Exploring the relationship between osteoporosis and polycystic ovary syndrome based on bioinformatics

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Abstract

Background: In recent years, clinical studies have found that there is a close relationship between osteoporosis and polycystic ovary syndrome. However, there are few literature on the pathogenesis of osteoporosis and polycystic ovary syndrome. In order to clarify their common pathogenic mechanism and provide potential targets for drugs to regulate them at the same time, bioinformatics methods are used to explore, so as to provide a new direction for the study of the relationship between diseases in the future.

Methods: To screen the targets of osteoporosis and polycystic ovary syndrome by GeneCards, Online Mendelian Inheritance in Man databases and Therapeutic Target Database to take the intersection of the two mappings and upload the intersection targets to the STRING database to construct protein-protein interaction network; to screen the core targets by degree value and import them to Metascape database for Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway analysis; and finally, to construct the visualization network of core targets and pathways by Cytoscape software. Ethical approval and informed consent of patients are not required because the data used in this study is publicly available and does not involve individual patient data or privacy.

Results: The core targets of polycystic ovary syndrome and osteoporosis were insulin gene, insulin-like growth factor 1, CTNNB1, serine/threonine kinase 1, signal transducer and activator of transcription 3, LEP, etc. The biological processes involved include the regulation of protein phosphorylation, cell proliferation and differentiation, hormone endocrine, reproductive system and skeletal system. The related pathways were concentrated in Foxo signaling pathway, HTLV-I infection, PI3K-AKT signaling pathway, MAPK signaling pathway and AGE-RAGE signaling pathway in diabetic complications.

Conclusions: There is a close relationship between osteoporosis and polycystic ovary syndrome in terms of target and molecular mechanism. This study used bioinformatics to clarify their targets and mechanisms, providing potential targets for drugs to regulate both diseases simultaneously and providing new directions to explore the relationship between the diseases.

Abbreviations: AKT1 = serine/threonine kinase 1, GO = Gene Ontology, IGF1 = insulin-like growth factor 1, INS = insulin gene, KEGG = Kyoto Encyclopedia of Genes and Genomes, PCOS = polycystic ovary syndrome, PPI = protein-protein interaction, STAT3 = signal transducer and activator of transcription 3.

Keywords: bioinformatics, disease interrelationship, osteoporosis, polycystic ovary syndrome

1. Introduction

Osteoporosis (OP) is a systemic skeletal disease characterized by a decrease in bone density and bone quality, and the associated factors are complex and diverse.[1] Polycystic ovary syndrome (PCOS) is a disease caused by endocrine abnormalities. The National Institutes of Health reported that the incidence of PCOS in women of reproductive age is about 4% to 10%.[2] 

Osteoporosis and polycystic ovary syndrome are two types of chronic diseases that are closely related, and a study of 11,106 women in Taiwan[3] found an increased incidence of fractures in patients with PCOS compared to healthy women; Piovezan et al[4] in a systematic evaluation of 31,838 women found that PCOS patients tended to have lower bone mineral density and osteocalcin; there is also literature[5,6] suggests an association...
between metabolic dysfunction and low vitamin D levels in patients with PCOS.

Therefore, it is urgent to clarify the relationship between osteoporosis and polycystic ovary syndrome and to reduce the risk of osteoporosis in patients with PCOS. A bioinformatics approach was applied to integrate the genetic data of osteoporosis and polycystic ovary syndrome to explore the association and provide a theoretical basis for drug intervention in both diseases simultaneously.

2. Materials and methods

2.1. Osteoporosis – polycystic ovary syndrome related target collection

The “osteoporosis” “polycystic ovary syndrome” as keywords retrieval Genecards (https://www.genecards.org/), Online Mendelian Inheritance in Man (http://www.omim.org/) Therapeutic Target Database (http://db.idrblab.net/tdtd) database screening of disease related targets. To improve the accuracy, the top 300 target genes with high Relevance Score were selected from the Genecards database, and the results were combined and de-weighted, and then the Uniprot database was used to screen the clearly identified target genes as the final targets for polycystic ovary syndrome and osteoporosis.

