Original Article

DOI: 10.5582/irdr.2022.01043

Comprehensive bioinformatics analysis of susceptibility genes for developmental dysplasia of the hip

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SUMMARY Developmental dysplasia of the hip (DDH) is a multifactorial disease, which occurs under environmental and genetic influence. The etiopathogenesis of DDH has not been fully explained. As research progresses, many candidate genes have been found to be closely related to the occurrence of DDH. In this study, we comprehensively examined 16 susceptibility genes of DDH using bioinformatics. *COL1A1* encodes the pro-alpha1 chains of type I collagen, which is the major protein component of the bone extracellular matrix (ECM). The genes displaying the most statistically significant co-expression link to *COL1A1* are *ASPN*, *TGFB1*, *DKK1*, *IL-6*, *TENM3* and *GDF5*. DKK1, FRZB and WISP3 are components of the Wnt signaling pathway. CX3CR1 and GDF5 regulate chondrogenesis through the canonical Wnt signaling pathway. ASPN could induce collagen mineralization through binding with collagen and calcium. Integrated bioinformatics analysis indicates that ECM, Wnt signaling pathway and TGF-β signaling pathway are involved in the occurrence of DDH. These provide a basis for further exploring the pathogenesis of DDH.

Keywords developmental dysplasia of the hip, bioinformatics, protein-protein interaction, susceptibility gene, Wnt signaling pathway

1. Introduction

Developmental dysplasia of the hip (DDH), also known as congenital hip dislocation or congenital hip dysplasia, is one of the most frequent skeletal anomalies in newborns (*1*). It is characterized by laxity of the joint capsule caused by mild or incomplete formation of the acetabulum, secondary deformity of the proximal femur and complete luxation (*2*). Although early screening and treatment can help DDH children recover better, there are still many with residual malformations, such as re-dislocation, femoral head necrosis, and residual acetabular dysplasia, which may then develop into adult osteoarthritis, and often requires joint replacement. The whole treatment cycle is long and brings a huge burden to the family. How to fundamentally prevent the occurrence of DDH is an urgent clinical issue to be solved. Hence, it is of great importance to explore the etiology and pathogenesis of DDH.

However, the multifactorial etiology and pathogenesis of DDH have not yet been sufficiently clarified. Many studies have shown that genetic, environmental, and mechanical factors play an important role in the occurrence of DDH (*3*). The theory of the autosomal dominant mode with incomplete penetrance is popular. So the genetic factors occupy an important position in the pathogenesis of DDH (*4*). Genes involved in osteogenesis and chondrogenesis and genes associated with the formation of joint structures and connective tissue contribute to the occurrence of this disorder (*2*).

To date, 16 genes with the highest correlation of DDH in different populations have been reported. These include *ASPN, BMS1, CX3CR1, COL1A1, DKK1, FRZB, GDF5, HOXB9, HOXD9, IL-6, PAPPA2, TBX4, TENM3, TGFB1, UQCC1*, and *WISP3(CCN6)* (*3,4*). Changes in some genes, such as *DKK1, WISP3, HOX, UQCC1, TENM3, CX3CR1, PAPPA2* and *FRZB*, directly lead to abnormal formation of fibrous, bone, and cartilage tissue (*5-13*). Abnormal interactions of IL-6 and TGFB1 also produce the same result (*14*). The *COL1A1* gene encodes

the alpha1 chain of collagen, which is the structural component of cartilage. The promoter variations (rs113647555) in *COL1A1* affect joint laxity (*15*). A positive correlation between *GDF5* polymorphisms and DDH has been demonstrated (*16*). TBX4 and ASPN also act as key regulators that affect the number of fibroblasts in tendons and fascia, resulting in relaxation around the hip joint and increasing the risk of dislocation (*17,18*). BMS1 (rs201298233) indirectly affects bone resorption and mineral density by participating in a large protein-protein interaction (PPI) network (*19*). Bioinformatics was used in this study to examine the relationship of 16 reported DDH susceptibility genes, with the expectation of gaining insight into the possible molecular mechanisms of DDH.

2. Materials and Methods

2.1. Phylogenetic analysis and visualization of gene structures

Sequences of *ASPN, BMS1, CX3CR1, COL1A1, DKK1, FRZB, GDF5, HOXB9, HOXD9, IL-6, PAPPA2, TBX4, TENM3, TGFB1, UQCC1*, and *WISP3(CCN6)* in Fasta format as well as their encoding protein sequences were derived from the NCBI database (*https://www.ncbi.nlm.nih.gov/*). The visualization to truly show the location of these 16 genes on the chromosome was performed by the "gene on genome from Fasta" tool of TBtools software. Multiple alignment of their protein sequences was performed using CLUSTAL 2.0 software. A phylogenetic tree was constructed through Molecular Evolutionary Genetic Analysis (MEGA) software. Motif detection of these 16 protein sequences was performed using the MEME tool (*https://meme-suite.org/meme/index.html*), with the number of motifs equal to 15 and classic mode parameters setting (*20*). The obtained motif mining results and gene structure in Fasta format were visualized in "amazing optional gene viewer" of TBtools software.

2.2. Prediction of coexisting proteins and PPI networks

The STRING (*https://cn.string-db.org/*) and the GeneMANIA (*https://genemania.org/*) online tools were used to analyze the interactions of the 16 proteins coded by DDH susceptibility genes. The STRING website was used to obtain the available protein association networks by using the query of Multiple Proteins by names and organism ("Homo sapiens"). The interaction relationship between these 16 proteins was obtained by setting the following parameters: meaning of network edge was set as evidence, text-mining, experiments, databases, co-expression, neighborhood, gene fusion and co-occurrence were all selected as active interaction sources, with a medium confidence value of 0.4 (*21*). In the GeneMANIA online tool, the types of interactions were revealed by choosing the organism "Homo sapiens", and co-expression, co-localization, physical interactions, shared protein domains and pathway were set.

2.3. Expressive tightness analysis of genes

Correlation expression analysis of DDH susceptibility genes was conducted by MEM-Multi Experiment Matrix (*https://biit.cs.ut.ee/mem/index.cgi*) to obtain the experimental research expression matrix of 16 genes (*22,23*). Genes were entered into the text field, A-AFFY-44 collection was chosen and *COL1A1* was used as the reference gene. Other procedures included setting 0.29 as StDev threshold for query gene, choosing StDev as dataset weight, and using 100 as the number of most variant datasets.

