Review Article

Genetics of Developmental Dysplasia of the Hip: Recent Progress and Future Perspectives

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Abstract

Developmental dysplasia of the hip (DDH) is the most common congenital orthopedic disorder in infants. DDH is a heterogeneous disorder, and the exact pathophysiology is incompletely understood; however, several environmental as well as genetic factors have been identified as an underlying player in its pathogenesis. Involvement of genetic factors in the pathogenesis is evident from the fact that DDH occurrence has been observed in families with multiple affected individuals. Here, we reviewed the current literature on DDH, specifically concentrating on the genetic aspects of isolated (nonsyndromic) form of DDH. It is observed that genetic association studies, as well as linkage study designs, have been used to identify the extent of involvement of genetic factors in DDH pathogenesis. Variants in genes involved in joint development and chondrogenesis including *HOXD9, ASPN, HOXB9, TGF-Beta 1, PAPPA2, DKK1,* and *GDF5* genes have been identified as associated with DDH through genetic association studies. Moreover, mutations in *CX3CR1* and *TENM3* have been identified using linkage analysis and exome sequencing. Although various approaches including association studies, linkage analysis, and targeted sequencing have been used to detect genetic factors underlying DDH, structural genetic variants underlying DDH have not been explored. Therefore, we propose that copy number variation analysis and interactome studies (interaction analysis using gene and protein data using molecular interaction search tools) can help in identifying new DDH-associated genes.

Keywords: Association studies, developmental dysplasia of the hip, exome sequencing, genes, linkage analysis, mutations

Introduction

Developmental dysplasia of the hip (DDH: MIM 142700) is a congenital orthopedic malformation of the hip joint leading to a distorted femoral head socket. Globally, DDH exceeds all other congenital orthopedic disorders in terms of disease prevalence.[1-3] Anatomically, DDH is hallmarked by aberrant acetabular and/or femoral growth, which is implicated in a typical dysplasia encompassing hip joint dislocation or subluxation leading to impaired joint functioning. The impaired articular surface apposition may lead to early arthritis.[4,5] Phenotypic spectrum of DDH manifests variability, which is linked to the degree of aberration found in the femoral head and acetabulum. DDH is a heterogeneous disorder where several environmental as well as genetic factors play their role in its pathogenesis.[1-6] Among environmental factors, several pregnancy-related physiological conditions such as lack of amniotic fluid (oligohydramnios), breech presentation whereby buttocks and/or feet of the newborn baby comes first during delivery, delivering for the first time (primiparity), and increased weight of the newly born baby have been associated with the DDH pathogenesis.[3,7-10] The mere fact that more than one member of a single family are affected by DDH[11-15] depicts a pivotal role of genetic factors underlying DDH pathogenesis.[16-19] A substantial increase in disease risk of DDH has been reported in Asian siblings of affected families, with double impact in case of female siblings compared to male siblings.[20,21] DDH has been divided into DDH1 (MIM 142700) and DDH2 (MIM 615612) based on genetic heterogeneity. The inheritance pattern in DDH1 is multifactorial and the disease phenotype has been mapped on chromosome 13q22,[13] whereas

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DDH2 shows autosomal dominant inheritance and has been mapped on chromosome 3p22.2.[4] Clinical heterogeneity of DDH can be seen in its syndromic forms, where it is shown up with other clinical disorders such as cardiac and renal malformations and club foot,[22] though mostly it is manifested as an isolated entity without any other additional disorders. This review focuses on the genetic aspects of isolated form of DDH. The disease incidence rate of DDH varies in different parts of the world, ranging normally 1.5–20 cases out of 1000 live births,[23] with even higher rates of DDH incidence for certain Mediterranean countries along with Italy and Japan.[24,25] This variation in incidence is because of different practicing parameters such as the time at which the DDH is evaluated clinically and the use of different diagnostic methods.[23] An incidence rate of 3.17–3.50 for every 1000 live births has been documented for the Middle East, based on certain hospital-based studies.[26-28] This figure may rise considerably provided a detailed population-based study at the national level is conducted in the Middle Eastern countries especially in the Kingdom of Saudi Arabia, where genetic disorders are relatively more prevalent due to a typical tribal culture and common consanguineous marriages.[29]

In this article, we have reviewed genetic studies on DDH that are available online in PubMed. Keywords including "developmental dysplasia of the hip" in combination with "genetics," "gene," "mutation," "congenital," and "inherited" were used to search for the relevant literature. We found that a variety of genetic approaches have been used to identify the genetic defects underlying DDH based on the type of samples available and the pattern of inheritance of the disease. We first discuss the approaches used to delineate the genetics of DDH, followed by brief details of the genetic variants reported in DDH cases and families.

