

# Apoptosis pathways and osteoporosis: An approach to genomic analysis

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## Abstract

**Background:** Osteoporosis is a disease of the bone system that causes a decrease in skeletal density and degrades skeletal tissue. Decreased bone quality, so that bones are easily broken, damaged and fractured, is an important public health problem. Previous studies have shown that the maintenance of adult bone mass is not only due to changes in bone marrow and bone cells. By regulating apoptosis, they change the lifespan of each individual. This study influences understanding of the function of apoptosis in the pathogenesis of osteoporosis and the importance of controlling the mechanisms of osteoporosis.

**Methods:** On the National Institute of Biotechnology Information website, Gene Expression Omnibus (GEO) microarray data and GSE551495 GEO profiles were collected. The gene set enrichment analysis tool was used to confirm the enrichment of genetic sets in relation to the gene set. The collection of C2 gene sets is compiled from the KEGG (<https://www.gsea-msigdb.org/gsea/msigdb/human/search.jsp> and <https://www.kegg.jp/kegg/>) online database and REACTOME (<https://www.gsea-msigdb.org/gsea/msigdb/human/search.jsp> and <https://reactome.org/>) pathway analysis. The Search Tool for the Retrieval of Interaction Genes (STRING) website was used to construct and select proteins and genes. The comparative toxicological genomic database (CTD) tools can be used to predict the relationship between apoptosis, osteoporosis-related genes and interactions between central genes and osteoporosis.

**Results:** These results generally expand our understanding of the path of apoptosis in osteoporosis. We have discovered genes CASP9, CASP8, CASP3, BAX and TP53 associated with osteoporosis. In activation of KEGG apoptosis and REACTOME, caspase activation through the extrinsic apoptotic signaling pathway is characterized by the identification of a subcollection of C2. Other STRINGs show the formation of protein networks and central gene selection, and CTD can accurately predict the relationship between these apoptosis pathways and central genes.

**Conclusions:** Our research has highlighted the importance of the osteoporosis pathway associated with osteoporosis apoptosis with several analytical approaches.

These results have broadened our understanding of the pathways of osteoporosis apoptosis. It is particularly possible to predict the sensitivity and vulnerability to osteoporosis.

#### KEY WORDS

apoptosis pathway, bone, GSEA, osteoporosis

## 1 | BACKGROUND

Osteoporosis (OP) is a disease of the bone system that causes a decrease in skeletal density and degrades the skeletal tissue. Decreased bone quality, so that bones are easily broken or damaged is an important public health problem.<sup>1</sup> Osteoporosis is one of the most common bone diseases in humans and an important public health problem. More than 200 million people live with osteoporosis.<sup>2</sup> According to the National Health and Nutrition Survey from Taiwan, the incidence is 38.3% among women and 23.9% among men.<sup>3</sup>

The cost of treating and preventing further bone loss in Australians with osteoporosis or osteopenia is approximately \$830 million or 30% of this.<sup>4,5</sup> It is indicated that osteoporosis will become a worldwide problem in the future.<sup>6</sup>

According to Pouresmaeili, there are risk factors for diseases involving bone formation pathways and osteoporosis fractures.<sup>7</sup> However, the interaction between lifestyle, environmental factors and genetic factors contributes to the mechanisms of complex public diseases such as osteoporosis.<sup>2,7,8</sup> Therefore, the genetic factor should be considered for early diagnosis as an effective biomarker.<sup>2,7,9</sup>

Emerging information on metabolic bone diseases suggests that the maintenance of adult bone mass is controlled not only by changes in osteoclasts and osteoblasts, but also by alteration of their respective lifespans by the regulation of apoptosis.<sup>9,10</sup> Furthermore, apoptosis of these cell types is evident in bone ageing.<sup>10–12</sup> This study will discuss the role of apoptosis in osteoporosis pathogenesis and the importance of controlling it in osteoporosis treatment and prevention.

## 2 | MATERIALS AND METHODS

### 2.1 | Multiple omics and genetic analysis of humans and animals

Multiple omics and genetic analysis are a complex and multidisciplinary approach that involves the use of high-throughput technologies to generate large amounts of genomic, transcriptomic, proteomic and metabolomic data. These datasets are then subjected to quality control and preprocessing steps, followed by data integration and statistical and bioinformatic analysis to uncover the underlying biological mechanisms driving complex traits and diseases in humans and animals.<sup>1</sup> The process requires expertise in molecular biology, bioinformatics and statistics.

To perform multiple omics and genetic analysis, samples are collected and processed to extract DNA, RNA, proteins or metabolites. The extracted molecules are then quantified and analysed using high-throughput sequencing, microarray or mass spectrometry techniques. The resulting raw data are subjected to quality control and preprocessing steps to remove artefacts, normalize the data and correct for batch effects. Data integration techniques are applied to combine the different omics datasets, followed by statistical and bioinformatic analyses to identify differentially expressed genes, proteins or metabolites, and perform pathway and network analyses.<sup>2</sup>

Multiple omics and genetic analysis is a powerful tool for gaining insight into the molecular basis of disease and developing new therapies and treatments. However, it requires a multidisciplinary approach and collaboration between experts in molecular biology, bioinformatics and statistics.<sup>1–3</sup>

### 2.2 | The collection and processing of microarray data

The data's gene expression profiles related to osteoporosis were collected from the Gene Expression Omnibus (GEO).<sup>13</sup> The database is located on the website of the National Institute of Biotechnology Information. For the key words, “osteoporosis”, “the pathway” and “the single cell” were used. The GEO accession number GSE51495 refers to a dataset titled “Peripheral Blood Mononuclear Cells and Cortical Bone-Derived Transcriptional Profiles (PBMC)” available at <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE51495>. In this experiment, the RNA of a non-human primate (*Papio hamadryas* ssp.) was isolated from a single cell in peripheral blood and cortical bone. Two samples from the same animals and 15 tissue samples were collected for research. Profile data were used to build a network (Supplementary Data S2).