2.4. Enrichment analysis of related genes

To explore interacting proteins for the above 16 different proteins, STRING was used. Experiment-based interacting proteins were acquired by setting the parameters as follows: meaning of network edges was set as evidence, active interaction sources were experiment-based only, high confidence value of 0.150, and no more than 50 interactors in 1st shell. As above, GeneMANIA was conducted to obtain interacting proteins for these 16 target proteins. Meanwhile, "Similar Gene Detection" module of GEPIA2 (*http://gepia2.cancer-pku.cn/#index*) was adopted to gain the top 20 correlated genes for these 16 queries (*24*). Interacted proteins predicted from STRING, GeneMANIA and GEPIA2 were compared by Venn analysis (*http://bioinformatics.psb.ugent.be/webtools/Venn/*).

By combing the above two sets of data, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was conducted using Database for Annotation, Visualization, and Integrated Discovery (DAVID) (*https://david.ncifcrf.gov/*) online tools, then visualized with "Cairo" (*https://cran.r-project.org/web/packages/Cairo/index.html*), "stringr" (*https://cran.r-project.org/web/packages/stringr/index.html*), and "ggplot2" (*https://cran.r-project.org/web/packages/ggplot2/index.html*) R packages. Gene Ontology (GO) enrichment of biological process (BP), cellular component (CC), and molecular function (MF) were visualized by "clusterProfiler" R package (*http://www.bioconductor.org/packages/release/bioc/html/clusterProfiler.html*). $P < 0.01$ was set as the statistical significance threshold value.

2.5. Genetic alteration analysis

For the analysis of alteration in *ASPN, BMS1, CX3CR1, COL1A1, DKK1, FRZB, GDF5, HOXB9, HOXD9, IL-6, PAPPA2, TBX4, TENM3, TGFB1, UQCC1*, and *WISP3(CCN6)*, the cBioPortal (*https://www.cbioportal.*

org/) browser was selected in "TCGA Pan Cancer Atlas Studies" module. The frequency and characteristics of three different types of alteration including mutated gene, amplification and copy number alteration (CNA) were analyzed in all tumors recorded by TCGA databases (*25,26*). The corresponding mutation sites of *PAPPA2* and *TENM3* were conducted through "mutations" module.

3. Results

3.1. Phylogenetic analysis and visualization of gene structures

The locations of DDH susceptibility genes are scattered and spread over 11 chromosomes. There are no collinear genes. *HOXD9* and *FRZB* are located on chromosome 2. *BMS1* and *DKK1* are located on chromosome 10. *HOXB9, COL1A1* and *TBX4* are located on chromosome 17. *GDF5* and *UQCC1* are located on chromosome 20. The other seven genes are located on chromosomes 1, 3, 4, 6, 7, 9, and 19, respectively. It is worth noting that *GDF5* and *UQCC1* are relatively close (Figure 1A). A previous study has shown that abnormal bone growth and development in humans is associated with common variants in the *GDF5-UQCC* region (27).

The motif structures of 16 proteins are quite different, which reflects the complexity of DDH at the protein macromolecule level. Pathogenically, HOXB9 and HOXD9, which belong to the same family, are structurally similar, which is also consistent with the gene structure results (Figure 1B). In addition to the gene structures and phylogenetic tree, we also compared the positions and numbers of exons and introns of 16 genes (Figure 1C). The results showed that there is a diversity of structures for DDH susceptibility genes, among which *TENM3* is the largest, *PAPPA2* is second, *DKK1* is the smallest, and there is no good evolutionary relationship among these genes.

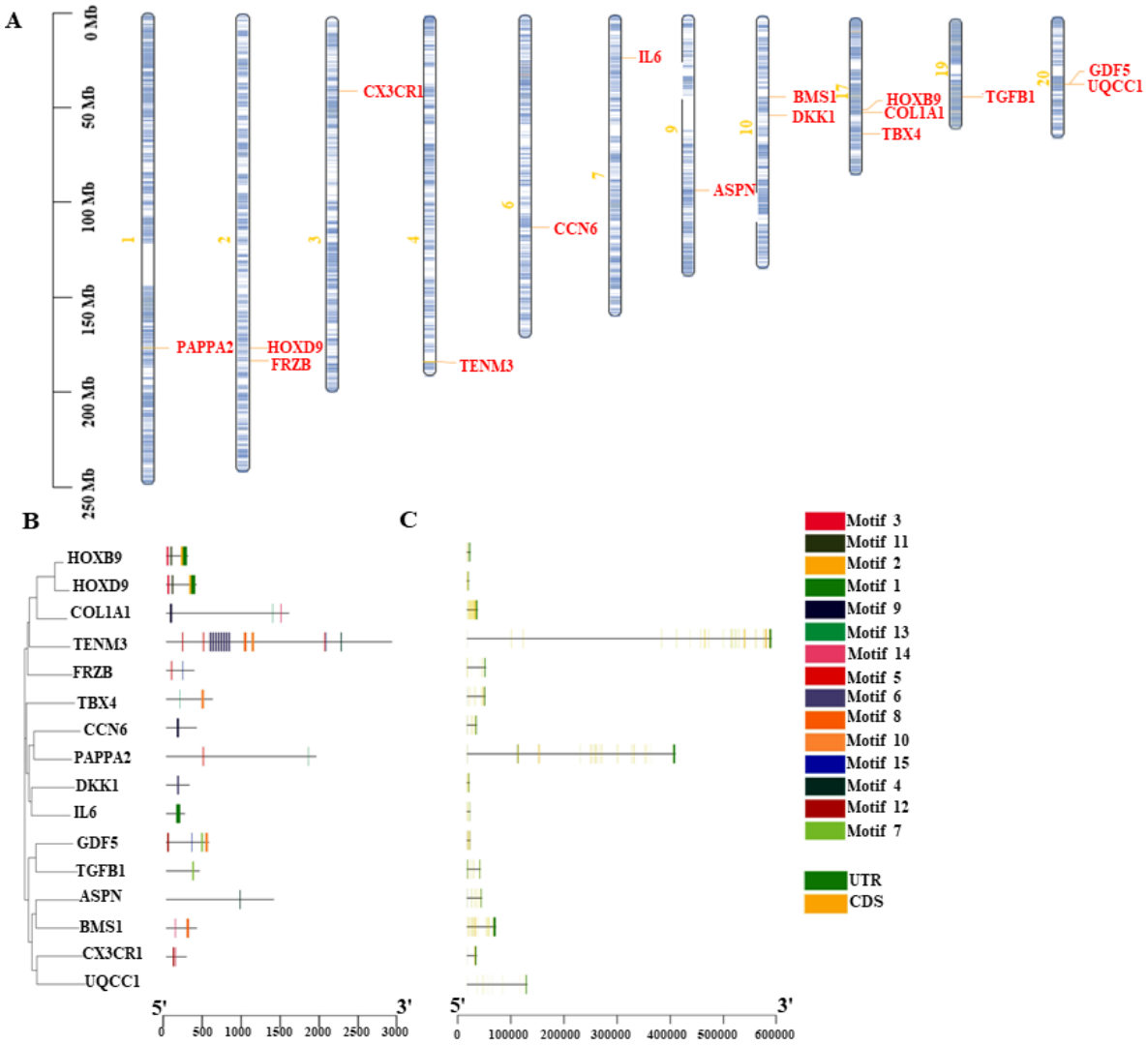

Figure 1. Phylogenetic analysis and visualization of chromosomal location and structures of 16 DDH susceptibility genes. (A) Chromosome location; **(B)** Phylogenetic analysis; **(C)** Gene structure.

