integrity is the core requirement to justify the interaction between genes and proteins. Cytoscape, a free software package was used for modeling, visualizing, and analyzing genetic and molecular interaction networks. In order to obtain the gene-gene interaction network a simple excel file containing attribute gene as source and target node along with the p score for each SNP was submitted as input, p-value works as edge attribute and helps in determining the path length between interacting nodes.

Cytoscape's software Core offers basic functionality to layout and probes the network; to visually assimilate the network with phenotypes, expression profiles, and additional molecular states; and to relate the network to databases of functional annotations. Cytoscape Core provides an extensible straightforward plug-in architecture, which allows swift development of supplementary computational analyses and topographies.

After obtaining a gene-gene interaction network the hub gene screening and analysis was performed followed by identification of putative complexes and functional modules.

Maximum of known biological networks contains few hub genes/proteins connected to the maximum nodes expect few hubs having least connections [20]. To identify key hub we used the scale free property of network and evaluated the hubs within the interacting network. Further to perform clustering plugin module MCODE was used followed by ontology analysis of hub gene. These investigative results can further confirm the molecular mechanism of osteoporosis and help in finding potentially essential genes.

Respective proteins interaction network was developed using STRING version 9.1 web interface. STRING is a database of well-known and anticipated protein interactions, comprising direct (physi-

cal) and indirect (functional) connotations. STRING quantitatively assimilates interaction data produced from four sources: High-Throughput Experiments, Genomic Context, Co-expression profiles (conserved) and Previous Knowledge. The database currently covers 9.6 million proteins from 2031 organisms. STRING was used to produce osteoporosis gene (protein) network.

2.4. Functional annotation by DAVID and KEGG pathway enrichment analysis

Understanding disease associated non-coding SNPs is a requisite step towards understanding molecular mechanism of multifaceted diseases. DAVID (The Database for Annotation, Visualization and Integrated Discovery)(https://david.ncifcrf.gov/home.jsp) comprises an integrated biological knowledgebase and analytic tools intended at systematically mining biological meaning from hefty gene/protein lists.

We used the DAVID to categorize overrepresented KEGG categories in pathways. We engrossed on biological pathway mode to explore KEGG (Kyoto Encyclopedia of Gene and Genome) pathways and performed functional annotation for the complete gene set. We obtained all of the metabolic and non-metabolic pathways and used the DAVID website to perform KEGG pathway cluster analysis for the complete gene set.

3. Results

3.1. Osteoporosis associated genes/SNPs

After running a complete search to the NCBI (PUBMED) and dbSNP, a total of 173 SNPs of 104 genes were found to be associated with osteoporosis, BMD or fractures with a significance threshold. Among these SNPs, 104 were mapped to introns, and 9 in 3′ and 5′ UTR, rest of them were either missense or upstream variant.

3.2. Ontology analysis

The results of osteoporosis GWAS-associated genes enrichment by gene ontology consortium and PANTHER is shown in (Fig. 1). Distribution of genes along with all three aspects of ontology is as follows.

Genes for Molecular function includes, binding (38 genes, 36.50%), receptor activity (25 genes, 24%), catalytic activity (19 genes, 18.30%), structural molecule activity (7 genes, 6.70%), nucleic acid binding transcription factor activity (6 genes, 5.80%), transporter activity (5 genes, 4.80%), enzyme regulator activity (3 genes, 2.90%), translation regulatory activity (1 gene, 1.00%). Genes for biological processes includes, cellular process (54 genes, 21.50%), metabolic process (31 genes, 12.40%), developmental process (30 genes, 12.00%), biological regulation (29 genes, 11.60%), response to stimulus (29 genes, 11.60%), immune system process (20 genes, 8%), multicellular organismal process (16 genes, 6.40%), localization (15 genes, 6%), apoptotic process (12 genes, 4.80%), cellular component organization or biogenesis (6 genes, 2.40%), biological adhesion (5 genes, 2.0%), reproduction (3 genes, 1.20%), and locomotion (1 genes, 0.40%). Genes for cellular components were found to be involved in cell part (16 genes, 31.40%), extracellular region (11 genes, 21.60%), membrane (9 genes, 17.60%), organelle (9 genes, 17.60%), extracellular matrix (4 genes, 7.80%) and macromolecular complex (2 genes, 3.90%).

3.3. Gene-gene and protein-protein interaction analysis

Gene-gene interaction analysis for osteoporosis associated GWAS produced 104 genes displayed a great interaction pattern. The gene-gene interaction was constructed using Cytoscape 3.4.0.