In general, various techniques, including genome-wide association studies, gene expression profiling, and pathway analysis, can be used to analyse genes and pathways related to osteoporosis. Genome-wide association studies compare the genomes of people with and without osteoporosis to find genetic variants related to the condition. To find genes that are differentially expressed, gene expression profiling measures the expression levels of hundreds of genes in bone tissue samples from people with and without osteoporosis. The identification of gene groups that participate in biological pathways related to osteoporosis is carried out by pathway analysis.<sup>3</sup>

However, in this study, a normal control group was sampled. The sample of *P. hamadryas* and its total RNA were taken from cortical bone and peripheral blood mononuclear cells of a monkey model of bone maintenance and turnover. In such circumstances, it is not possible to obtain bone tissue samples from healthy subjects (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE51495>).

Therefore, we used gene set enrichment analysis (GSEA) in this study. GSEA analysis can provide information on the biological pathways and processes that are dysregulated between control and experiment groups, and can also identify potential therapeutic targets for various diseases. However, it is important to note that the GSEA analysis has some limitations, such as the choice of gene sets and the sensitivity to the ranking metric used.<sup>14</sup>

### 2.3 | GSEA of osteoporosis

Related to osteoporosis pathways, the set of genes was investigated using the GSEA tool.<sup>14,15</sup> The enrichment of gene expression was evaluated between samples without osteoporosis and with osteoporosis (series GSE551495).

GSEA is a widely used computational method for identifying differentially expressed gene sets between two groups of samples, such as control and experimental sets. GSEA is based on the principle that genes work together in functional pathways and sets, and the expression of a gene set can be more informative than the expression of individual genes.

To perform GSEA analysis, the first step is to obtain gene expression data from both control and experimental groups. Gene expression data can be obtained using various high-throughput technologies, such as microarrays or RNA sequencing. Next, the gene expression data are normalized and preprocessed to remove batch effects and other technical artefacts.

After preprocessing, GSEA analysis involves three main steps: the selection of a set of genes, the ranking of genes and statistical analysis. Gene set selection involves choosing a set of genes that are involved in a particular biological process or pathway of interest. Gene classification involves classifying all genes according to their expression differences between the control and experimental groups. Finally, statistical analysis involves testing whether the members of a gene set are enriched at the top or the bottom of the ranked gene list using a statistical test such as the Kolmogorov-Smirnov test.

GSEA tools can help to understand the entire genome expression profile.<sup>12</sup> The KEGG (<https://www.gsea-msigdb.org/gsea/msigdb/human/search.jsp> and <https://www.kegg.jp/kegg/>) and REACTOME (<https://www.gsea-msigdb.org/gsea/msigdb/human/search.jsp> and <https://reactome.org/>) enrichment pathways of GSE551495 were identified by GSEA analysis based on expression levels. In addition, GSEA includes three types of data: first, expression data in a limited text menu; second, clinical information or samples in file format; and finally, the gene set or module gene in file format.<sup>16</sup> Analysis was performed using a GSEA analysis tool that detects osteoporosis module genes from profile data from the GSE 551495 series. The parameters

used were that the enrichment statistic was weighted, the metric for ranking genes was Signal2Noise, the maximum size was 500, excluding larger sets, the minimum size was 15, excluding smaller sets gene sets, with a nominal *p*-value of 5% and a false discovery rate of 25%.

Analysis of gene group enrichment confirmed that the gene group was in good condition. Canonical pathways (CP) in the collection of C2 provide essential insights into the interconnected molecular networks and signaling cascades underlying various biological processes. These genes were obtained by installing the GSEA algorithm and GSEA v4.3.2 in Windows software. In addition, MSigDB licenses also specified the specific terms of these gene sets. Further, the collection included gene sets compiled from an online pathway database and biomedical literature from various sources. Each gene set page lists the source of the gene set. The study used several genes to enrich online databases by KEGG (<http://www.pathway.jp>) and REACTOME (<http://www.REACTOME.org>).

### 2.4 | Analysis of the KEGG pathway analysis and the REACTOME pathway

The analysis of KEGG and REACTOME pathways is widely used. In this study, the C2 subcollection was utilized for the CP gene set and the analysis of the REACTOME pathways. The KEGG gene set data (<http://www.gsea-msigdb.org/gsea/msigdb/human/genesets.jsp?collection=CP:KEGG>; [ftp://broadinstitute.org://pub/gsea/gene\\_sets/c2.cp.kegg.v2022.1.Hs.symbols.gmt](ftp://broadinstitute.org://pub/gsea/gene_sets/c2.cp.kegg.v2022.1.Hs.symbols.gmt)) and REACTOME (<http://www.gsea-msigdb.org/gsea/msigdb/human/genesets.jsp?collection=CP:REACTOME>; [ftp://broadinstitute.org://pub/gsea/gene\\_sets/c2.cp.REACTOME.v2022.1.Hs.symbols.gmt](ftp://broadinstitute.org://pub/gsea/gene_sets/c2.cp.REACTOME.v2022.1.Hs.symbols.gmt)) were obtained from the GSEA website and GSEA v4.3.2 for Windows software (<http://www.gsea-msigdb.org/gsea/downloads.jsp>). These pathways were imaged in KEGG (<http://www.pathway.jp>) and REACTOME (<https://REACTOME.org/>).

### 2.5 | Creation of protein networks and the selection of central genes

The Search Tool for the Retrieval of Interaction Genes<sup>17</sup> (STRING; <http://string.embl.de/>) was used. The differentially expressed gene (DEG) criteria for the creation of a protein-protein interaction network (PPI) were 2, the cutoff time of node = 0.2, and the cutoff time = 2.