3.2. Prediction of coexisting proteins and PPI networks

PPI analysis conducted by STRING indicated that co-expression is the most common among all interactions of 16 analyzed proteins, and it is worth noting that *COL1A1* encoding protein has a co-expression relationship with four proteins, namely ASPN, GDF5, DKK1, and IL6 (Supplementary Table S1, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=98*, Figure 2A). The highest score between *COL1A1* encoding protein and ASPN protein was 0.38. The prediction results of the GeneMANIA database showed that these 16 proteins were associated with TSR1, DKK2, DKK3, RSPO1, TENM2, DKK4, GTF3A, ITGA11, TENM1, KREMEN2, TENM4, IL17A, MED12, HOXC9, HOXA9, PAPPA, COL1A2, FZD8, CSF3 and VEGFD, a total of 20 proteins (Table 1, Figure 2B). It is worth noting that TGFB1 and GDF5 share common domains. TGFB1 and GDF5 are members of the TGF-β superfamily, and both act as important regulators in bone and cartilage formation in DDH-related pathways (*3*).

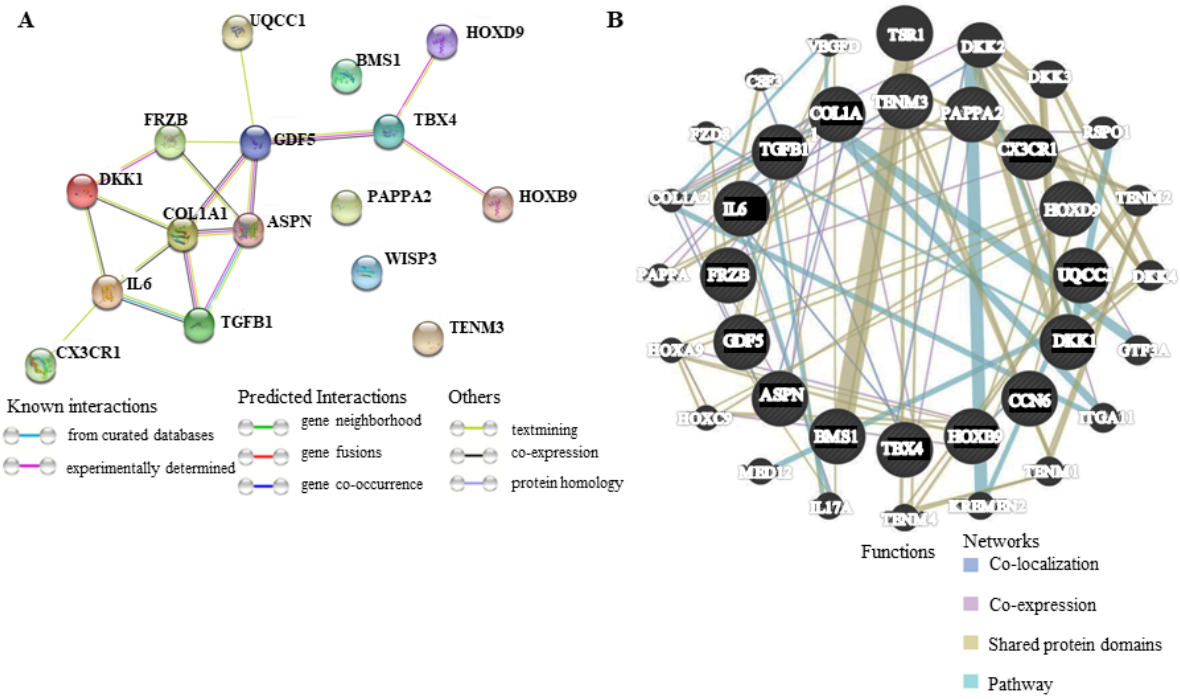

Figure 2. Predicted protein-protein interaction. (A) The interaction networks between 16 DDH susceptibility genes; **(B)** Protein interaction networks between susceptibility genes and 20 related genes.

Table 1. Top 20 encoding genes of interacted proteins indicated by GeneMANIA

Gene	Description	HGNC	Rank
TSR1	TSR1 ribosome maturation factor	25542	1
DKK2	dickkopf WNT signaling pathway inhibitor 2	2892	2
DKK3	dickkopf WNT signaling pathway inhibitor 3	2893	3
RSPO1	R-spondin 1	21679	4
TENM2	teneurin transmembrane protein 2	29943	5
DKK4	dickkopf WNT signaling pathway inhibitor 4	2894	6
GTF3A	general transcription factor IIIA	4662	7
ITGA11	integrin subunit alpha 11	6136	8
TENM1	teneurin transmembrane protein 1	8117	9
KREMEN2	kringle containing transmembrane protein 2	18797	10
TENM4	teneurin transmembrane protein 4	29945	11
IL17A	interleukin 17A	5981	12
MED12	mediator complex subunit 12	11957	13
HOXC9	homeobox C9	5130	14
HOXA9	homeobox A9	5109	15
PAPPA	pappalysin 1	8602	16
COL1A2	collagen type I alpha 2 chain	2198	17
FZD8	frizzled class receptor 8	4046	18
CSF3	colony stimulating factor 3	2438	19
VEGFD	vascular endothelial growth factor D	3708	20

3.3. Expressive tightness analysis of genes

The correlation matrix for expression data of the 16 genes was obtained from the MEM-Multi Experiment Matrix open database. We used *COL1A1* as the reference standard. The results showed that there were higher expression densities between *COL1A1* and 6 genes: *ASPN*_(219087_at), *TGFB1*_(203085_s_at), *DKK1*_(204602_at), *IL-6*_(205207_at), *TENM3*_(219523_s_at) and *GDF5*_(206614_at). The scores were 1.3E-34, 1.49E-25, 2.57E-24, 6.67E-22, 1.31E-17 and 7.61E-12, respectively (Figure 3). It indicates that *ASPN, TGFB1, DKK1, IL-6, TENM3, GDF5* and *COL1A1* were more closely expressed in the corresponding experimental projects. Although the expression of other genes was correlated, the expression affinity was not significant.