SOST with highest degree 11 was found to be the hub gene (Table 1) with closeness centrality value of 0.33441558. LRP5 a direct network associate to the SOST gene also showed the second highest value for degree i.e. 10 (Fig. 2).

Further to validate the role of SOST, respective protein interaction network was developed using STRING Version 10.0 with default settings. The protein-protein interaction network for 104 osteoporosis genes showed substantially reliable interaction (Fig. 3).

The network shows hub proteins with resilient connection SOST, WNT3A, FRZB, LRP5, DKK2, and RUNX2. These proteins are involved in Wnt signaling pathway [21].

3.4. Functional annotation

Total 104 unique genes were submitted as gene list, and among them, 95 genes were present in DAVID database. These 95 DAVID selected genes were then converted to DAVID gene IDs using Gene Accession Conversion Tool. Based on the enrichment score the submitted gene list was classified into three main groups with enrichment score values of 7.88, 4.26, and 2.38.

To the group 1 (Table 2) functionally related genes showed similarity score as high as 0.77 and as low as 0.36. Sclerostin showed high kappa score and was also directly associated with the group 1 genes with the highest enrichment score of 7.88 (Table 3), so it can be deduced that SOST could play a crucial role in osteoporosis.

In group 2 (Table 4) genes, tumor necrosis factor family showed a strong association to osteoporosis with an enrichment score of 4.26. Further analysis of functionally related genes showed a Kappa value of 0.67 for NFKB activator.

In group 3 (Table 5), with an enrichment score of 2.38 and Kappa score of 0.97, glutathione transferase family (GST) showed a significant association to osteoporosis.

3.5. KEGG pathway analysis

We conducted pathway enrichment analysis for the selected gene set using DAVID Version 6.7. KEGG pathways with $P < 0.01$ were considered as significant. A total of 4 pathways with p-values less than 0.01 were found to be significant. The most significant pathway was Cytokine-cytokine receptor interaction pathway with a *p*-value of 8.69E-5 which includes 12 genes from the given gene set. This pathway also showed the minimal FDR (0.0958977) value. The additional significant pathways included Neuroactive ligand-receptor interaction (*P*-value=3.44E-04, FDR=0.37874578), Wnt signaling pathway (*P*-value=0.005418103, FDR=5.81929673), Adipocytokine signaling pathway (*P*-value=0.005670277, FDR=6.08248497). A total of 35 genes were involved in these pathways (Fig. 4).

Related term analysis was further conducted in four significant pathways which showed a similarity score (Kappa score) of 1 to Wnt signaling pathway (Fig. 5).

3.6. Screening analysis of hub protein

After calculating the hub degree of the interacting network, we found that SOST has highest hub degree. First neighbor to the hub gene includes following genes GHRH, GHR, SMOC1, HSBG, GALNT3, FTO, TNFSF11, GBT4, CLDN14, and TNFRSF1B.

STRING was then used to find hub protein cluster in which hub protein module existed (Fig. 6).

Successively, GO functional analysis (Table 6) for the cluster gene set was done (GO ID with significant FDR were chosen) which revealed that Wnt signaling pathway was the most significant. Wnt signaling pathway has always been reported as a crucial pathway in skeletal homeostasis [22,23].