### 2.6 | Central gene interaction with osteoporosis

Comparative toxicological genomic databases (CTDs) can accurately predict the correlation between drugs, disease and genes (<http://ctdbase.org/>).<sup>18</sup> A common genetic factor was found between apoptosis, CTD genes and predicted genes. The cross-section of apoptosis genes and CTD genes is considered a central gene. Furthermore,

estimated indices and reference numbers have been isolated for central genes associated with CTD osteoporosis. The interactions between central genes and osteoporosis were analysed by CTD.

### 3 | RESULTS

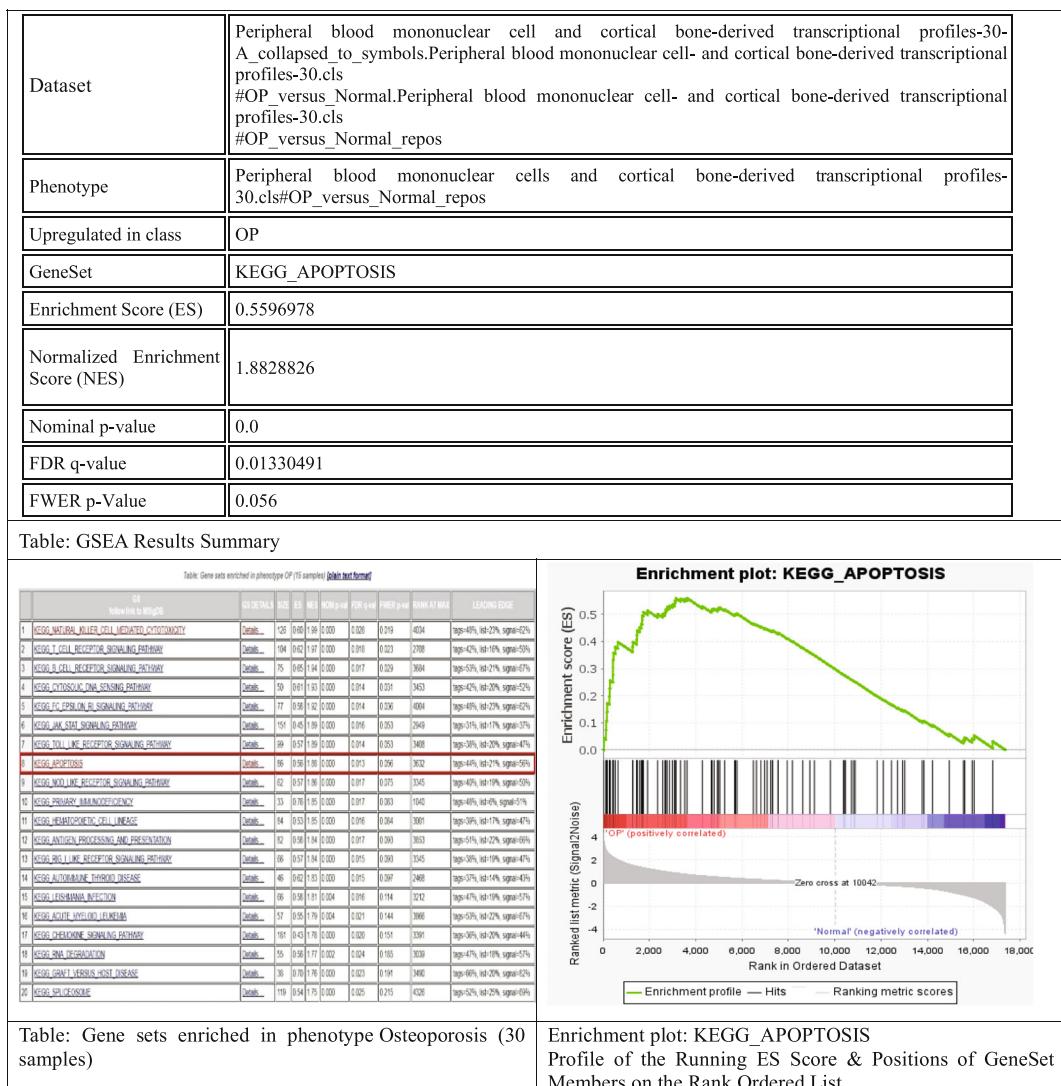
#### 3.1 | The basic characteristics of the sample

This study used a non-human primate model (*P. hamadryas* ssp.) to investigate the relationship between the PBMC transcriptome and the cortical bone transcriptome, with a focus on identifying genes and signaling pathways relevant to osteoporosis pathogenesis. A total of 15 animals were used for the study and both PBMC and cortical bone tissue were collected from each animal.

The sample of this study did not provide detailed information on the demography of the animals used, such as age, sex or weight (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE51495>).

However, given that the animals were used as a model for maintenance and turnover, it is likely that they were selected based on their similarity to humans in these aspects. The study did not specify any inclusion or exclusion criteria for the animals.

In terms of sample size, 15 animals can be considered a relatively small sample size for a study aiming to comprehensively characterize the relationship between the PBMC transcriptome and the bone transcriptome. However, the use of a non-human primate model with a well-established and genetically well-characterized bone maintenance and turnover system may have allowed for a more targeted and efficient approach to the study. In general, although the study did not provide extensive information on the demographic and inclusion/exclusion criteria of the animals used, it used a non-human primate model with a targeted approach to investigate the relationship between PBMC and bone transcriptomes for the identification of genes and signaling pathways relevant to osteoporosis pathogenesis (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE51495>).



**FIGURE 1** Detailed rank-ordered genes and results of the KEGG apoptosis pathway in gene set enrichment analysis (GSEA) analysis.