3.4. Enrichment analysis of related genes

To ensure the reliable protein-protein interaction predication, experiment-based interacting proteins for the 16 DDH related proteins were analyzed by STRING and GeneMANIA (Figure 4A, Supplementary table S2, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=99*). Correlated proteins for the 16 proteins were predicted by GEPIA2 (Supplementary table S2, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=99*). Venn analysis demonstrated that three proteins, including CSF3, RSPO1 and COL1A2, were predicted by both GEPIA2 and GeneMANIA. LTBP1 and IL6ST were identified from the intersection analysis of STRING and GEPIA2 (Figure 4B).

KEGG pathway enrichment analysis suggested that the analyzed DDH susceptibility genes were mainly enriched in Wnt, TNF and TGF-β signaling pathways, signaling pathways regulating pluripotency of stem cells, ribosome biogenesis, regulation of actin cytoskeleton, focal adhesion, ECM-receptor interaction, and so on. Most notably, genes enriched in ribosome biogenesis in eukaryotes were greater than 20 and -log10 (*p*-value) greater than 12 (Figure 4C).

GO analysis demonstrated the enriched biological process, which included ncRNA processing, ribonucleoprotein complex biogenesis, ribosome biogenesis, rRNA metabolic process, rRNA processing, and so on (Figure 4D). Cellular components were enriched in 90s preribosome, collagen-containing extracellular matrix, collagen trimer, preribosome, small-subunit processome (Figure 4E). Molecular functions were mainly enriched in extracellular matrix structural constituent, glycosaminoglycan binding, growth factor binding, snoRNA binding, and transforming growth factor β-activated receptor activity (Figure 4F). Notably, the main function of BMS1 is related to eukaryotic ribosome biosynthesis. At present, there are few studies on the correlation between BMS1 and DDH. One study has shown that variants of the *BMS1* gene are associated with alterations in bone resorption and mineral density (*19*).

3.5. Genetic alteration for genes

Prevalence and characteristics of genetic alteration of *ASPN, BMS1, CX3CR1, COL1A1, DKK1, FRZB, GDF5, HOXB9, HOXD9, IL-6, PAPPA2, TBX4, TENM3, TGFB1, UQCC1*, and *WISP3(CCN6)* in 33 types of cancer in TCGA database were acquired. A total of 10,967 samples originating from 10,953 patients were tested for five different types of genetic alteration, including mutations, fusions, amplifications, deep deletions, and multiple alteration. Mutation was the predominant type in most tumors as indicated (Figure 5A). After observing the mutations of every gene, it was found that the highest mutation of 25.9% for *PAPPA2* was identified in melanoma (Figure 5B and 5C). The mutation frequency of *TENM3* in melanoma was as high as 24.8% (Figure 5D and 5E). In addition, the KEGG enrichment results also revealed that the related genes of these 16 genes are highly involved in cancer pathways. We found that the change of arginine to leucine or histidine at position 324 of FRZB was identified in esophageal adenocarcinoma, endometrioid carcinoma and lung adenocarcinoma. A variant of *FRZB* (rs7775), with a cysteine replacement at position 324, was reported in DDH (*13*). Polymorphism at the same locus leads to the different clinical symptoms of the disease. Similarly, glutamine to lysine change at position 56 was identified in skin melanoma. Polymorphism of *CCN6* (rs1230345), resulting in a glutamine to histidine change, was associated with DDH development (*6*). Notably, fusion mutations of *UQCC1* and *GDF5* lead to the development of lung squamous cell carcinoma. Mutations in *GDF5* affect transcriptional processes that ultimately affect joint angles to exacerbate DDH progression (*27*).

4. Discussion

Mild acetabular dysplasia or severe hip dislocation during infancy and early childhood development is defined as DDH. DDH could cause notable pain and osteoarthritis by early adulthood (*28*). It is associated with a variety of risk factors, such as female gender, intrauterine breech, and positive family history (*29*). Postural is one of the risk factors. About 2% to 3% of normal newborns are breech births, but breech birth rate in children with DDH is as high as 16% (*30*). One in 35 breech-birth girls are DDH patients (*31*). DDH is more likely to occur in newborns wrapped in knee and hip extension position. On the contrary, if hip abduction flexion is kept, the incidence is lower (*32*). In DDH rabbit model, the thickening of acetabular cartilage in young rabbits and fibrosis in adult rabbits were found. The expression of integrin β, type I collagen and type II collagen were changed in the process of cartilage thickening and