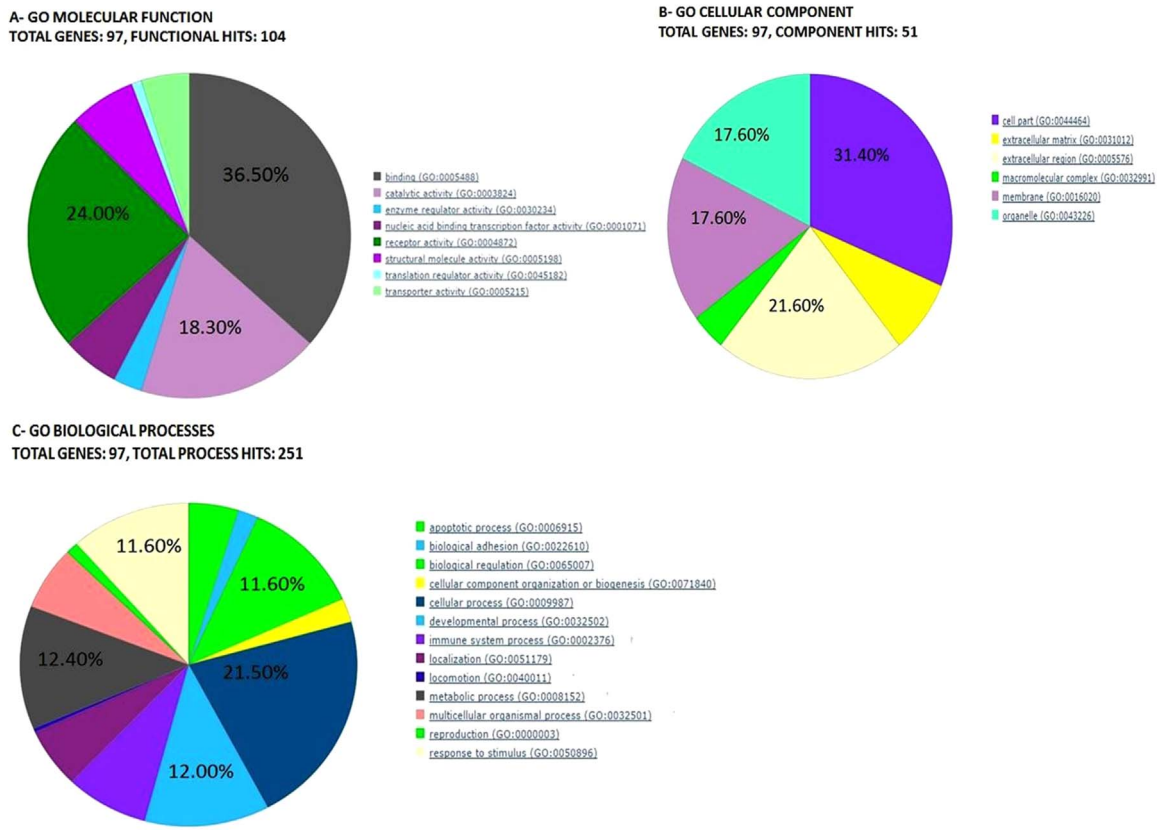

Fig. 1. Gene Ontology analysis for Molecular function, Biological process and Cellular component.

Table 1
Gene-Gene interaction network characteristics.

Nodes	104
Edges	151
Clustering coefficient	0.22
Network diameter	8
Network density	0.028
Network heterogeneity	0.609
Average no. of neighbors	2.90
Highest degree	11 (SOST)

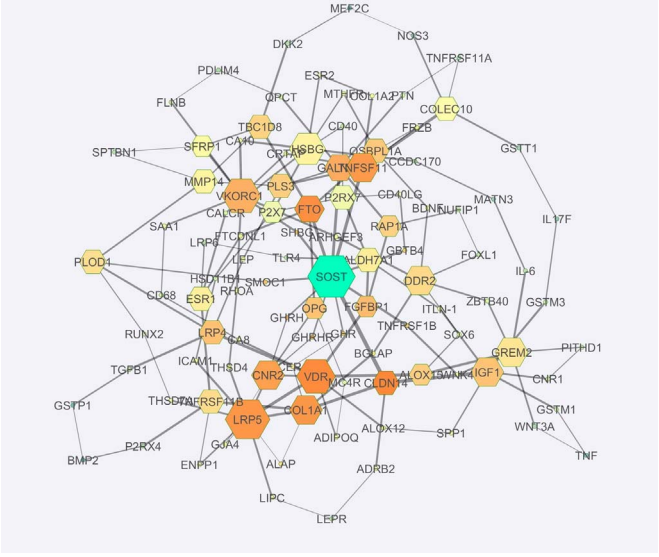

Fig. 2. Gene-Gene interaction network for selected gene set produced using cytoscape.

4. Discussion and conclusion

To discover disease heterogeneity, function and mechanisms of osteoporosis GWAS-associated SNPs and genes were characterized. We conducted GO and pathway analyses, gene-gene interaction analysis, protein-protein interaction analysis and KEGG pathway analysis to look into the insights of osteoporosis GWAS-associated genes. Our analyses exposed polymorphism in SOST and LRP5 as significantly conservative SNPs. The identification and characterization of genes related to osteoporosis have a central and pragmatic relevance, as it is presently a common disease.

