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Title: Genetic of Legg-Calvé-Perthes Disease: A Review

Running Title: Genetic of LCPD

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Abstract

Legg-Calvé-Perthes (LCPD), a juvenile hip disorder, caused by blood flow impairment to the femoral head. In severe LCPD cases, the femoral head may develop a flattening deformity. Furthermore, if LCPD is diagnosed at the later stages, it causes early osteoarthritis of the hip. The etiology of LCPD is complex and embraces both genetic and epigenetic factors. This review is an attempt to summarize the current scholarship on the role of these genetic variants in the incidence of LCPD. Among the genetic causes of this disease are mutations in the genes of thrombophilia factors such as FV Leiden, and anticardiolipin antibodies. The mutation of COL2A1, TRPS1, eNOS genes are the other agents. Moreover, the clinical symptoms of avascular necrosis may be indiscernible in patients with Gaucher’s disease or LCPD and the differential diagnosis is a challenge.

Keywords: Legg-Calvé-Perthes disease, Genetics; Osteoarthritis, Thrombophilia Factors, eNOS
1. Context:

Legg-Calvé-Perthes disease (LCPD) or femoral head ischemia (OMIM#150600) is a childhood hip disorder induced by the loss of blood flow to the femoral head and the cells within the bone tissue begin to die. It is also known as the cause of femoral head osteonecrosis(2). One prevalent complication of LCPD is a lasting femoral head deformity so that 50% of patients having hip pain in early adulthood and developing disabling osteoarthritis before the age of 60 due to the femoral head deformity. Generally, children above the age of 6 diagnosed with LCPD are more likely to develop hip problems in their adulthood. Diagnosis at younger age may lead to a better prognosis (3). The treatment is successful when the femur head is renewed with a new blood supply and re-ossified. During the healing, the bony epiphysis undergoes broad remodeling and repair. Extensive resorption of necrotic bone is presumed to be related to the collapse of the femoral head; therefore, balanced bone formation is expected to help preserve the form of the femoral head in the course of the healing process(4).

LCPD was first discovered about a century ago by three medical practitioners, Legg, Calvé, and Perthes (5). The disease affects 1 in 740 males and 1 in 3500 females in the age range of 2 to 14. The prevalence rate of the disease is between 0.4/100000 to 29.0/100000 in children below 15 years of age and children between 4 to 8 years of age are most vulnerable to this disease. Furthermore, white people are more likely to be affected by LCPD (6, 7). Multiple genetic and environmental factors may be involved in the LCPD. Some risk factors or conditions include passive smoke inhalation, adverse socioeconomic status, attention deficit hyperactivity disorder (ADHD), low birth weight, and psychological burden, obesity and high plasma level of leptin, familial history and genetics, coagulation disturbance, inflammation markers, and apoptosis factors (8).
2. Evidence Acquisition:

In order to investigate genetic agents and gene mutations that can cause LCPD (9), several articles published to 2020 were reviewed in this paper. These articles mainly focused on gene mutations, polymorphisms or variants. This review was motivated by the role of polymorphisms and mutations in different family genes in LCPD.

We searched for manuscripts published in English using the search terms “genetics” AND “Legg-Calvé-Perthes”, “genetics” AND “Osteoarthritis”, and more general search terms such as “genome wide association” , “polymorphisms”; “femoral head ischemia”, and “LCPD”. We also reviewed specific websites including OMIM, Genetics Home Reference, and Gene Review. We complemented PubMed searches with Google scholar and Science Direct searches.

3. Results:

As shown in Table 1, the main findings of these articles are summarized here:

3.1. Thrombophilia Factors: Markers of Inflammation

Thrombophilia factors play an important role in the inhibition of hemorrhage. The abnormality of blood coagulation, whether hereditary or acquired, makes an individual prone to venous or arterial thrombosis (10). The emergence of thrombotic complications is contingent upon several factors. An inherited abnormality, hereditary thrombophilia is caused by genetic mutation in the genes coding for either coagulation factors like FV and prothrombinor anticoagulants like protein C or protein S, and a lupus anticoagulant may be occasionally included (11). Factor V Leiden thrombophilia (FV Leiden) is caused by particular mutations in the FV gene, which change the structure of FV protein and subsequently disrupt its function in the coagulation and anticoagulation pathways (12). The risk of thrombosis in heterozygous people for FV gene polymorphisms is four to five times
higher than the non-carriers. Homozygosity, nevertheless, leads to a nine to twelve-fold increase in thrombosis risk (13).