2.2. Target mapping and protein-protein interaction (PPI) analysis of osteoporosis – polycystic ovary syndrome

The target genes of osteoporosis and polycystic ovary syndrome were imported into the BMK Cloud (http://www.biocloud.net/) platform to obtain the intersecting genes, and the intersecting genes were uploaded to the STRING database (https://string-db.org/cgi/input.pl) to construct a PPI network. Download the PPI network in tsv format and import it into Cytoscape 3.8.0 software to better visualize the protein-protein interaction relationships. Using Network analyzer plug-in, adjust the size and color of the targets according to the node connectivity (degree), and adjust the thickness and color of the connecting lines according to the combined score, and finally select the targets with the degree value ≥ 18 to make the core target map of osteoporosis – polycystic ovary syndrome.

2.3. Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis and visualization network of osteoporosis – polycystic ovary syndrome

The target genes of osteoporosis and polycystic ovary syndrome were imported into Metascape database (http://metascape.org/)[11] and set P < .01 and enrichment factor > 1.5 for GO and KEGG analysis. The first 40 GO, KEGG pathways of the two diseases were selected and intersected by logP order, and finally 17 (GO), 19 (KEGG) interaction pathways were screened (Fig. 4, Table 1, Table 2), and imported into Cytoscape 3.8.0 software to construct the interaction pathway map of osteoporosis – polycystic ovary syndrome.

3. Results

3.1. Results of target collection for osteoporosis – polycystic ovary syndrome

We obtained 30,020,315 targets related to polycystic ovary syndrome and 3,002,942 targets related to osteoporosis from GeneCards, Online Mendelian Inheritance in Man and Therapeutic Target Database, respectively; 2,921,765 effective targets for polycystic ovary syndrome and 281,138 effective targets for osteoporosis were obtained after screening by Uniprot database. A total of 433 effective target genes for polycystic ovary syndrome and 281 effective target genes for osteoporosis were obtained after combined de-duplication.

3.2. Target mapping of osteoporosis – polycystic ovary syndrome and results of PPI analysis

Target genes mapping osteoporosis and polycystic ovary syndrome, a total of 70 intersecting genes were obtained (Fig. 1). The intersection genes were uploaded to the STRING database, and the minimum interaction score was set to 0.7 and the free nodes were hidden to generate the PPI interaction network (Fig. 2). The results showed that a total of 69 targets were interlinked, and the network had 363 edges with an average node degree of 10.5 and an average clustering coefficient of 0.552. We downloaded its TSV file and imported into Cytoscape 3.8.0 software, 17 core targets with degree value ≥ 18 were screened (Fig. 3). The obtained core targets directly or indirectly affect osteoporosis and polycystic ovary syndrome.

3.3. Results of GO, KEGG analysis in osteoporosis – polycystic ovary syndrome

The GO, KEGG signaling pathways of osteoporosis and polycystic ovary syndrome were obtained from the Metascape database, respectively. And the first 40 GO, KEGG pathways of the two diseases were selected in ascending logP order, and finally 17 (GO), 19 (KEGG) interaction pathways were screened (Fig. 4, Table 1, Table 2), and imported into Cytoscape 3.8.0 software to construct the interaction pathway map of osteoporosis – polycystic ovary syndrome (Fig. 5).

4. Discussion

4.1. Results and analysis

Recent studies have found that the two types of diseases, polycystic ovary syndrome and osteoporosis, are interrelated and affect each other. Clinical studies PCOS patients

![Image](https://example.com/image.png)

Figure 1. Intersection target of OP and PCOS.
were found to have different degrees of bone mineral density loss, which is closely related to leptin resistance and hyperinsulinemia. Therefore, in this study, bioinformatics was used to find the intersection and common pathways in the vast gene network of the two diseases to provide potential targets for drug therapy. A total of 70 intersecting targets were found between polycystic ovary syndrome and osteoporosis, accounting for about 10.38%, with core targets such as insulin gene (INS), insulin-like growth factor 1 (IGF1), CTNNB1, Serine/threonine kinase 1 (AKT1), signal transducer and activator of transcription 3 (STAT3), LEP, etc.; The biological processes involved include the regulation of protein phosphorylation, cell proliferation and differentiation, hormone endocrine, reproductive system and skeletal system; KEGG pathway analysis showed that the Foxo signaling pathway, HTLV-I infection, PI3K-AKT signaling pathway, MAPK signaling pathway and AGE-RAGE signaling pathway in diabetic complications and many other signaling pathways.