A. Genetic Association Studies

Detection of association of genetic variants in a specific gene or genomic region with a given phenotype is usually carried out using the technique of genetic association. In this approach, genetic markers are genotyped in a group of DNA samples from patients (cases) and normal individuals (controls). Nowadays, single-nucleotide polymorphism (SNP) markers are extensively used for genotyping. Screening of SNPs determines the association of a phenotype with a particular area of a chromosome. This association is helpful in exposing the underlying genes for a particular trait.[30] The International Human HapMap Project has generated human genome haplotypes, which are used in genotyping SNPs throughout the genome. This approach is termed as genome-wide association studies (GWASs).

In GWAS, the whole genome is screened using SNPs; therefore, a good number of patients as well as control samples from the same population are required.[31,32] In the context of DDH, recruiting enough patients for GWAS always remains a challenge. This led to comparatively less efficiency of GWAS in detecting DDH-specific susceptibility genetic variants. However, few case–control studies have led to the identification of genetic variants underlying DDH phenotype in several populations. Susceptible variants in genes involved in joint development and chondrogenesis have been associated with DDH. These include variants in *HOXD9, ASPN, HOXB9, TGF-Beta 1*, *PAPPA2, DKK1*, and *GDF5* genes.[10,33-39]

B. Genetic Linkage Studies

Studying the inheritance pattern of a phenotype in a family and detection of genetic markers closely segregating with the disease phenotype has been used extensively to identify the underlying genetic variants in several diseases. This approach has identified mutations in several genes as a causative factor of the genetic diseases. Earlier, identification of chromosomal parts segregating with a disease phenotype within a family was accomplished using microsatellite markers. Nowadays, SNP markers are genotyped throughout the genome to detect linkage. This approach is termed as genome-wide linkage analysis (GWLA). GWLA approach is not very effective in complex genetic disorders as compared to single-gene disorders.[40,41] In DDH, owing to complex inheritance fashion and incomplete penetrance of the potentially pathogenic variants, GWLA has not successfully mapped chromosomal regions except in few studies.[3,4,15] Moreover, a variable spectrum of DDH phenotype is also observed in different members of a single family manifesting its phenotypic heterogeneity.[42] The chromosomal regions identified in linkage to DDH using GWLA approach include regions on chromosome 3p22.2-p22.1, 4q35, 13q22, 16p, and 17q21.32.[4,13,15,43]

C. Structural Genomic Variations

Structural genomic aberrations, which lead to a change in the diploid pattern of the genome, in the form of deletions or duplications, are known as copy number variants (CNVs). Moreover, chromosomal rearrangements typically larger than 1 kb are also classified as structural genomic variations. Different genome analytic approaches such as whole-genome SNP genotyping, array comparative genomic hybridization, and high-throughput DNA sequencing are generally used to detect CNVs. Currently, high-resolution microarrays having probes for both the SNPs and CNVs are used as a gold standard to identify the CNVs.[44] Disease-causing CNVs manifest disease phenotypes by either distorting the coding region of the gene or increasing the number of the already existing gene or by giving rise to a new gene from the fusion of two genes. At present, above 6,359,956 CNVs are enlisted in the database of genomic variants (2016). Likewise, 22,358 disease-causing CNVs (gross insertions and deletions) are documented in the Human Gene Mutation Database (2019). These CNVs have been implicated in several genetic disorders such as schizophrenia, bipolar disorders, and autism.[45-48] As far as DDH is concerned, both sporadic and familial, no study has been conducted to identify CNVs in DDH. Any such study may come up with novel results.