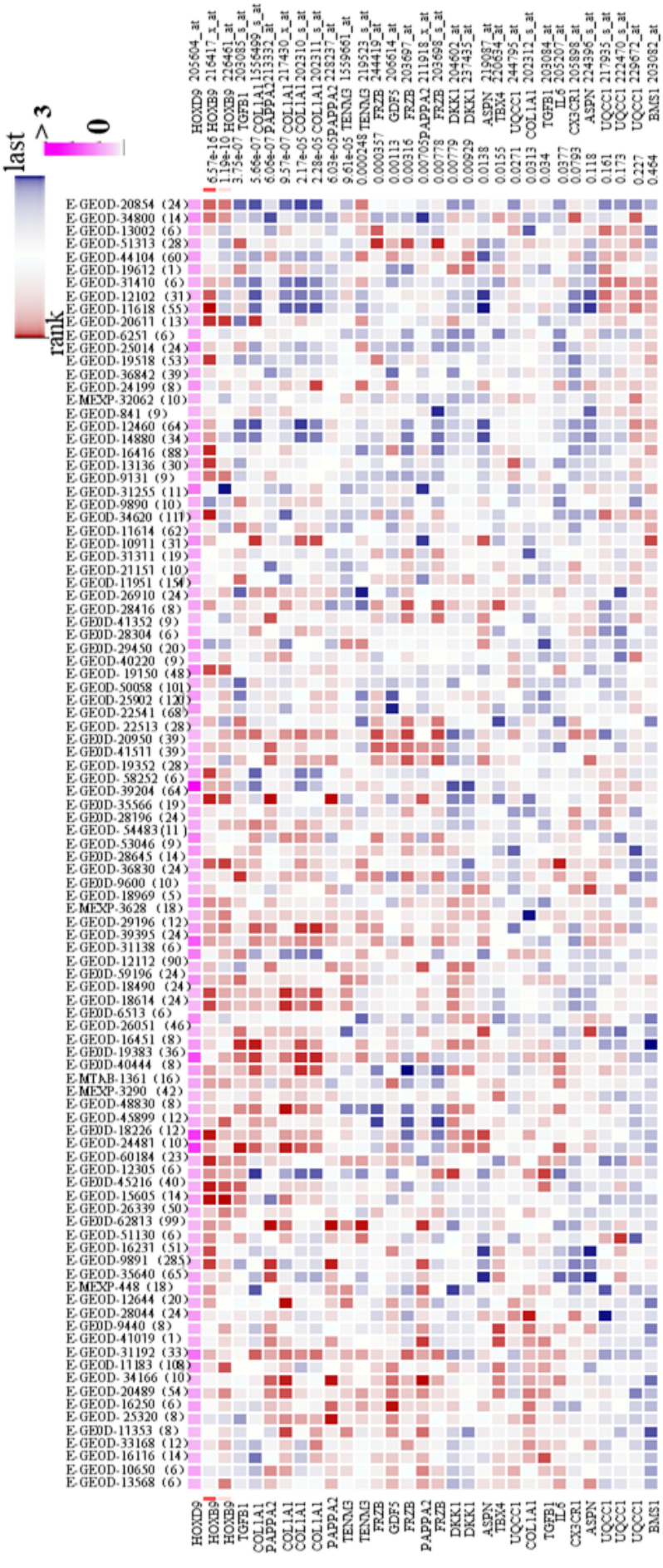

Figure 3. Co-expressed 16 susceptibility genes predicted by MEM. *COL1A1* was set as the reference gene.

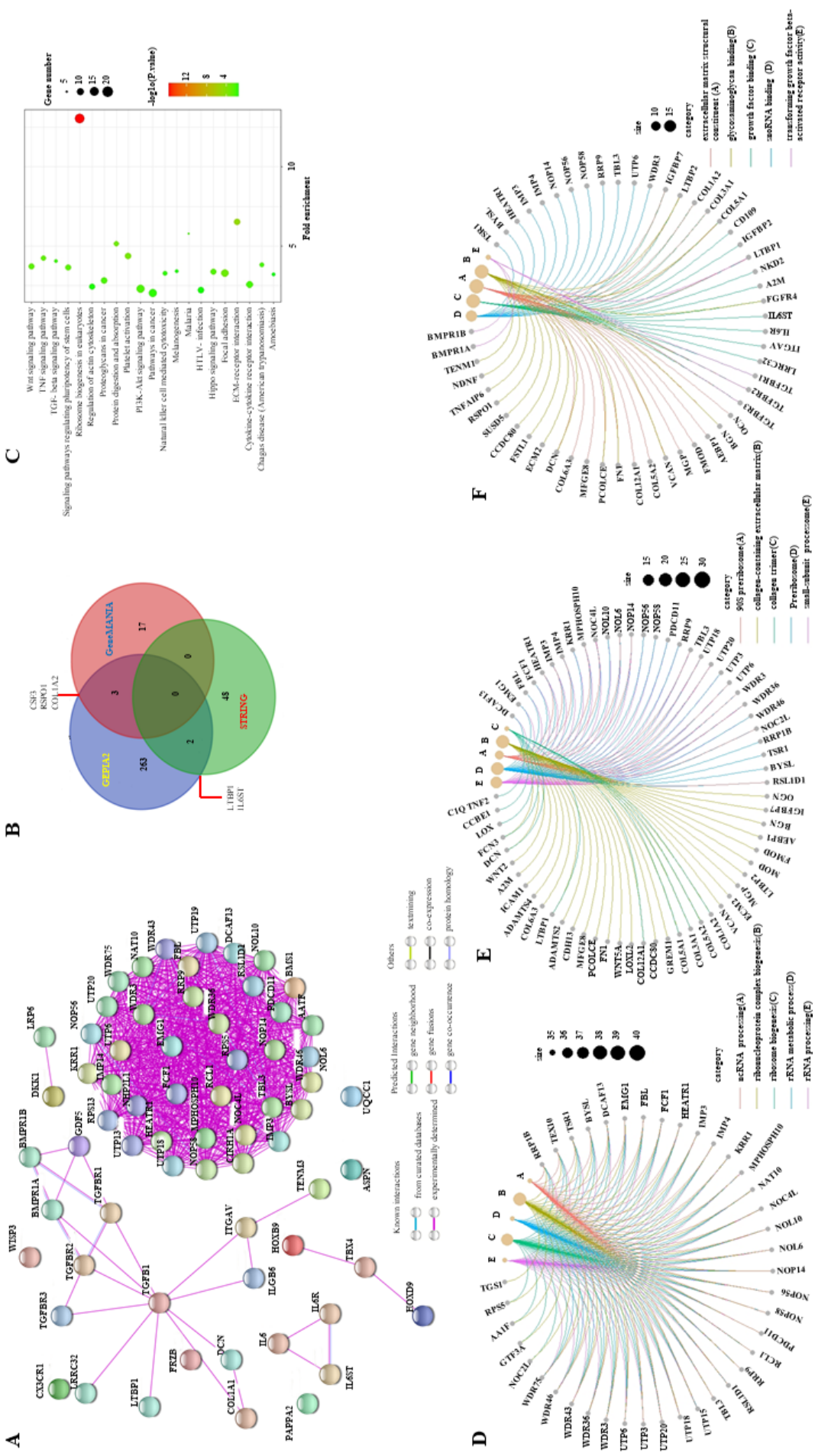

Figure 4. Protein-protein interaction and enrichment analysis. (A) PPI network as analyzed by STRING; **(B)** Venn diagrams showing overlap of the number of proteins predicted by STRING, GeneMANIA, and GEPIA2; **(C)** KEGG pathway enrichment analysis for interacted proteins; **(D-F)** Enrichment analysis of biological process (BP), cellular component (CC) and molecular function (MF).