Our present study acknowledged 187 genes (identified upon manual curation through NCBI-PUBMED) in osteoporosis. We constructed gene-gene and protein-protein interaction network for 104 genes for which 173 SNPs were reported and recognized the substantial genes of osteoporosis along with their molecular functions. GO enrichment function analysis was expressively characterized by molecular functions which involves binding and receptor activity which supports results of related studies [20,22]. The results of KEGG pathway enrichment divulge that cytokine-cytokine receptor interaction pathway (cell signaling), neuroactive ligand-receptor interaction pathway (growth) and Wnt signaling pathway (cancer) pathways were significant metabolic pathways for osteoporosis-related genes. Our interactome module (gene-gene and protein-protein) analysis unveils SOST as hub gene/protein accompanied by LRP5. Additionally, VDR gene cluster with significant interaction degree was found in propinquity [24]. Direct neighbor to SOST (GHRH, GHR, SMOC1, HSBG, GALNT3, FTO, TNFSF11, GBT4, CLDN14, and TNFRSF1B) along with VDR neighbors can also provide a potential insight about osteoporosis heterogeneity and could prove to be the key regulatory genes of the disease.

SOST expressed in osteocytes codes for a protein sclerostin which is a key regulator to Wnt/b-catenin signaling and Wnt/b-catenin signal-

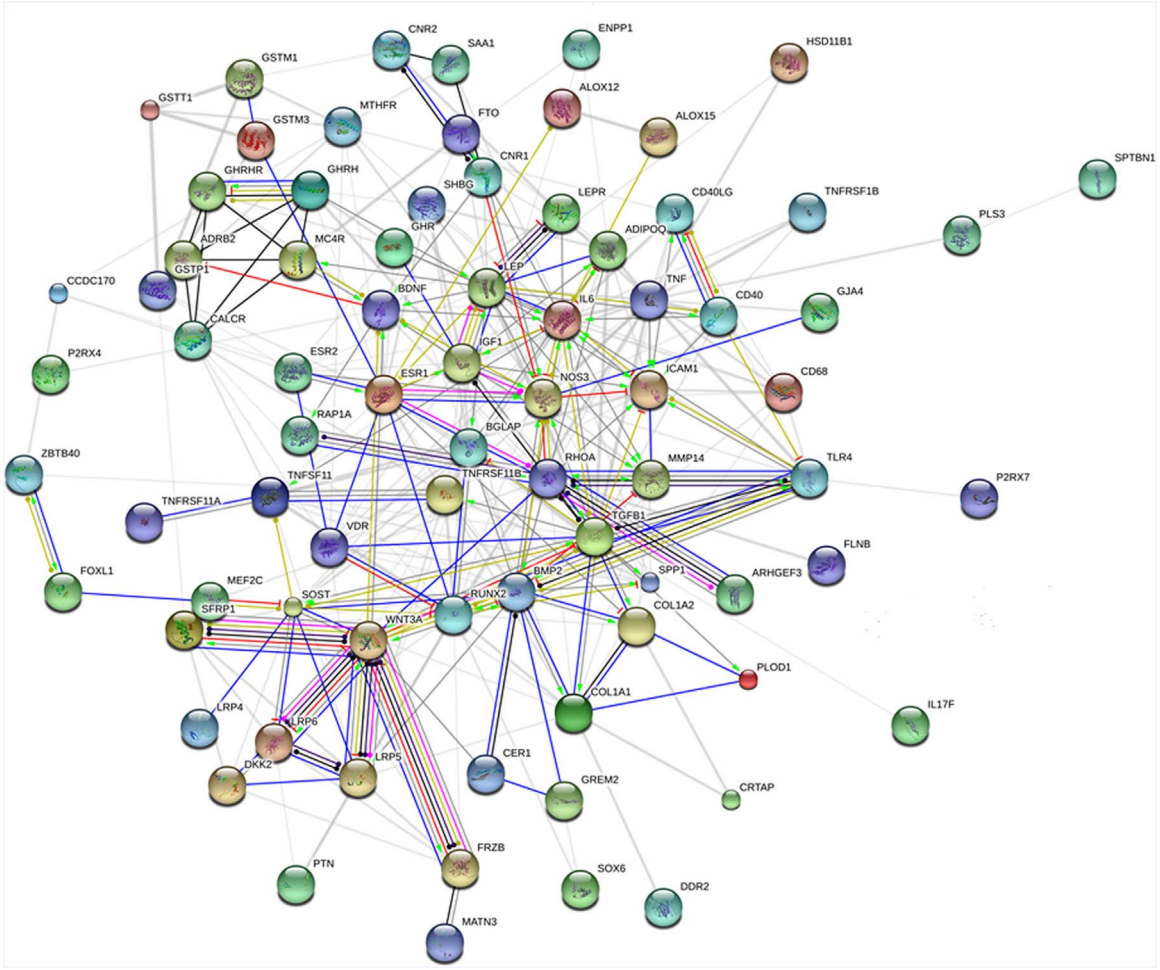

Fig. 3. Protein-Protein interaction network produced using STRING to validate the role of SOST, in this WNT pathway members can be seen in close vicinity along with SOST as a regulatory member.