D. DNA Sequencing

Sequencing coding part of the human genome or even the complete human genome can be used to detect mutations underlying DDH. Sequencing of complete protein-coding regions (exons) of the genome is termed as whole-exome sequencing (WES). WES covers almost 3% of the human genome. Another DNA sequencing approach, called whole-genome sequencing (WGS), determines the order of all the nucleotides in an individual's DNA and can determine variations in any part of the genome. With the advent of high-throughput next-generation sequencing technique, the dream of sequencing the entire coding regions of the genome (WES) and WGS in a matter of days with accuracy and reliability has changed into reality. The very first implication of WES in the identification of mutations underlying a genetic disorder was first reported in 2009.[49] There onward, this powerful technique has successfully been implicated in finding the underlying genetic variants in a variety of genetic disorders ranging from skeletal abnormalities, neurological malformations, skin disorders, and retinal dystrophies.[50-53] Feldman *et al.* in 2013[4] recruited a large family with multiple individuals affected with DDH. WES of the family led to the identification of a genetic variant in a chemokine receptor gene (*CX3CR1*). Previously, this nonsynonymous variant (rs3732378) was thought to be an SNP. The authors have shown that this variant is indeed a pathogenic variant using different pathogenicity prediction software such as PolyPhen-2 and SIFT. Based on the presence of phenotypic variability among the affected members of a DDH family, the authors have also suggested the role of a modifier variant that might have been involved in disease heterogeneity. This indicates the need for further investigations and validations of the variant (rs3732378) in *CX3CR1* gene. Recently, the same group has reported a mutation in teneurin 3 (*TENM3*) in a large family with multiple DDH-affected individuals.[54]

Single and Small Genomic Variations in Developmental Dysplasia of the Hip

Using the above-mentioned approaches, many genetic variants were found affiliated with DDH or as an underlying genetic defect in DDH. Variants in genes such as *CX3CR1, TENM3*, *PAPPA2, COL2A1, HOXD9, GDF-5*, and *TGFB1* have been identified as increasing the susceptibility of having DDH.[55]

Chemokine-CX3C motif-receptor 1

Chemokine-CX3C motif-receptor 1 (CX3CR1) is a member of a group of about 45 proteins of human chemokine family that play a significant role in human health and diseases development. Chemokines are small protein molecules that express in response to injury or infection and bind to and subsequently activate chemokine receptors. This led to the leukocyte adhesion to the vessel wall, leukocyte trafficking, changes in the morphology, and chemotaxis to the site of injury or infection.[56] Moreover, activation of chemokine receptor via chemokine binding also plays a role in different biological processes such as extracellular matrix remodeling and tumor metastasis besides differentiation and activation.[57-60] Furthermore, HIV and malarial parasite use chemokine receptors to invade the host cells.[61,62]

Using an approach of linkage analysis and WES, Feldman *et al*.[25] identified a variant in CX3CR1 as an underlying genetic defect in a large family from Utah. DNA samples from 71 members of a family were collected, and DNA from four severely affected members was exome sequenced. A previously reported population polymorphism (rs3732378) in CX3CR1 was identified as a pathogenic variant. Moreover, few sporadic DDH cases were screened, and the same variant was detected.[21,25] In continuation of their previous work, Feldman *et al*. generated a *CK3CR1* knocked down mice and compared the resultant phenotype with the wild-type mice. Computerized tomography (CT) was used to evaluate the hips of both the knocked down and wild-type mice at the age of 5 and 8 weeks, respectively. An inclined treadmill was used to evaluate the gait of 8-week-old mice. The authors showed that *CX3CR1* ablation affects acetabular morphology and gait.[63]

In a case–control study, Li *et al*. found that variants in *CX3CR1* increase the susceptibility to DDH. The assumption is based on genotyping data from 689 DDH patients, in which two *CX3CR1* variants (rs3732378 and rs3732379) were genotyped.[21]

Teneurin transmembrane protein 3

TENM3 encodes a transmembrane protein, which belongs to the tenascin superfamily. Members of this family play a variety of functions. TENM3 predominantly functions in the development of the visual system.[64,65] TENM3 mutations have been reported to cause different disease conditions including motor developmental delay, ocular coloboma, microphthalmia, and intellectual disability.[66-78] Although developing nervous system harbors high levels of TENM3, *TENM3* mRNA can also be found in prechondrogenic mesenchymal cells. Therefore, it could be involved in the initial phase of differentiation of chondrogenic cells.[69] Recently, the mutation in *TENM3* has been found in a family segregating DDH.[54] A mouse model was generated for *TENM3* mutation depicting late left glenoid fossa and acetabular development. Moreover, it was found that the bone marrow cells from *TENM3* mutant mouse overexpress *MMP13* with or without BMP2 stimulation. It is known that higher levels of MMP13 lead to cartilage degeneration.[70] Based on the above observations, the authors hypothesized that mutated TENM3 may slow chondrogenesis.[54]