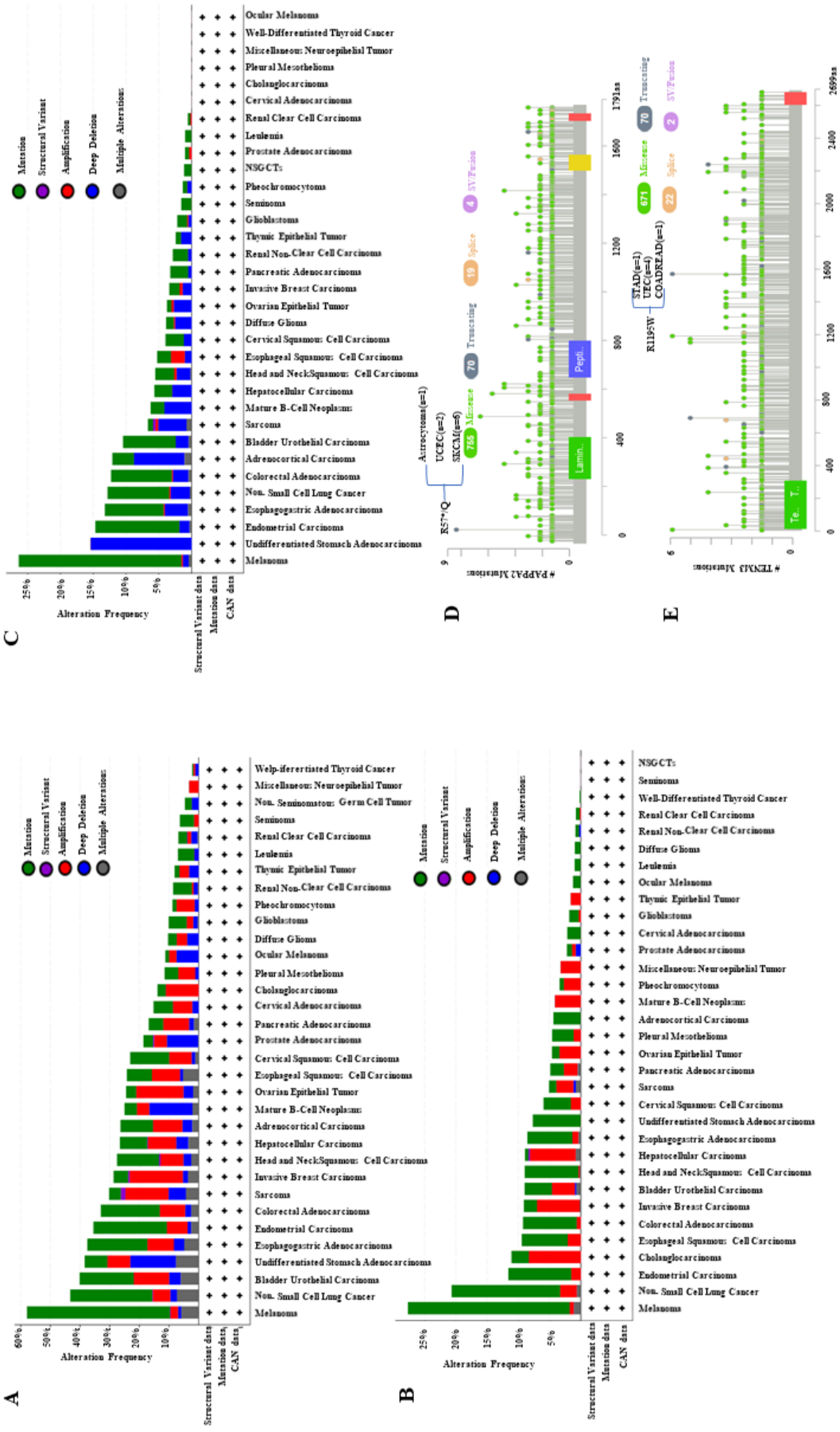

Figure 5. Genetic alteration for 16 DDH susceptibility genes in different tumors of TCGA using the cBioPortal tool. (A) Mutation types; **(B-C)** The mutation features and mutation site of PAPPA2; **(D-E)** The mutation features and mutation site of *TENM3*.

fibrosis, suggesting that mechanical conduction signal pathway is involved in the degeneration of acetabular cartilage (*33*). Meanwhile, both the ratio of different types of collagen and the size of collagen fibrils changed, possibly due to the abnormal collagen metabolism (*34*). Collagen is one of the main components of extracellular matrix and it provides stability to the matrix. Variation in the *COL1A1* gene promoter is associated with DDH in Chinese Han (*15*). *ASPN* encodes a cartilage extracellular protein that belongs to the small leucine-rich proteoglycan family (SLRP). It binds collagen and calcium and induces collagen mineralization (*35*). In *ASPN*-/- mice, biomechanical phenotype was changed, along with relatively thinner collagen fibrils, higher expression of collagen genes, increased chondroitin/dermatan and versican proteoglycans, and increased amount of decorin and biglycan protein (*36*). Following the comprehensive bioinformatics analysis, we proposed that the interaction of collagen and ASPN contributes to the mechanical change and plays a role in DDH cartilage degeneration. Enriched KEGG and GO analysis indicated that DDH susceptibility genes are involved with ECM pathway, collagen-containing ECM and glycosaminoglycan binding. Other proteins, like integrin, were identified, from PPI analysis, to interact with DDH susceptibility genes. Integrins participate in cell-cell and cell-matrix interactions. Integrin-ECM was reported in osteogenesis and the inhibition of chondrogenesis (*37*).

Wnt signaling pathway is one of the main pathways enriched in DDH. FRZB is a secreted protein, functioning as a modulator of Wnt signaling through direct interaction with Wnts. FRZB was reported to regulate chondrocyte maturation and long bone development. Its expression in DDH joint tissue was significantly higher than that in the control group (*13*). FRZB mediated the cell adhesion pathway and cell spreading by regulating integrin expression (*37*). Polymorphisms rs2242070 and rs3768842 of *FRZB* were involved in DDH (*37*). DKK1 binds to the LRP6 co-receptor and inhibits canonical beta-catenin-dependent Wnt signaling pathway, which is critical for chondrogenesis and joint formation (*38*). WISP3 is a member of the WNT1 inducible signaling pathway (WISP) protein subfamily, which belongs to the connective tissue growth factor (CTGF) family. It is the pathogenic gene for progressive pseudorheumatoid dysplasia, a joint disease characterized by degeneration of the cartilage between bones (*1*). Meanwhile, CX3CR1 regulates chondrocyte proliferation and apoptosis through the Wnt signaling pathway and this is associated with the inflammatory reaction of osteoarthritis (*39*). GDF5 is a ligand of the TGF-β superfamily, which could induce chondrogenesis in rat limb bud cells (*40*). GDF5 regulates MMP13 expression *via* DKK1 mediated Wnt/β-catenin signaling pathway in chondrocytes (*41*). RSPO1, as one of the interaction proteins predicted, can affect the differentiation process of osteoblasts and chondrocytes by stimulating the Wnt signaling pathway, maintaining articular cartilage homeostasis and joint formation (*42,43*). Similar to DKK1, it has an important role in tissue repair and fibrosis (*44*). Additionally, it was reported to activate TGF-β signaling and suppress the tumorigenesis of colon cancer (*45*).