Table 2
Gene group 1 of functionally related genes produced by Functional annotation using DAVID.

Sr. No.	ID	Gene family
1	783053	Frizzled related protein
2	803546	Gremlin 2, Cysteine knot superfamily
3	805261	Cerberus 1, Cysteine knot superfamily
4	784005	Sclerosteosis
5	822680	Dickkopf homolog 2
6	786401	Secreted frizzled related protein 1

Table 3
Kappa score for functionally related genes to group 1.

Functionally related gene	Kappa score (similarity score)
Dickkopf homolog 2 (xenopuslaevis)	0.77
Sclerosteosis	0.68
Germline2, cysteine knot superfamily, homolog (xenopuslaevis)	0.64
Frizzled related protein	0.57

Table 4
Gene group 2 of functionally related genesproduced by Functional annotation using DAVID.

Sr. No.	ID	Gene family
1	825920	Tumor necrosis factor receptor superfamily, member 1B
2	802013	Thrombospondin, type 1, domain containing 7A
3	776184	Tumor necrosis factor receptor superfamily, member 11a NFKB activator
4	809821	CD68 molecule

Table 5
Gene group 3 of functionally related genesproduced by Functional annotation using DAVID.

Sr. No.	ID	Gene family
1	809178	Glutathione S-transferase mu 3 (brain)
2	825037	Glutathione S-transferase theta 1
3	780298	Glutathione S-transferase mu 1
4	817433	Glutathione S-transferase pi 1

ing/canonical Wnt signaling and plays a critical role in skeletal homeostasis [25]. In bone, Wnt/b-catenin signaling is required for osteoblastic growth and differentiation [26–28]. Polymorphisms in SOST have also been associated with BMD in some population-based studies [29]. A sequence of studies investigates the relationship between the SOST genotypes and BMD, and the results are erratic. We find the SOST polymorphism is significantly associated with BMD [8]. Second associated hub protein LRP5 have also been proved to be involved in both low and high bone mass in a different mutated form [30,31]. Sclerostin is an inhibitor of the Wnt/beta-catenin signaling pathway as it is a competitive inhibitor to LRP5 [32–34], thus over study identifying SOST and LRP5 as hub gene/protein supports other studies

Category	Term	RT	Genes	Count	%	P-Value	Benjamini
KEGG_PATHWAY	Cytokine-cytokine receptor interaction	RT		12	12.9	8.7E-5	8.4E-3
KEGG_PATHWAY	Neuroactive ligand-receptor interaction	RT		11	11.8	3.4E-4	1.7E-2
KEGG_PATHWAY	Wnt signaling pathway	RT		7	7.5	5.4E-3	1.6E-1
KEGG_PATHWAY	Adipocytokine signaling pathway	RT		5	5.4	5.7E-3	1.3E-1
KEGG_PATHWAY	Glutathione metabolism	RT		4	4.3	1.6E-2	2.7E-1
KEGG_PATHWAY	Focal adhesion	RT		7	7.5	2.0E-2	2.8E-1
KEGG_PATHWAY	Metabolism of xenobiotics by cytochrome P450	RT		4	4.3	2.7E-2	3.1E-1
KEGG_PATHWAY	Drug metabolism	RT		4	4.3	2.9E-2	3.0E-1
KEGG_PATHWAY	Asthma	RT		3	3.2	3.9E-2	3.5E-1
KEGG_PATHWAY	Allograft rejection	RT		3	3.2	5.7E-2	4.4E-1
KEGG_PATHWAY	TGF-beta signaling pathway	RT		4	4.3	6.7E-2	4.6E-1
KEGG_PATHWAY	Toll-like receptor signaling pathway	RT		4	4.3	9.5E-2	5.5E-1
KEGG_PATHWAY	Intestinal immune network for IgA production	RT		3	3.2	9.8E-2	5.4E-1

Fig. 4. KEGG pathway analysis for the selected gene set.

stating the role of Wnt associated biomolecules and verifies the accuracy of the present study. Accompanying SOST, VDR showed a significant presence in network architecture as the modulation in VDR affects the transcription and expression of genes associated with calcium uptake and bone mass formation [31]. In our present study, the pathway enrichment investigations of osteoporosis GWAS-associated SNPs/genes only confirmed well-known cytokine regulated pathways and Wnt signaling pathways. Although, earlier studies have provided many other genes shown to be associated with osteoporosis, apparently, our procedural limitations in the acute processing of existing databases restricted us to state the finding of limited genes only. One of the essential part of our work was curating genetic variants from online data stores as it is vital that curation produces 100% specificity and accuracy. As such, the main limitation to our work was manual curation of data from online databases. Curating genetic variants data from enormous databases of gene variants defined in literature may not give candid information primarily due to issues like expression and nomenclature patterns. In addition to this, such extensive stores of gene variants are present as atypical names in literatures and the online search tools that are largely used may not be adequate for finding them.