WNT1-inducible signaling pathway protein 3

WNT1-inducible signaling pathway (WISP) protein subfamily comes under the umbrella of connective tissue growth factor family. WISP3 encodes a member of WISP family. Genes that belong to this family are involved in regulating cell growth and differentiation.[71,72] Mutations in the WISP3 gene have been known to cause an autosomal recessive form of progressive pseudorheumatoid dysplasia (PDD). PDD is a skeletal disorder characterized by damaged hip joint as a result of continuous degeneration and loss of articular cartilage.[71,73]

Recently, a case–control study, including 386 DDH patients and 558 healthy individuals, identified an SNP (rs69306665) upstream of WISP3 gene in association with DDH.[1]

Ubiquinol-cytochrome c reductase complex assembly factor 1

Ubiquinol-cytochrome c reductase complex assembly factor 1 (UQCC1) is reported to be expressed in developing cartilaginous cells called chondrocytes, which are responsible for the secretion of the matrix by the cartilage.[74] This gene has previously been reported as a candidate gene for phenotypes such as height, testicular germ cell tumor, and spine bone size.[75-77] Keeping in view the importance of UQCC in the chondrification process, an associated role of UQCC with DDH had been suggested. The results of GWASs revealed 12 variants in UQCC1 gene linked with DDH. Following GWAS, a case–control study was conducted to evaluate the association of UQCC gene in DDH manifestation. The results confirmed the association of UQCC1 variant (rs6060373) with DDH phenotype in the Han Chinese population.[30]

Asporin

A cartilaginous protein called Asporin (ASPN) is encoded by the gene ASPN. ASPN is involved in the regulation of cartilage development process, chondrogenesis.[78-80] ASPN is reported to bind with bone morphogenetic protein 2 (BMP2) and subsequently block the downstream BMP/Smad signaling pathway.[81,82] BMP2 is an important growth factor of transforming growth factor β1 (TGF-β1) family and plays a significant role in the proliferation and differentiation of osteoblast and perichondrial cells.[83,84] The gene comprises a repeat region of aspartic acid, which is linked with skeletal anomalies, owing to its polymorphism.[79]

Skeletal abnormalities such as lumbar disc malformation, rheumatoid arthritis, and osteoarthritis of the hip region are affiliated with allelic polymorphism of the ASPN gene.[85-87] A recent study identified loss of copy number variations in ASPN gene on chromosome 9 at 9q22.31 and found that the CNVs in the region are associated with severe acetabular dysplasia.[87] Moreover, a case–control study established the association of ASPN polymorphism and DDH.[34] The authors showed that polymorphism in ASPN actually affects TGF-β1 to cause DDH.

Transforming growth factor-beta 1

TGF-β1-encoded protein was found to be involved in different developmental processes such as growth and proliferation of cells followed by their differentiation. The protein is also considered to play a regulatory role for certain other growth factors.[88-91] To evaluate the role of TGF-β1 in DDH, a carefully designed case–control study was conducted. The cases included were osteoarthritis patients secondary to DDH and the controls were osteoarthritis patients without any DDH manifestation. This investigation identified a potential interaction between TGF-β1 and interleukin 6 (IL6). Moreover, this study found an association of variants in both TGF-β1 and IL6 with DDH.[6]

Growth differentiation factor 5

Proteins involved in the formation of bone skeleton are coded by *GDF5* and lie under the umbrella of a superfamily of proteins known as TGF-β. Binding different receptors in signal transduction pathways, these proteins regulate expression of the genes.[92] *GDF5* is involved in the morphogenesis of different cell types including neural and bone formation-related cell types beside teeth and fat cell types.[93] Any change in the sequence of this gene will lead to skeletal-related anomalies.[94-106]

As far as the hip is concerned, GDF5 has been implicated in the development of an embryonic limb/skeletal system, in particular development of articular cavities and cartilage.[93,106-109] GDF5 is an important player involved in hip development and joint formation.[108] Several GWAS established the link between common SNPs spanning a 130 kb interval containing GDF5 and osteoarthritis.[110-112] Moreover, various reproducible studies showed a strong association of GDF5 with DDH and hip dislocation.[33,113-115]

Pregnancy-associated plasma protein-A2

Protein generated by *PAPPA2* interacts with binding protein 5 of insulin-like growth factor (IGF) and is considered to regulate the IGF.[116,117] Pregnancy-associated plasma protein-A2 (PAPPA2) is identified to have a major regulatory role in the growth process, so the effect of PAPPA2 on bone shape and size has been suggested.[118] Deletion of PAPPA2 in mice leads to shorter femur length.[119] In humans, an association between DDH and a PAPPA2 SNP has been reported.[36] Others have replicated the results of previous studies.[120]

Homeobox genes

Homeobox (HOX) gene-encoded transcription factors occupy a key position in the development of the vertebrate skeleton. This group contains 39 genes and are mapped to four gene loci HOXA-D.[121] HOX genes regulate target genes by binding the targeted DNA region using its homeodomain.[122] For instance, anomalies related to lower limbs are related to 5' truncation of HOXC genes.[123] In the following part, we discuss only those genes having known association with DDH.