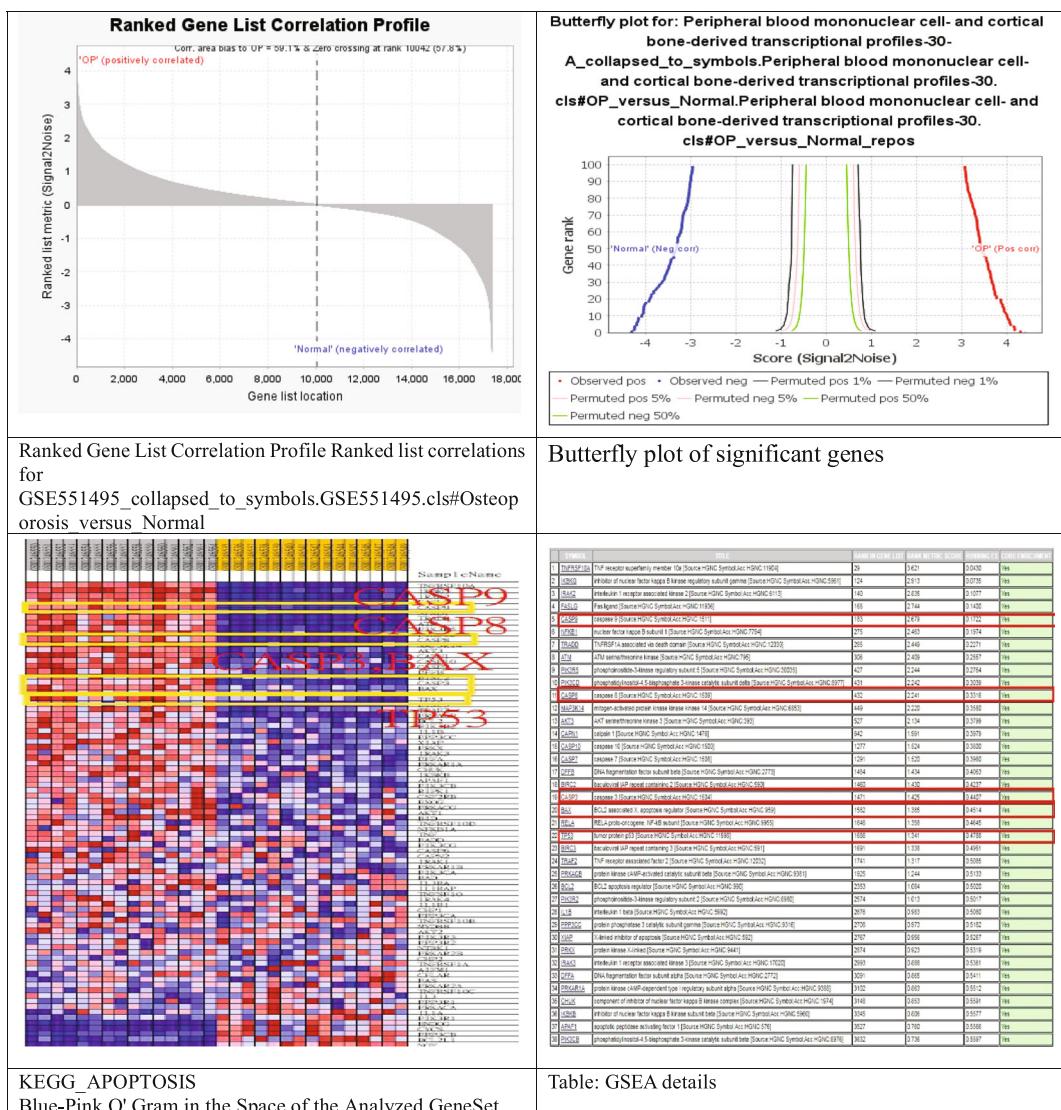


FIGURE 1 (Continued)

### 3.2 | Analysis of KEGG and REACTOME in GSEA using C2 collection in various osteoporosis

Enrichment analysis against gene sets was used with tools<sup>14,19</sup> to identify C2 as the canonical pathway. This article selects the following gene sets from the online database. KEGG and REACTOME used for gene expression were selected between non-osteoporosis and osteoporosis cases (series GSE551495).

In this study, there were gene markers for the OP vs. normal comparison, and the dataset had 17,360 characteristics (genes). Furthermore, with a correlation area of 59.1%, the markers for the OP phenotype made up 10,042 (57.8%) of the total markers. On the other hand, 7318 (42.2%) of the markers had phenotypic associations with the normal phenotype, with a correlation area of 40.9%.

For details of gene sets and signal analysis in the KEGG pathway, the details can refer to the KEGG database and its associated resources. All sets of pathways were curated from the online

databases. The KEGG pathway modules were enriched with sets corresponding to osteoporosis patients. The enrichment value is 0.5596978, the nominal enrichment value is 1.8828826 and the nominal p-value is 0.0.0. Therefore, we chose the KEGG apoptosis pathway (Figure 1 and Table 2). The results of the analysis show a graphical view of the enrichment score of a set of genes. All detailed gene lists are in rank order for all datasets and heat maps, and correlation profiles are provided for all datasets and gene lists (Figure 1 and Table 1) (Supplementary Data S1).

Second, we discuss the details of the gene sets and signals used in the analysis of the REACTOME pathway and the results of REACTOME caspase activation through the extrinsic apoptotic signaling pathway.