TGFB1 and IL-6 are pro-inflammatory cytokines, which take part in the pathogenesis of hip osteoarthritis (*46*). They are involved in the bone remodeling process (*47*). The *HOX* genes encode a conserved family of transcript factors that control morphogenesis and embryonic skeletal formation through endochondral ossification (*48*). A former study has shown that some *HOX* genes encode transcription factors that are important to skeletal development and play a role in embryonic limb development (*49*). Their specific role in the DDH is still unknown. In osteoarthritis, HOTAIR, an lncRNA HOX transcript antisense RNA, could enhance the expression of SGTB by acing as miR-1277-5p sponge, and hence regulates LPS-induced chondrocyte apoptosis and inflammation (*50*).

Through comprehensive bioinformatic analysis, we identified the interactions among susceptibility genes and signaling pathways correlated with DDH. The results in this study can eventually provide novel clues for understanding the molecular mechanisms underlying the pathogenesis of DDH.

Funding: This work was supported by a grant from the Project to Promote Academics of Shandong First Medical University (no. 2019LJ001).

Conflict of Interest: The authors have no conflicts of interest to disclose.

References

1. Gkiatas I, Boptsi A, Tserga D, Gelalis I, Kosmas D, Pakos E. Developmental dysplasia of the hip: a systematic literature review of the genes related with its occurrence. EFORT Open Rev. 2019; 4:595-601.
2. Harsanyi S, Zamborsky R, Krajciova L, Kokavec M, Danisovic L. Developmental dysplasia of the hip: a review of etiopathogenesis, risk factors, and genetic aspects. Medicina (Kaunas). 2020; 56:153.
3. Harsanyi S, Zamborsky R, Kokavec M, Danisovic L. Genetics of developmental dysplasia of the hip. Eur J Med Genet. 2020; 63:103990.
4. Kenanidis E, Gkekas NK, Karasmani A, Anagnostis P, Christofilopoulos P, Tsiridis E. Genetic predisposition to developmental dysplasia of the hip. J Arthroplasty. 2020; 35:291-300.
5. Liu S, Tian W, Wang J, Cheng L, Jia J, Ma X. Two single-nucleotide polymorphisms in the *DKK1* gene are associated with developmental dysplasia of the hip in the Chinese Han female population. Genet Test Mol Biomarkers. 2014; 18:557-561.
6. Zhang J, Yan M, Zhang Y, Yang H, Sun Y. Association analysis on polymorphisms in *WISP3* gene and developmental dysplasia of the hip in Han Chinese population: a case-control study. Gene. 2018; 664:192-

195.
7. Hao Z, Dai J, Shi D, Xu Z, Chen D, Zhao B, Teng H, Jiang Q. Association of a single nucleotide polymorphism in HOXB9 with developmental dysplasia of the hip: a case-control study. J Orthop Res. 2014; 32:179-182.
8. Tian W, Zhao L, Wang J, Suo P, Wang J, Cheng L, Cheng Z, Jia J, Kan S, Wang B, Ma X. Association analysis between *HOXD9* genes and the development of developmental dysplasia of the hip in Chinese female Han population. BMC Musculoskelet Disord. 2012; 13:59.
9. Sun Y, Wang C, Hao Z, Dai J, Chen D, Xu Z, Shi D, Mao P, Teng H, Gao X, Hu Z, Shen H, Jiang Q. A common variant of ubiquinol-cytochrome c reductase complex is associated with DDH. PLoS One. 2015; 10:e0120212.
10. Feldman G, Kappes D, Mookerjee-Basu J, Freeman T, Fertala A, Parvizi J. Novel mutation in Teneurin 3 found to co-segregate in all affecteds in a multi-generation family with developmental dysplasia of the hip. J Orthop Res. 2019; 37:171-180.
11. Li L, Wang X, Zhao Q, Wang E, Wang L, Cheng J, Zhang L, Wang B. CX3CR1 polymorphisms associated with an increased risk of developmental dysplasia of the hip in human. J Orthop Res. 2017; 35:377-380.
12. Jia J, Li L, Zhao Q, Zhang L, Ru J, Liu X, Li Q, Shi L. Association of a single nucleotide polymorphism in pregnancy-associated plasma protein-A2 with developmental dysplasia of the hip: a case-control study. Osteoarthritis Cartilage. 2012; 20:60-63.
13. Baker-Lepain JC, Lynch JA, Parimi N, McCulloch CE, Nevitt MC, Corr M, Lane NE. Variant alleles of the Wnt antagonist FRZB are determinants of hip shape and modify the relationship between hip shape and osteoarthritis. Arthritis Rheum. 2012; 64:1457-1465.
14. Kolundžić R, Trkulja V, Mikolaučić M, Kolundžić MJ, Pavelić SK, Pavelić K. Association of interleukin-6 and transforming growth factor-β1 gene polymorphisms with developmental hip dysplasia and severe adult hip osteoarthritis: a preliminary study. Cytokine. 2011; 54:125-128.
15. Zhao L, Tian W, Pan H, Zhu X, Wang J, Cheng Z, Cheng L, Ma X, Wang B. Variations of the *COL1A1* gene promoter and the relation to developmental dysplasia of the hip. Genet Test Mol Biomarkers. 2013; 17:840-843.
16. Rouault K, Scotet V, Autret S, Gaucher F, Dubrana F, Tanguy D, El Rassi CY, Fenoll B, Férec C. Evidence of association between GDF5 polymorphisms and congenital dislocation of the hip in a Caucasian population. Osteoarthritis Cartilage. 2010; 18:1144-1149.
17. Wang K, Shi D, Zhu P, Dai J, Zhu L, Zhu H, Lv Y, Zhao B, Jiang Q. Association of a single nucleotide polymorphism in Tbx4 with developmental dysplasia of the hip: a case-control study. Osteoarthritis Cartilage. 2010; 18:1592-1595.
18. Shi D, Dai J, Zhu P, Qin J, Zhu L, Zhu H, Zhao B, Qiu X, Xu Z, Chen D, Yi L, Ikegawa S, Jiang Q. Association of the D repeat polymorphism in the *ASPN* gene with developmental dysplasia of the hip: a case-control study in Han Chinese. Arthritis Res Ther. 2011; 13:R27.
19. Zhu LQ, Su GH, Dai J, *et al*. Whole genome sequencing of pairwise human subjects reveals DNA mutations specific to developmental dysplasia of the hip. Genomics. 2019; 111:320-326.
20. Bailey TL, Johnson J, Grant CE, Noble WS. The MEME Suite. Nucleic Acids Res. 2015; 43:39-49.
21. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, Simonovic M, Doncheva NT, Morris JH, Bork P, Jensen LJ, Mering CV. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res. 2019; 47: 607-613.
22. Adler P, Kolde R, Kull M, Tkachenko A, Peterson H, Reimand J, Vilo J. Mining for coexpression across hundreds of datasets using novel rank aggregation and visualization methods. Genome Biol. 2009; 10:R139.
23. Kolde R, Laur S, Adler P, Vilo J. Robust rank aggregation for gene list integration and meta-analysis. Bioinformatics. 2012; 28:573-580.
24. Tang Z, Kang B, Li C, Chen T, Zhang Z. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. Nucleic Acids Res. 2019; 47:556-560.
25. Cerami E, Gao J, Dogrusoz U, *et al*. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012; 2:401-404.
26. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, Cerami E, Sander C, Schultz N. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal. 2013; 6:pl1.
27. Sanna S, Jackson AU, Nagaraja R, *et al*. Common variants in the GDF5-UQCC region are associated with variation in human height. Nat Genet. 2008; 40:198-203.
28. Schmitz MR, Murtha AS, Clohisy JC. Developmental dysplasia of the hip in adolescents and young adults. J Am Acad Orthop Surg. 2020; 28:91-101.
29. Yang S, Zusman N, Lieberman E, Goldstein RY. Developmental dysplasia of the hip. Pediatrics. 2019; 143: e20181147.
30. Muller GM, Seddon HJ. Late results of treatment of congenital dislocation of the hip. J Bone Joint Surg Br. 1953; 35-b:342-362.
31. Ramsey PL, Lasser S, MacEwen GD. Congenital dislocation of the hip. Use of the Pavlik harness in the child during the first six months of life. J Bone Joint Surg Am. 1976; 58:1000-1004.
32. Klisić P, Zivanović V, Brdar R. Effects of triple prevention of CDH, stimulated by distribution of "baby packages". J Pediatr Orthop. 1988; 8:9-11.
33. Li T, Ma R. Increasing thickness and fibrosis of the cartilage in acetabular dysplasia: a rabbit model research. Chin Med J (Engl). 2010; 123:3061-3066.
34. Skirving AP, Sims TJ, Bailey AJ. Congenital dislocation of the hip: a possible inborn error of collagen metabolism. J Inherit Metab Dis. 1984; 7:27-31.
35. Kalamajski S, Aspberg A, Lindblom K, Heinegård D, Oldberg A. Asporin competes with decorin for collagen binding, binds calcium and promotes osteoblast collagen mineralization. Biochem J. 2009; 423:53-59.
36. Maccarana M, Svensson RB, Knutsson A, Giannopoulos A, Pelkonen M, Weis M, Eyre D, Warman M, Kalamajski S. Asporin-deficient mice have tougher skin and altered skin glycosaminoglycan content and structure. PLoS One. 2017; 12:e0184028.
37. Xu R, Zhang F, Lu J, Wang K, Pan P, Sun Y, Zhang Y. Secreted frizzled-related protein 3 was genetically and functionally associated with developmental dysplasia of the hip. Aging. 2021; 13:11281-11295.
38. Witte F, Dokas J, Neuendorf F, Mundlos S, Stricker S.