HOXD genes encompass nine genes, namely HOXD1, 3, 4, 8, 9, 10, 11, 12, and 13, which are closely located at chromosome 2q24.1-q33.1. These genes have their role known in skeletal system development, particularly in the development of a limb.[124] By creating different knockdown mutant models for HOXD genes in mice and chicken led to the finding that any perturbance in the expression pattern of HOXD genes will lead to change in shape and size of the targeted skeletal part.[125,126] The positioning of the hip joint in proximal limb part suggest a coincidence between hip area and area of expression of 9th pairs of HOX gene. Keeping in view these facts, a hypothesis was made regarding a possible role of HOXD in DDH manifestation. To verify this hypothesis, a genetic association study was designed to assess the association between SNPs of HOXD9 gene and female Han Chinese population with DDH symptoms. The findings of the study revealed the connection

between HOXD9 SNPs (rs711819) and Han Chinese population with DDH. The studies also reported HOXD9 as a novel disease candidate gene for DDH.[37] Follow-up and functional studies are required to clearly elucidate the exact molecular mechanism through which this part of the genome is associated pathogenically with DDH.

HOXB9 is a member of the Abd-B HOX family and has implications in cellular developmental processes such as proliferation and subsequent differentiation. As far as DDH pathogenicity is concerned, HOXB9 gene appears with contradictory results. In one of the studies where genetic linkage analysis was done for a Chinese DDH family, DDH was shown to be linked to chromosome 17 at position 17q21 where HOXB9 gene is located.[127] However, these findings were contradicted in a case–control study in a European population where no association was developed between SNPs of HOXB9 and DDH.[128] Another study where whole-genome screening established linkage to a specific location on chromosome 17 where HOXB9 gene is also present.[15] Looking up at these contradictory reports, a case–control study on the Han Chinese population was conducted to evaluate the association between HOXB9 and DDH manifestation. Two tag SNPs (rs8844 and rs2303486) were used in the study. This study established an association of HOXB9 SNP (rs2303486) and DDH.[38]

T-box 4

Transcription factors that regulate different developmental mechanisms are encoded by T-box 4 (TBX4), one of the T-box genes.[129] Expression of Tbx4 in growing hindlimb in chicken and mouse models indicates a connection between this gene and regulation and specification of limb development.[130,131] Microduplications involving TBX4 are associated with clubfoot in humans.[132] Moreover, mutations in TBX4 cause a small patella syndrome, a skeletal dysplasia, in which cartilage and bone growth processes are affected. As a result, individuals with small patella syndrome have malformations of the pelvis and feet.[133] Likewise, limb tissue-specific Tbx4 mutant mice showed skeletal abnormalities such as hypoplastic pelvis, femurs, and fibula.[134] Keeping in mind the significant role played by Tbx4 in skeletal development, especially hindlimb development, it has been assumed that Tbx4 SNPs might cause susceptibility to DDH phenotype. To evaluate this assumption, all SNPs of Tbx4 were evaluated and two SNPs (rs3744438 and rs3744448) were found to be associated with DDH.[135]

Developmental dysplasia of the hip-associated genes

Some DDH-associated genes such as *ASPN*, *GDF5, TGFB1,* and *HOX* are interrelated functionally. However, genes such as *TENM3, CX3CR1,* and *PAPPA2* are not related to each other and with other DDH genes. Similarly, *HSPG2* and *ATP2B4* gene mutations are known in familial DDH; however, they are not functionally interacting with other DDH known genes.[2] Interaction analysis using gene and protein data using molecular interaction search tools such as MIST[136] and GeneMANIA[137,138] was carried out to identify functional interaction between DDH-associated genes and to detect new candidate gene. For instance, using molecular interactome analysis, we identified several members of keratin-associated (KRTAP) genes that directly interact with DDH-associated gene. KRTAP members identified in this analysis include *KRTAP1-1, KRTAP1-5, KRTAP2-3, KRTAP3-1, KRTAP3-2, KRTAP4-2, KRTAP4-11, KRTAP4-12, KRTAP5-7, KRTAP5-9, KRTAP6-2, KRTAP10-8, KRTAP10-11, KRTAP12-2, KRTAP17-1,* and *KRTAP19-5*. The KRTAP proteins form a matrix of keratin intermediate filaments which contribute to the structure of hair fibers. KRTAP family members appear to have unique, family-specific amino- and carboxyl-terminal regions. Their association with disease conditions has not been described yet. Mutations in these genes might contribute to the development of DDH. We found that most of the DDH genes interact with each other either directly through protein–protein interaction or indirectly through transcription factors. Interactome analysis using gene and protein data identified several important genes as well [Figure 1].