For details of the gene sets and signals used in the analysis of the REACTOME pathway, the gene sets of the pathway were curated from the online databases. The sets corresponding to the modules of the REACTOME pathway module were enriched in osteoporosis

**TABLE 1** Gene set enrichment analysis (GSEA) results summary.

Dataset	Peripheral blood mononuclear cells and cortical bone-derived transcriptional profiles 30-A_collapsed_to_symbols.
Phenotype	Peripheral blood mononuclear cells and cortical bone-derived transcriptional profiles-30.cls #OP_versus_Normal.
Upregulated in class	Peripheral blood mononuclear cells and cortical bone-derived transcriptional profiles-30.cls#OP_versus_Normal_repos
GeneSet	KEGG_APOPTOSIS
Enrichment score (ES)	0.5596978
Normalized enrichment score (NES)	1.8828826
Nominal <i>p</i> -value	0.0
False discovery rate (FDR) <i>q</i> -value	0.01330491
FWER <i>p</i> -value	0.056

Abbreviation: FWER, familywise error rate.

patients. The enrichment score (ES) was 0.60537034, the normalized enrichment score (NES) was 1.8757209, and the nominal *p*-value was 0.0. Therefore, we chose REACTOME caspase activation through the extrinsic apoptotic signaling pathway (Figure 2 and Table 2). The results show a graphical representation of the gene enrichment scores. All detailed gene lists for all features in the dataset and heat map and correlation profiles of the list of genes ordered by rank for all features in the dataset are provided in Figure 1 and Table 2 (Supplementary Data S1).

### 3.3 | Creation of protein networks and selection of central genes

The STRING website tool creates PPI and identifies key genes associated with osteoporosis: CASP9, CASP8, CASP3, BAX and TP53 genes (<http://string.embl.de/>; Figure 3; Supplementary Data S1).

The PPI network predicted 86 genes from the location of KEGG apoptosis. Therefore, the PPI analysis occurred in STRING v11.5, and the genes selected for enrichment are shown as red circles.

Additionally CASP9, CASP8, CASP3, BAX, TP53 and ESR1 genes were identified using the CTD website tool (<http://ctdbase.org/>) and identified as central genes that can effectively predict the inference score and the reference count of osteoporosis. The value for CASP9 was 98.29, for CASP8 it was 78.03, for CASP3 it was 110.32, for BAX it was 105.02, for TP53 it was 27.02 and for ESR1 it was 77.49 (Table 3).

## 4 | DISCUSSION

Apoptosis has been revealed over the past three decades as an important biological process in normal physiology and the pathogenesis of various diseases.<sup>10,20,21</sup> Cell death is one of the fundamental processes in the life cycle of a cell. The balance between cell division and cell death is a crucial factor in the maintenance and development of multicellular organisms. Transformed and unregulated apoptosis has pathological consequences and changes in embryonic development, neurodegenerative diseases, cancer and osteoporosis can occur.<sup>10,20,22</sup> In addition, apoptosis is involved in abnormal biological modifications and morphology, including cell destruction, DNA fragmentation and membrane rupture.<sup>12</sup>

Apoptosis is controlled by two different signaling pathways: the first is the death receptor and the second is the Bcl-2 protein. Both pathways activate a proteolytic enzyme called caspase, which cleaves specific substrates and induces the morphology of apoptosis by influencing the morphology of apoptosis.<sup>23</sup> On the other hand, apoptosis includes the caspase-dependent extrinsic pathway, the intrinsic BAX pathway and the physiological functions of p53 in cell cycle arrest and apoptosis.<sup>10,20,21</sup>

In this study, we found two pathways. There was KEGG apoptosis and activation of REACTOME caspase activation via the extrinsic apoptotic signaling pathway.

Firstly, KEGG and GSEA used the KEGG apoptosis pathway to demonstrate that apoptosis is a cell death mechanism controlled by genes that regulate tissue homeostasis. The main pathways for apoptosis are the external pathway (of cells and other members and ligands) and the internal pathway (related to mitochondria) in the cytoplasm. Extrinsic pathways are activated by death receptors and activate signal cascades. Furthermore, caspase-8 is released directly from caspase-3 activation and stimulates mitochondrial chromosome c. Caspase-3 activation causes degradation of the protein needed to live and function in the cell. Internal pathways occur when several apoptotic stimulations cause mitochondrial c-cytochrome (dependent on caspase-8 activation). Cytochrome c interacts with Apo-1 and caspase-9 and promotes the activation of caspase-3. Recently, the endoplasmic reticulum (ER) has been shown to be the third subcellular part of apoptosis. Changes in Ca<sup>2+</sup> homeostasis and accumulation of protein defects in the ER cause stress in the ER. Long-term ER stress could cause caspase-12 and/or Bcl-2-associated death promoter (BAD) activation and apoptosis ([http://www.gsea-msigdb.org/gsea/msigdb/human/geneset/KEGG\\_APOPTOSIS](http://www.gsea-msigdb.org/gsea/msigdb/human/geneset/KEGG_APOPTOSIS); <https://www.kegg.jp/entry/map04210>).

Therefore, in the KEGG apoptosis pathway, we have 86 genes that we selected, including five genes—CASP9, CASP8, CASP3, BAX and TP53—associated with osteoporosis apoptosis and enriched by GSEA analysis.

Second, in REACTOME, caspase activation occurs via the extrinsic apoptotic signaling pathway ([http://www.gsea-msigdb.org/gsea/msigdb/human/geneset/REACTOME\\_CASPASE\\_ACTIVATION\\_VIA\\_EXTRINSIC\\_APOPTOTIC\\_SIGNALLING\\_PATHWAY](http://www.gsea-msigdb.org/gsea/msigdb/human/geneset/REACTOME_CASPASE_ACTIVATION_VIA_EXTRINSIC_APOPTOTIC_SIGNALLING_PATHWAY); <https://www.REACTOME.org/content/detail/R-HSA-5357769>). We have 26 genes that we screened, including CASP9, CASP8 and CASP3, for gene-