As an endocrine disease with complex etiology, polycystic ovary syndrome is closely related to obesity and insulin resistance, and the INS is also considered to be one of the candidate genes for the development of PCOS. The INS is also considered as one of the candidate genes for PCOS. Studies have confirmed that decreased insulin receptor activity and PI3K activity lead to the decrease of glucose uptake rate. Therefore, PCOS patients are prone to metabolic abnormalities such as obesity and decreased glucose tolerance. Meanwhile, the high glucose environment alters osteoblast activity and induces osteoblast apoptosis, leading to reduced bone mineral density and increased fracture risk. IGF1, an osteogenic differentiation factor, upregulates the expression of protein kinase and alkaline phosphatase mesenchymal stem cells and participates in cartilage anabolism and repair. Animal experiments found that different concentrations of IGF1 play a positive role in regulating the expression of bone genes in cartilage layer and subchondral bone, and the bone thickness of IGF1 knockout
mice is significantly lower than that of normal mice, which confirmed the importance of IGF1 in bone growth; meanwhile, the occurrence of hyperandrogenism in PCOS is closely related to hyperinsulinemia, 44–77% of PCOS patients can be combined with hyperinsulinemia, and the higher insulin stimulates IGF1, promotes the release of LH, and enhances the activity of 17α-hydroxylase in follicular membrane cells, which increases the synthesis and secretion of androgens, thus causing the development of hyperandrogenemia.[23]; AKT1 is an isomer of Akt, mediates many downstream pathways regulated by PI3K,[24] regulates the growth of follicles, the proliferation of granulosa luteal cells and the dynamic balance between bone formation and bone resorption. Some studies[25] speculate that the high expression of AKT1 is one of the reasons affecting the quality of follicles and the function of granulosa luteal cells in patients with PCOS. There are also literature[26,27] reported that activated AKT1 can control the proliferation and differentiation of osteoblasts and osteoclasts through the RANK/RANKL signaling pathway and PI3K/AKT signaling pathway to affect bone metabolism, and Mukherjee et al[28] also found that osteoblast formation was significantly inhibited by culturing AKT1 knockout bone marrow stromal cells or mesenchymal stem cells. The STAT3 dimer formed by the dimerization of STAT3 can activate STAT,[29] promote the transcription of STAT3 target genes, form JAK2/STAT3 signal pathway mediated by IL-6,[30] inhibit the expression of TNF, VEGF and other inflammatory factors, and improve leptin resistance in PCOS patients.[31] Meanwhile, STAT3 dimer can also promote the proliferation and differentiation of Th17 cells, promote the secretion of IL-17 and TNF-α.[32] It affects the formation of osteoclasts and bone resorption process, and inhibits the apoptosis of osteoblasts by promoting the expression of apoptosis inhibitory protein Bcl-2.[33]

Foxy signaling pathway, as one of the pathways mediating the inflammatory response, can improve insulin resistance, regulate glucose metabolism in PCOS patients.[34] It can prevent the effect of high glucose environment on osteoblast apoptosis, and regulate the free oxygen concentration in osteoblasts to promote osteoblast differentiation.[35] It can also reduce the damage to cartilage caused by inflammation. He Tingting et al[36] found that the level of anti-apoptotic gene Foxo1 in PCOS patients was lower than that in normal women, suggesting that the low expression of Foxo may be one of the reasons for ovarian granulosa cell autophagy and follicular development disorder in PCOS patients. As an important pathway to regulate cell proliferation, differentiation, metabolism and other basic functions, PI3K/AKT signaling pathway can regulate the function of ovarian granulosa cell, affect hormone levels in vivo, and also affect bone metabolism by controlling the proliferation and differentiation of osteoblasts and osteoclasts, and is a key pathway to affect the functional coordination between osteoblasts and osteoclasts.[27] Mei Shao et al[37] found that PI3K/AKT signaling pathway could inhibit apoptosis and autophagy of ovarian granulosa cells in PCOS patients, promote cell differentiation and proliferation, and improve polycystic ovary syndrome. Animal experiments[38] demonstrated that activation
Figure 4. Barplot histogram of signal pathway of OP and PCOS.