Conclusion

Development dysplasia of the hip is clinically and genetically a heterogeneous disorder. It encompasses a broad range of disorders including minor acetabular dysplasia to irreducible hip dislocation. The pathophysiology of the DDH is incompletely understood. Mild hip subluxations and dislocation escape the early diagnosis due to lack of optimal timing for clinical examination, imaging, and appropriate use of imaging.

Genetic studies have helped in identifying the molecular markers for DDH. Association studies and linkage analysis succeeded in identifying several candidate genes, such as *PAPPA2, COL2A1, HOXD9, GDF-5, TGFB1*, *CX3CR1*, and *TENM3* as DDH-associated genes. It is believed that these genes play a significant role in the pathogenesis of DDH. A genetic screening program can be established for the screening for mild forms of DDH. Genetic screening program also has the potential to detect the DDH earlier in life.

Some of the DDH genes discussed in this review are part of the same signaling pathways, or they interact with each other through protein–protein interaction. For instance, *ASPN*, *GDF5, TGFB1,* and *HOX* genes are interrelated functionally. However, some genes such as *TENM3, CX3CR1,* and *PAPPA2* are not related to each other and with other DDH genes. Similarly, *HSPG2* and *ATP2B4* gene mutations are known in familial DDH; however, they are not functionally interacting with other DDH known genes. Using DDH known genes, molecular interaction search tools have been used to identify other interacting genes in DDH-related signaling pathways. Interactome analysis has the potential to identify new potential candidate genes for studies aiming at identifying genetic variants underlying DDH.

Identification of candidate genes would pave the way for early detection of DDH. Timely detection helps in preventing further disability including osteoarthritis and movement impairment

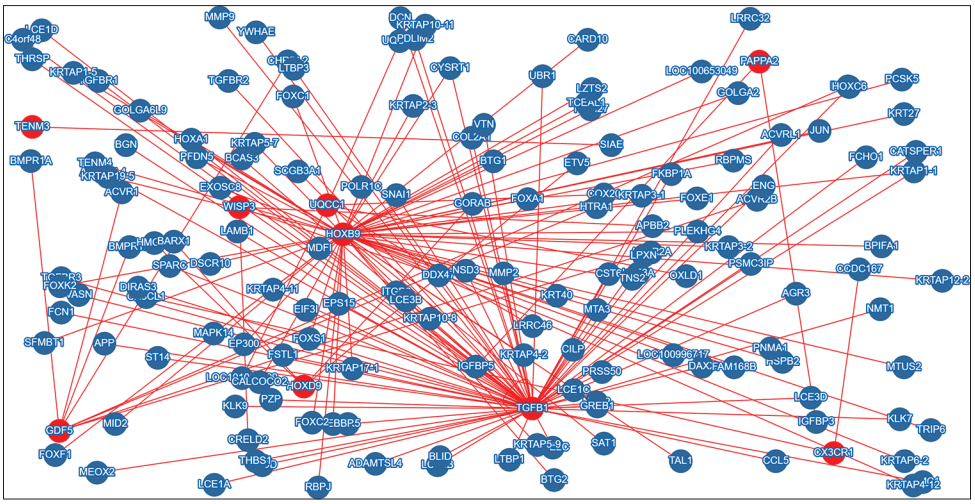

Figure 1: Output of molecular interaction search tool. Developmental dysplasia of the hip-associated genes were used as a training set to identify molecular interactions between developmental dysplasia of the hip genes. Other potential candidate genes have also been identified

and improves the psychological health and quality of life in affected children.

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Conflicts of interest

There are no conflicts of interest.

Authors' contribution

KIK conceived the idea. JAH and SB performed literature survey and wrote the initial draft. SB carried out molecular interaction studies. KIK edited the initial draft and provided guidelines for improvement of the initial draft. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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