Dataset	Peripheral blood mononuclear cell and cortical bone-derived transcriptional profiles-30-A_collapsed_to_symbols.Peripheral blood mononuclear cell- and cortical bone-derived transcriptional profiles-30.cls #OP_versus_Normal.Peripheral blood mononuclear cell- and cortical bone-derived transcriptional profiles-30.cls #OP_versus_Normal_repos
Phenotype	Peripheral blood mononuclear cell and cortical bone-derived transcriptional profiles-30.cls#OP_versus_Normal_repos
Upregulated in class	OP
GeneSet	REACTOME_CASPASE_ACTIVATION_VIA_EXTRINSIC_APOPTOTIC_SIGNALLING_PATHWAY
Enrichment Score (ES)	0.60537034
Normalized Enrichment Score (NES)	1.8757209
Nominal p-value	0.0
FDR q-value	0.036123637
FWER p-Value	0.237

Table: GSEA Results Summary

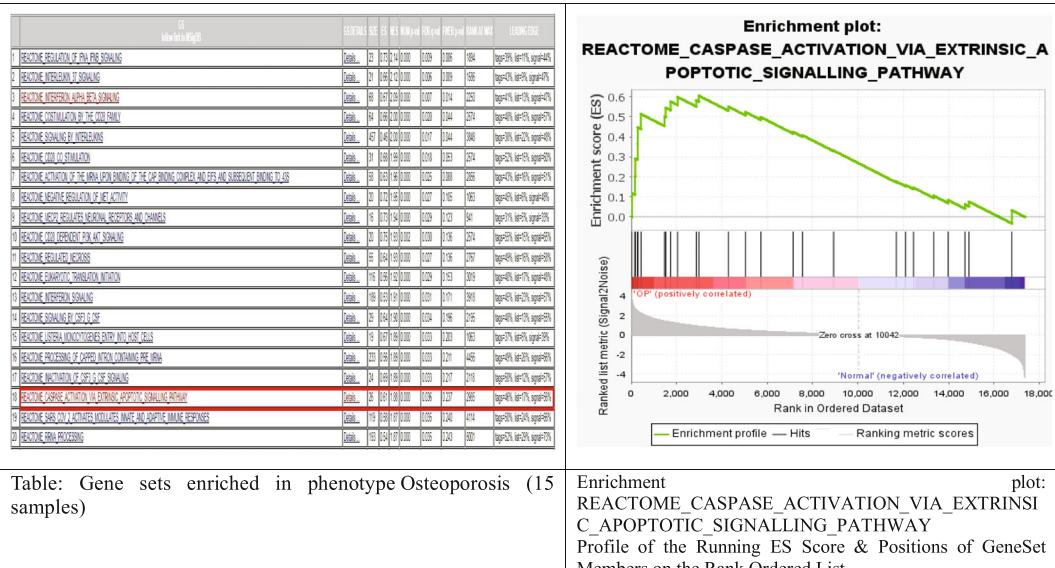


Table: Gene sets enriched in phenotype Osteoporosis (15 samples)

Enrichment plot: REACTOME\_CASPASE\_ACTIVATION\_VIA\_EXTRINSIC\_APOPTOTIC\_SIGNALLING\_PATHWAY  
Profile of the Running ES Score & Positions of GeneSet Members on the Rank Ordered List

**FIGURE 2** Detailed rank-ordered genes and results of REACTOME caspase activation through the extrinsic apoptotic signaling pathway in GSEA analysis.

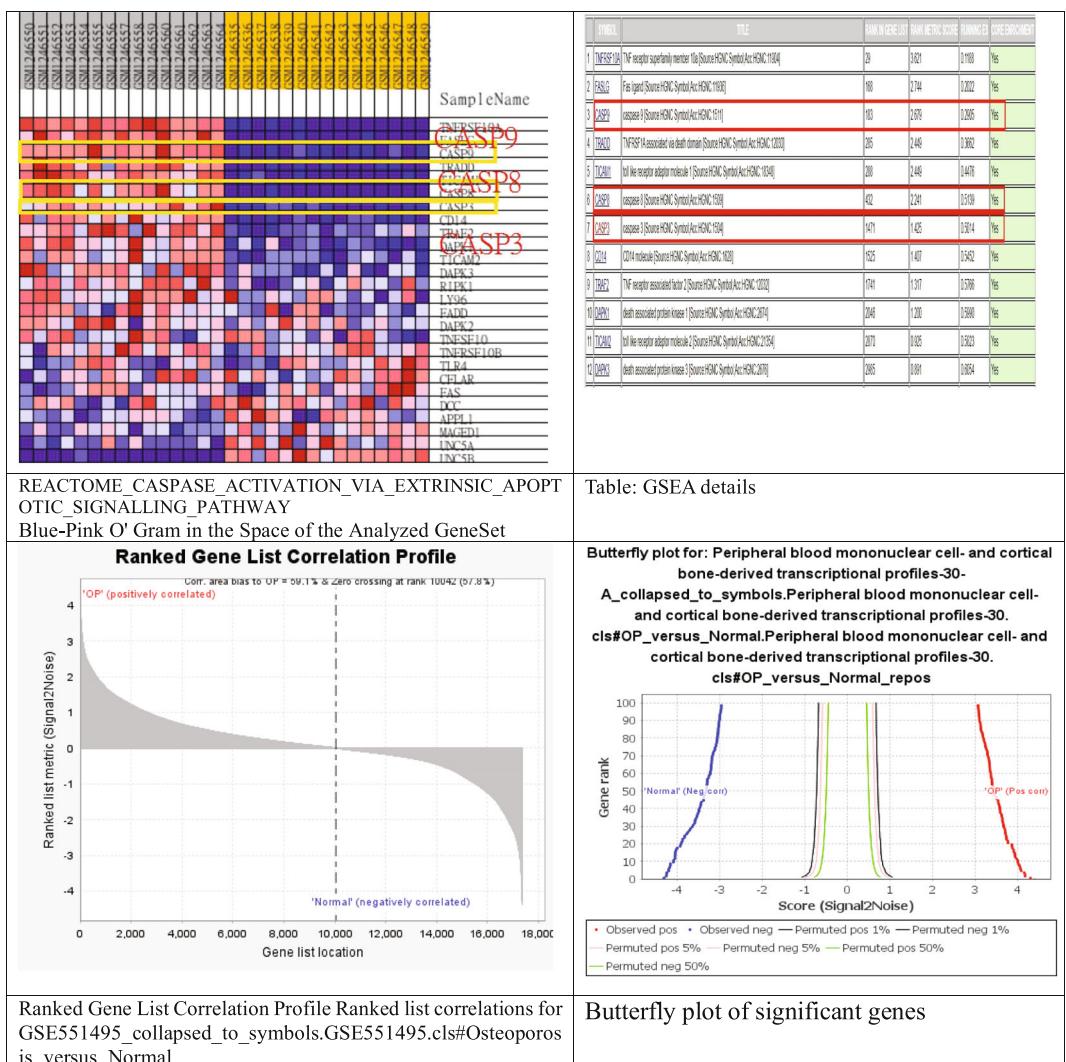
related apoptosis of osteoporosis because they are enriched in the results of GSEA analysis.