Nevertheless, exploring these pathways may aid to unravel potential targets which in turn might instigate the development of novel agents to tackle diseases of low bone mass, such as osteoporosis. Thus, our computational portrayal of osteoporosis GWAS-associated SNPs emphasized genes (proteins) and pathways that are vital for osteoporosis pathophysiology which may assist in improving our understanding of the disease etiology thereby contributing towards the discovery of potential treatment targets. Our results also justify a rationale for further experimental tests in the future to explore osteoporosis disease heterogeneity.

5. Executive summary

- Osteoporosis gene interactome was analyzed.
- Relevant gene data was collected from NCBI.
- Gene ontology analysis, cytoscapeinteractome analysis and func-

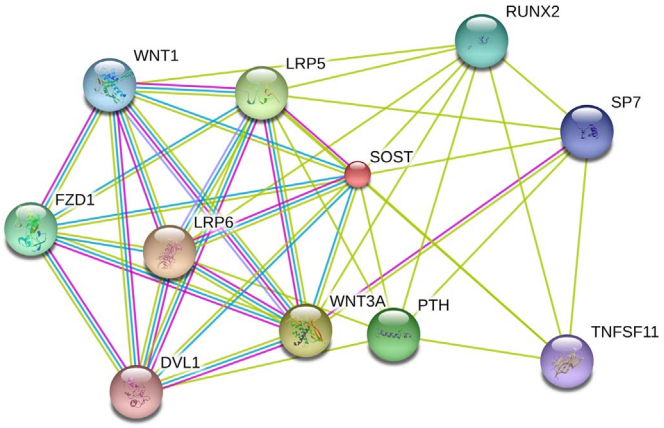

Fig. 6. Hub protein i.e. SOST screening analysis to show direct neighbors.

Table 6
GO functional analysis of hub protein SOST.

GO TERM	GO ID	DESCRIPTION	GENE COUNT	FDR
BP	GO:0045893	Positive regulation of transcription, DNA-templated	11	3.46e−10
BP	GO:0060070	Canonical Wnt signaling pathway	6	2.45e−09
BP	GO:0016055	Wnt signaling pathway	7	6.9e−09
MF	GO:0005109	Frizzled binding	5	1.27e−08
MF	GO:0001664	G-protein coupled receptor binding	6	4.04e−07
MF	GO:0042813	Wnt activated receptor activity	3	4.52e−05
KEGG	04310	Wnt signaling pathway	6	9.53e−09
KEGG	05217	Basal cell carcinoma	4	2.18e−06
KEGG	04916	Melanogenesis	4	1.4e−05
KEGG	04390	Hippo signaling pathway	4	5.67e−05

Similarity Score: Very High (0.75-1) High (0.5-0.75) Moderate (0.25-0.5) Low (<0.25)

#	Category	Term	Kappa
1	KEGG_PATHWAY	Wnt signaling pathway	1.00
2	GOTERM_BP_FAT	regionalization	0.82
3	GOTERM_BP_FAT	anterior/posterior pattern formation	0.82
4	GOTERM_BP_FAT	Wnt receptor signaling pathway	0.75
5	GOTERM_BP_FAT	pattern specification process	0.75
6	GOTERM_BP_FAT	gastrulation	0.71
7	SP_PIR_KEYWORDS	wnt signaling pathway	0.69

Fig. 5. Related term analysis for most significant pathways showing maximum Kappa score for WNT pathway.

tional annotation of genes exposed 3 genes, namely, SOST, LRP5 and VDR to be significantly associated with osteoporosis.
- Direct neighbors to the hub gene (SOST) could prove to be the key players in dealing with the disease in near future.

Conflict of interest

The authors confirm that this article content has no conflicts of interest.