Table 1
19 KEGG interaction paths between OP and PCOS.

| Gene Ontology | Path description | Log \( P \) |
|---------------|------------------|-----------|
| hsa05200      | Pathways in cancer | -41.7856  |
| ko04933       | AGE-RAGE signaling pathway in diabetic complications | -29.8879  |
| ko05224       | Breast cancer     | -25.2937  |
| ko05142       | Chagas disease (American trypanosomiasis) | -24.7953  |
| hsa05205      | Proteoglycans in cancer | -24.213   |
| hsa04068      | Foxo signaling pathway | -20.2934  |
| hsa05166      | HTLV-I infection  | -19.8598  |
| hsa04151      | PI3K-Akt signaling pathway | -19.4415  |
| hsa05161      | Hepatitis B       | -19.0402  |
| hsa04010      | MAPK signaling pathway | -18.3932  |
| hsa01522      | Endocrine resistance | -15.1646  |
| ko04932       | Non-alcoholic fatty liver disease (NAFLD) | -14.4383  |
| hsa04917      | Prolactin signaling pathway | -13.974   |
| hsa04550      | Signaling pathways regulating pluripotency of stem cells | -13.0563  |
| hsa04211      | Longevity regulating pathway | -12.9737  |
| hsa05210      | Colorectal cancer  | -12.6346  |
| hsa04066      | HIF-1 signaling pathway | -12.9944  |
| hsa04630      | Jak-STAT signaling pathway | -12.2563  |
| ko05212       | Pancreatic cancer  | -12.1551  |

KEGG = Kyoto Encyclopedia of Genes and Genomes, OP = osteoporosis, PCOS = polycystic ovary syndrome.

Table 2
17 GO interaction paths between OP and PCOS.

| Gene Ontology | Path description | Log \( P \) |
|---------------|------------------|-----------|
| GO:0002009    | Morphogenesis of an epithelium | -59.0308  |
| GO:0048732    | Gland development   | -53.7721  |
| GO:0039239    | Tube morphogenesis  | -52.9034  |
| GO:0048608    | Reproductive structure Development | -45.3505  |
| GO:0061458    | Reproductive system development | -45.1488  |
| GO:0009725    | Response to hormone | -41.8798  |
| GO:0001934    | Positive regulation of protein Phosphorylation | -37.9615  |
| GO:0050678    | Regulation of epithelial cell proliferation | -37.8068  |
| GO:0001501    | Skeletal system development | -37.5617  |
| GO:0043549    | Regulation of kinase activity | -37.2621  |
| GO:1901699    | Cellular response to nitrogen compound | -37.0043  |
| GO:0071417    | Cellular response to organonitrogen compound | -35.7585  |
| GO:1901652    | Response to peptide | -33.5401  |
| GO:0008285    | Negative regulation of cell population proliferation | -32.9009  |
| GO:0007167    | Enzyme linked receptor protein signaling pathway | -31.6865  |
| GO:0045596    | Negative regulation of cell differentiation | -30.2734  |

GO = Gene Ontology, OP = osteoporosis, PCOS = polycystic ovary syndrome.
of PI3K/AKT signaling pathway can bidirectionally regulate osteoblast differentiation and osteoclast apoptosis. Dina et al\[39\] and Tao Ling et al\[40\] found that the activation of PTHrP gene in HTLV-I infected patients can affect bone mineral deposition, promote osteolysis and cause osteoporosis. MAPK signaling pathway is an important pathway that regulates the proliferation and differentiation of osteoclasts and osteoblasts, and can also promote the osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs), accelerate calcium deposition, and prevent the occurrence of osteoporosis.\[41\] The high insulin status of PCOS patients leads to abnormal activation of MAPK signaling pathway, which affects the growth and differentiation of ovarian granulosa cells. Xu Jinbang et al\[42\] found that the artificial cycle of acupuncture and drugs can regulate the mitotic activity of ovarian granulosa cells through MAPK signaling pathway, and improve the reproductive endocrine disorders of PCOS patients.

5. Conclusions

In this study, we searched for common targets and pathways of action between polycystic ovary syndrome and osteoporosis through bioinformatics, and expressed the interrelationship between them more clearly through visualization network, which also provides potential targets for drugs to regulate both diseases simultaneously. It is found that insulin resistance, aggravation of glucose metabolism disorder and imbalance of hormone level in polycystic ovary syndrome will affect the proliferation and differentiation of osteoblasts and osteoclasts. Foxo signal pathway, PI3K/AKT signal pathway and JAK2/STAT3 signal pathway all play a direct or indirect role. Of course, with the continuous development of bioinformatics and disease databases, the reliability and accuracy of data will continue to improve, and further studies are needed to make the conclusions more reliable.

Author contributions

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