Rendina et al. used the results of the study in hard tissues (that is, red femurs) and used caspas-9, a basic protein involved in apoptosis. Its downstream target is caspase-3 (casp3), but ovariectomy (OVX) negatively regulates gene expression and diet therapy changes this response. These results provide some evidence of dry plumes in the current study, and there is a discrepancy between Casp9 and Casp3, the subsequent markers of apoptosis. In mineralized bone and bone marrow, the regulation of apoptosis transcription may be different.<sup>24</sup>

Furthermore, caspases-8 is the main initiator of caspases in the death receptor. Active caspas-8 binds to several cell proteins, including procaspas-3, and activates and completes cell death programmes.<sup>25</sup>

Yadav et al. observed the activation of extrinsic and intrinsic mitochondrial apoptotic pathways suggested by caspas-8, -9 and -3, and BH3-interacting domain death agonist (Bid) activation. All of this is accompanied by a reduction in the regulation of Bcl-xL antiapoptotic proteins and an increase in the regulation of Bak pro-apoptotic proteins. In addition to down-regulation of BAX expression and the promotion of activation, the expression of CASP3 and CASP9 can lead to apoptosis.<sup>26</sup>

The results demonstrated that MLO-Y4 and irisin, when utilized in a bone model, effectively controlled caspase-9 and caspase-3, leading to the inhibition of apoptosis.<sup>27,28</sup> This is interesting because osteoclasts are more susceptible to apoptosis near microdamage sites. They are also associated with an increase in apoptotic biomarkers in the expression (caspase-3, BAX).<sup>27,29</sup>

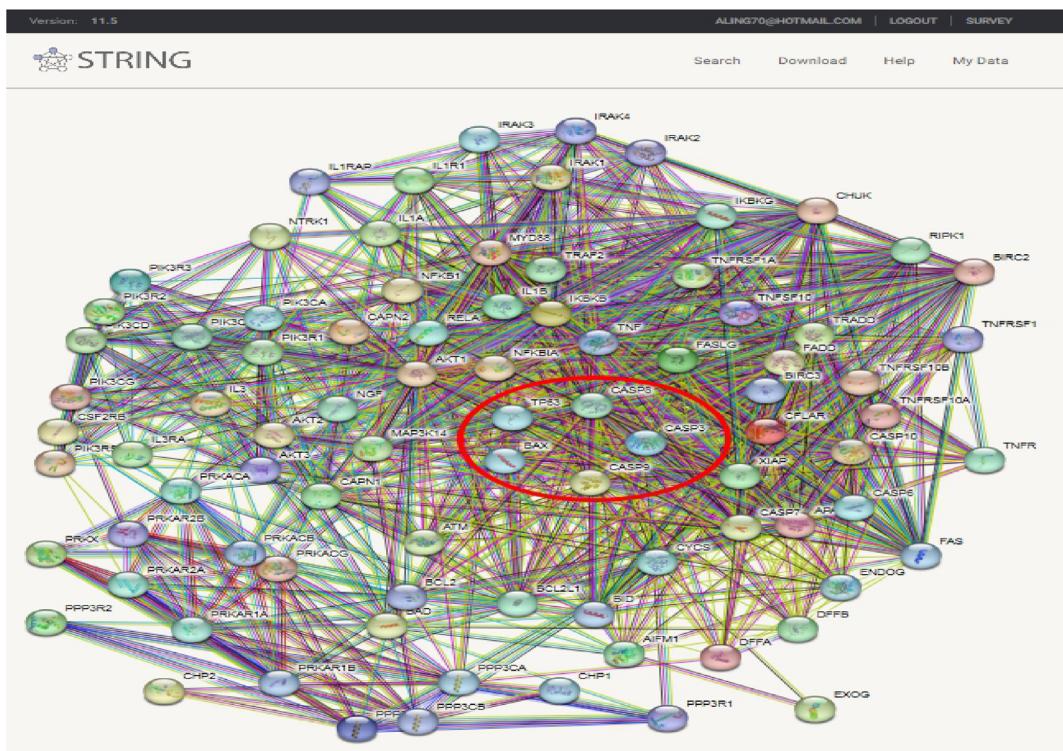


**FIGURE 2** (Continued)

**TABLE 2** GSEA results summary.

Phenotype	Peripheral blood mononuclear cells and cortical bone-derived transcriptional profiles-30.cls#OP_versus_Normal_repos
Upregulated in class	OP
GeneSet	REACTOME_CASPASE_ACTIVATION_VIA_EXTRINSIC_APOPTOTIC_SIGNALLING_PATHWAY
ES	0.60537034
NES	1.8757209
Nominal <i>p</i> -value	0.0
FDR <i>q</i> -value	0.036123637
FWER <i>p</i> -value	0.237

Abbreviation: FWER, familywise error rate.