References

[1] A.L. Barabasi, N. Gulbahce, J. Loscalzo, Network medicine: a network-based approach to human disease, Nat. Rev. Genet. 12 (2011) 56–68.
[2] J. Xu, Y. Li, Discovering disease-genes by topological features in human protein-protein interaction network, Bioinformatics 22 (2006) 2800–2805.
[3] S.R. Cummings, L.J. Melton, Epidemiology and outcomes of osteoporotic fractures, Lancet 359 (2002) 1761–1767.
[4] T.D. Rachner, S. Khosla, L.C. Hofbauer, Osteoporosis: now and the future, Lancet 377 (2011) 1276–1287.
[5] S.H. Ralston, A.G. Uitterlinden, Genetics of osteoporosis, Endocr. Rev. 31 (2010) 629–662.
[6] S.Y. Hwang, J.W. Putney Jr., Calcium signaling in osteoclasts, Biochim. Et Biophys. Mol. Cell Res. 1813 (2011) 979–983.
[7] P. Pietschmann, M. Rauner, W. Sipos, K. Kerschan-Schindl, Osteoporosis: an age-related and gender-specific disease – a mini-review, Gerontology 55 (1) (2009) 3–12.
[8] H. Zhang, J.W. He, C. Wang, Z. Zhang, H. Yue, W.W. Hu, J.M. Gu, Y.Q. Hu, M. Li, W.Z. Fu, Z.L. Zhang, Associations of polymorphisms in the SOST gene and bone mineral density in postmenopausal Chinese Women, Osteoporos. Int. 25 (12) (2014) 2797–2803.
[9] P.R. Ebeling, L.M. Atley, J.R. Guthrie, H.G. Burger, L. Dennerstein, J.L. Hopper, J.D. Wark, Bone turnover markers and bone density across the menopausal transition, J. Clin. Endocrinol. Metab. (1996) 813366–813371.
[10] H.W. Goderie-Plomp, M. van der Klift, W. de Ronde, A. Hofman, F.H. de Jong, H.A. Pols, Endogenous sex hormones, sex hormone-binding globulin, and the risk of incident vertebral fractures in elderly men and women: the Rotterdam Study, J. Clin. Endocrinol. Metab. (2004) 893261–893269.
[11] H.W. Daniell, S.R. Dunn, D.W. Ferguson, G. Lomas, Z. Niazi, P.T. Stratte, Progressive osteoporosis during androgen deprivation therapy for prostate cancer, J. Urol. 163 (1) (2000) 181–186.
[12] A. Falahati-Nini, B.L. Riggs, E.J. Atkinson, W.M. O'Fallon, R. Eastell, S. Khosla, Relative contributions of testosterone and estrogen in regulating bone resorption and formation in normal elderly men, J. Clin. Investig. (2000) 1061553–1061560.
[13] Y. Gao, W.P. Qian, K. Dark, G. Toraldo, A.S. Lin, R.E. Guldberg, R.A. Flavell, M.N. Weitzmann, R. Pacifici, Estrogen prevents bone loss through transforming growth factor beta signaling in T cells, Proc. Natl. Acad. Sci. USA (2004) 10116618–10116623.
[14] D.R. Nyholt, SECA: snp effect concordance analysis using genome-wide association summary results, Bioinformatics 30 (2014) 2086–2088.
[15] M. Oti, B. Snel, M.A. Huynen, H.G. Brunner, Predicting disease genes using protein-protein interactions, J. Med. Genet. 43 (2006) 691–698.
[16] A.L. Price, N.J. Patterson, R.M. Plenge, M.E. Weinblatt, N.A. Shadick, D. Reich, Principal components analysis corrects for stratification in genome-wide association studies, Nat. Genet. 38 (2006) 904–909.
[17] A.M. Parfitt, A.R. Villanueva, J. Foldes, D.S. Rao, Relations between histologic indices of bone formation: implications for the pathogenesis of spinal osteoporosis, J. Bone Miner. Res. (1995) 10466–10473.
[18] K.I. Goh, M.E. Cusick, D. Valle, B. Childs, M. Vidal, A.L. Barabasi, The human disease network, Proc. Natl. Acad. Sci. USA 104 (2007) 8685–8690.
[19] T. Ideker, R. Sharan, Protein networks in disease, Genome Res. 18 (2008) 644–652.
[20] Y. Chen, J. Zhu, P.Y. Lum, X. Yang, S. Pinto, D.J. MacNeil, C. Zhang, J. Lamb, S. Edwards, S.K. Sieberts, A. Leonardson, Variations in DNA elucidate molecular networks that cause disease, Nature 452 (7186) (2008) 429–435.