Comprehensive expression analysis of all Wnt genes and their major secreted antagonists during mouse limb development and cartilage differentiation. Gene Expr Patterns. 2009; 9:215-223.
39. Sun Y, Wang F, Sun X, Wang X, Zhang L, Li Y. CX3CR1 regulates osteoarthrosis chondrocyte proliferation and apoptosis *via* Wnt/β-catenin signaling. Biomed Pharmacother. 2017; 96:1317-1323.
40. Hötten GC, Matsumoto T, Kimura M, Bechtold RF, Kron R, Ohara T, Tanaka H, Satoh Y, Okazaki M, Shirai T, Pan H, Kawai S, Pohl JS, Kudo A. Recombinant human growth/differentiation factor 5 stimulates mesenchyme aggregation and chondrogenesis responsible for the skeletal development of limbs. Growth Factors. 1996; 13:65-74.
41. Enochson L, Stenberg J, Brittberg M, Lindahl A. GDF5 reduces MMP13 expression in human chondrocytes *via* DKK1 mediated canonical Wnt signaling inhibition. Osteoarthritis Cartilage. 2014; 22:566-577.
42. Sharma AR, Choi BS, Park JM, Lee DH, Lee JE, Kim HS, Yoon JK, Song DK, Nam JS, Lee SS. Rspo 1 promotes osteoblast differentiation *via* Wnt signaling pathway. Indian J Biochem Biophys. 2013; 50:19-25.
43. Tian E, Zhan FH, Walker R, Rasmussen E, Ma Y, Barlogie B, Shaughnessy JD, Jr. The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma. N Engl J Med. 2003; 349:2483-2494.
44. Lee YH, Sharma AR, Jagga S, Lee SS, Nam JS. Differential expression patterns of rspondin family and leucine-rich repeat-containing g-protein coupled receptors in chondrocytes and osteoblasts. Cell journal. 2021; 22:437-449.
45. Zhou X, Geng L, Wang D, Yi H, Talmon G, Wang J. R-spondin1/LGR5 activates TGFβ signaling and suppresses colon cancer metastasis. Cancer Res. 2017; 77:6589-6602.
46. Malemud CJ. Anticytokine therapy for osteoarthritis: evidence to date. Drugs Aging. 2010; 27:95-115.
47. Tat KS, Padrines M, Théoleyre S, Heymann D, Fortun Y. IL-6, RANKL, TNF-alpha/IL-1: interrelations in bone resorption pathophysiology. Cytokine Growth Factor Rev. 2004; 15:49-60.
48. Rux DR, Wellik DM. *Hox* genes in the adult skeleton: Novel functions beyond embryonic development. Dev Dyn. 2017; 246:310-317.
49. Sauvegarde C, Paul D, Bridoux L, Jouneau A, Degrelle S, Hue I, Rezsohazy R, Donnay I. Dynamic pattern of HOXB9 protein localization during oocyte maturation and early embryonic development in mammals. PLoS One. 2016; 11:e0165898.
50. Wang B, Sun Y, Liu N, Liu H. LncRNA HOTAIR modulates chondrocyte apoptosis and inflammation in osteoarthritis *via* regulating miR-1277-5p/SGTB axis. Wound Repair Regen. 2021; 29:495-504.

Received March 27, 2022; Revised May 3, 2022; Accepted May 10, 2022.

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Released online in J-STAGE as advance publication May 18, 2022.