**FIGURE 3** Creation of protein networks and selection of central genes.

**TABLE 3** The comparative toxicological genomic database (CTD) of osteoporosis-associated osteoporosis.

Disease	Gene symbol	Inference score	Reference count
Osteoporosis	CASP9	98.29	60
Osteoporosis	CASP8	78.03	43
Osteoporosis	CASP3	110.32	83
Osteoporosis	BAX	105.02	65
Osteoporosis	TP53	27.02	21
Osteoporosis	ESR1	77.49	49

However, *in vitro* experiments have shown that tomatidine inhibits p53 expression and plays a biological role in reducing apoptosis.<sup>30</sup> Furthermore, p53 regulates the start of the cell cycle as a transcription factor.<sup>30,31</sup> Furthermore, p53 proteins can receive multiple signals and respond. The determination of cell division depends on the protein. When cells are irreversibly damaged, p53 participates in the initialization process and causes apoptosis.<sup>30,32</sup> Furthermore, Br regulates p53 and plays a role in osteoblast differentiation.<sup>30,33</sup> In conclusion, the p53 protein has been found to be essential for osteoblast death, indicating its necessity in bone marrow death.<sup>30,34</sup>

Lastly, mention must be made of the apoptosis pathway affected by the ESR1 gene in the estrogen receptor signaling pathway. Although not found in GSEA, in experiments the summary of GSE 55149 showed that the estrogen receptor signaling pathways were highly expressed in PBMC.

In bone, a lack of estrogen is associated with rapid osteoblast apoptosis and sensitivity to osteoporosis fractures. The study suggested that the basis of the fight against osteoporosis may be the prevention of osteoblast apoptosis. The main events of the process are suppressing the expression of the apoptosis gene and suppressing caspase-3/7.<sup>22</sup> Consequently, estrogen protection effects can be associated with preventing apoptosis and programmed cell death in osteoblasts that form bone.<sup>8,22</sup> It is interesting to note that estrogen suppresses the transcription of ER1 in the ITPR1 gene. The channel of calcium release in the cell encodes the key regulator of early apoptosis.<sup>22</sup>

Summarizing the experiments in GSE 55149, it was determined that there is a high degree of overlap between cortical bone genetic expression and PBMC, and genes in the signaling pathways of OP-related ranked osteoclastic and estrogen receptors are highly

expressed. (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE51495>). However, we did not get these pathways in the GSEA analysis.

According to the study, people with *ESR1* rs2982573 are less likely to develop osteoporosis when they consume no less than three cups of coffee a week, according to the Taiwan Biobank.<sup>35</sup> In fact, the estrogen signal is exerted through *ESR1*. Conditional mice induced osteoclast apoptosis and induced bone formation by suppressing bone resorption in *ESR1* knock-out mice.<sup>8</sup> Furthermore, an important mechanism of the estrogen effect on the association of osteoblasts with osteoblasts is the regulation of longevity by inhibiting cell apoptosis. Through apoptosis inhibitors, estrogen can prolong the lifetime of osteoblasts, allowing bone formation to follow bone absorption.<sup>22</sup> In previous studies in isolated bone marrow, compared with healthy young women, postmenopausal bone samples showed higher levels of inflammatory receptors than cell death caused by the Fas ligand.<sup>22,23,36</sup>

Furthermore, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and flow cytometric analyses have shown that miR-22 suppresses cell survival and promotes cell apoptosis. Specific bone marrow proteins, alkaline phosphatase, and in MC3T3E1, miR-22, alizarin red, were found to be expressed, and these proteins reduced osteogenic development. Furthermore, Luciferase reporter assays and *in silico* analysis were used to identify connections directly between MiR-22 and its potential targets.<sup>37</sup> Previous studies have shown that in mouse models, the OVX C57BL/6J cascade + osteoblasts are more abundant, mainly in the posterior cortex of the long bone axes. This shows that apoptosis caused by estrogen deficiency in the osteocyte can be detected locally in the cortex. It is also necessary to activate endocortical reconstruction after loss by apoptosis. All of the results indicate that a lack of estrogen causes osteocyte apoptosis and bone loss.<sup>27,38</sup>

Previous studies investigating molecular mechanisms caused by western diffusion have shown that ER-transfected cells increase the expression of activated caspase 3. Furthermore, the ER-SP1 complex is closely bound to the nearest and furthest point, affecting the expression of activated caspase 3 and dependent on the ligand.<sup>39</sup>

In conclusion, based on our research, we have found that the osteoporosis path and the associated apoptosis path are important for diagnosing osteoporosis. We have used several analytical methods to study these pathways, which has broadened our understanding of how they are related to osteoporosis. However, our study has some limitations that need to be addressed in future research.

First, we used a sample of *P. hamadryas* in PBMCs and cortical bone, which may not be representative of human osteoporosis. Therefore, further studies using human samples are needed to confirm our findings. Furthermore, while our study provides important information on osteoporosis apoptosis, more *in vitro* and *in vivo* research is needed to establish the link between osteoporosis and these pathways.

Furthermore, our study only focused on the diagnosis of osteoporosis and did not predict the risk of developing the disease or vulnerability to it. Therefore, future research should investigate these aspects to better understand how to prevent and treat osteoporosis.

The conclusion of our research highlights the importance of the osteoporosis path and associated apoptosis path for diagnosing

osteoporosis. However, more research is needed to confirm our findings to better understand the risk of developing osteoporosis and vulnerability to the disease.

## AUTHOR CONTRIBUTIONS

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## CONFLICT OF INTEREST STATEMENT

The author states that there is no potential conflict of interest.

## DATA AVAILABILITY STATEMENT

The authors claim that all data describing the results of the study can be found in the text of the article or in *Supporting information* documents. The GEO Profile dataset is the GSE51495 series.

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## SUPPORTING INFORMATION

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