[21] P.V. Bodine, J. Billiard, R.A. Moran, H. Ponce-de-Leon, S. McLarney, A. Mangine, M.J. Scrimo, R.A. Bhat, B. Stauffer, J. Green, G.S. Stein, The Wnt antagonist secreted frizzled-related protein-1 controls osteoblast and osteocyte apoptosis, J. Cell. Biochem. (2005).
[22] M.K. Chang, I. Kramer, H. Keller, J.H. Gooi, C. Collett, D. Jenkins, S.A. Ettenberg, F. Cong, C. Halleux, M. Kneissel, Reversing LRP5-dependent osteoporosis and sost deficiency–induced sclerosing bone disorders by altering WNT signaling activity, J. Bone Miner. Res. 29 (1) (2014) 29–42.
[23] T.P. Hill, D. Spater, M.M. Taketo, W. Birchmeier, C. Hartmann, Canonical Wnt/beta-catenin signaling prevents osteoblasts from differentiating into chondrocytes, Dev. Cell 8 (2005) 727–738.
[24] S.L. Ferrari, R. Rizzoli, D.O. Slosman, J.P. Bonjour, Do dietary calcium and age explain the controversy surrounding the relationship between bone mineral density and vitamin D receptor gene polymorphisms, J. Bone Miner. Res. (1998) 13363–13370.
[25] J. He, H. Zhang, C. Wang, Z. Zhang, H. Yue, W. Hu, J. Gu, W. Fu, Y. Hu, M. Li, Y. Liu, Associations of serum sclerostin and polymorphisms in the SOST gene with bone mineral density and markers of bone metabolism in postmenopausal Chinese women, J. Clin. Endocrinol. Metab. 99 (4) (2014) E665–E673.
[26] D.A. Glass, P. Bialek, J.D. Ahn, M. Starbuck, M.S. Patel, H. Clevers, M.M. Taketo, F. Long, A.P. McMahon, R.A. Lang, G. Karsenty, Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation, Dev. Cell 8 (5) (2005) 751–764.
[27] I. Kramer, C. Halleux, H. Keller, M. Pegurri, J.H. Gooi, P.B. Weber, J.Q. Feng, L.F. Bonewald, M. Kneissel, Osteocyte Wnt/β-catenin signaling is required for normal bone homeostasis, Mol. Cell. Biol. 30 (12) (2010) 3071–3085.
[28] S.L. Holmen, C.R. Zylstra, A. Mukherjee, R.E. Sigler, M.C. Faugere, M.L. Bouxsein, L. Deng, T.L. Clemens, B.O. Williams, Essential role of beta-catenin in postnatal bone acquisition, J. Biol. Chem. 280 (2005) 21162–21168.
[29] A.G. Uitterlinden, P.P. Arp, B.W. Paeper, P. Charmley, S. Proll, F. Rivadeneira, Y. Fang, J.B. van Meurs, T.B. Britschgi, J.A. Latham, R.C. Schatzman, Polymorphisms in the sclerosteosis/van Buchem disease gene (*SOST*) region are associated with bone-mineral density in elderly whites, Am. J. Hum. Genet. (2004) 751032–751045.
[30] R.D. Little, C. Folz, S.P. Manning, P.M. Swain, S.C. Zhao, B. Eustace, M.M. Lappe, L. Spitzer, S. Zweier, K. Braunschweiger, Y. Benchekroun, A mutation in the LDL receptor–related protein 5 gene results in the autosomal dominant high–bone-mass trait, Am. J. Hum. Genet. 70 (1) (2002) 11–19.
[31] X. Li, Y. Zhang, H. Kang, W. Liu, P. Liu, J. Zhang, S.E. Harris, D. Wu, Sclerostin binds to LRP5/6 and antagonizes canonical Wnt signaling, J. Biol. Chem. (2005) 28019883–28019887.
[32] L.M. Boyden, J. Mao, J. Belsky, L. Mitzner, A. Farhi, M.A. Mitnick, D. Wu, K. Insogna, R.P. Lifton, High bone density due to a mutation in LDL-receptor-related protein 5, N. Engl. J. Med. (2002) 3461513–3461521.
[33] Wei He, M. Liu, X. Huang, Z. Qing, W. Gao, The influence of vitamin D receptor genetic variants on bone mineral density and osteoporosis in Chinese postmenopausal women, Dis. Markers (2015).
[34] M.A. Koay, P.Y. Woon, Y. Zhang, L.J. Miles, E.L. Duncan, S.H. Ralston, J.E. Compston, C. Cooper, R. Keen, B.L. Langdahl, A. MacLelland, Influence of LRP5 polymorphisms on normal variation in BMD, J. Bone Miner. Res. (2004) 191619–191627.