Prothrombin (a vitamin-K dependent protein), a precursor to thrombin, is converted by the prothrombinase complex in the diffusion phase of coagulation (14). This protein is coded by the F2 gene. G20210A mutation in F2 gene augments prothrombin serum concentrations, which raises the risk of thrombosis (15). Asymptomatic carriers of this mutation possess identical incidence rates of venous thrombosis as FV Leiden carriers (13). Protein C (PC) is a vitamin-K dependent anticoagulant protein that is generated in the liver and activated by thrombin. The activated form of this protein plays a mediating role in the inactivation of FVa and FVIIIa (16). Several different mutations trigger deficiencies in PC with a decrease in protein activity or quantity. Afterward, the inactivation of factor Va and VIIIa decrease, which leads to a higher risk of thrombosis (17). An anticoagulant protein, protein S (PS) is chiefly derived from liver enzymes and acts as a cofactor for APC. Then by augmenting of APC, they inactivate factor Va and VIIIa (18). About 60% of total PS that circulates in plasma, is bound to C4b binding protein, and only the remaining 40% is free PS with anticoagulant properties (19).

Because of blood supply problems in LCPD, many studies have investigated the association between thrombophilia factors and this disease. Evidence from a case-control study on 90 children with LCPD, a pilot study on a group of German children and a cohort of Ashkenazi Jews patients suggested that none of the thrombophilia gene variants (Factor V Leiden, Prothrombin gene, and Methylenetetrahydrofolate reductase: MTHFR variants) had any impacts on LCPD (20-22). In addition, two different studies on Iranian and Serbian children showed that there was no link between MTHFR (677C > T and 1298A > C) and Tumor necrosis factor-alpha: TNF-α (-308G > A and -238G > A) polymorphisms and disease. Furthermore, in the evaluation of other coagulation factors including PAI-1 as well as another
inflammation factor, IL-3, the results were identical (23, 24). One study evaluated the association of TLR4 (Asp299Gly, Thr399Ile) and IL-6 (G-174C, G-597A) variants with LCPD in Serbian population. There were no significant differences between intervention and control groups in terms of TLR4 gene polymorphisms. Moreover, heterozygotes for the IL-6 variants had a lower chance of developing disease. Also, complete linkage disequilibrium was observed between these gene polymorphisms (25). In another study on Iranian population, the association of IL-6 -174G>C and -572G>C Polymorphisms with the risk of LCPD was assessed. The homozygous genotype of IL-6 -174 G>C variant (CC) was positively associated with the increased risk of Perthes disease and the IL-6 -572G>C variant was not associate with the incidence of this disease in Iranian children (26).

On the other hand, it was found that factor V Leiden mutation (G1691A, Arg506Gln) increased the risk of disease. It was the only inherited risk factor among hypercoagulability factors related to the incidence of LCPD. In contrast, no association was observed between prothrombin II (G21210A) polymorphism and MTHFR (C677T) mutation and the development of LCPD. Similar results were reported in a study on a group of Brazilian LCPD children and several other studies (22, 27, 28). In the same vein, the results of a research suggested that two thrombophilia risk factors, the factor-V Leiden mutation and anticrodiolipin antibodies, were associated with LCPD (29).

The relationship between LCPD and beta fibrinogen gene (G-455-A) polymorphism was also analyzed in a case-control study. The cases had a greater chance of exposure to passive smoking than controls. Although the odd ratios were not statistically significant, the interplay of passive smoking and this polymorphism was found to influence the incidence of LCPD (30). The level of protein C, protein S and anticrodiolipin antibody was also measured in different studies. A significant increase in the risk of LCPD and a drop in the levels of PC
and PS as well as a direct association between anticardiolipin antibodies IgG, or IgM were detected in this disease (29, 31)

3.2. Collagen Type II Alpha 1 Chain (COL2A1) Gene

Pro-alpha1(II) chain, a component of type II collagen, encoded by COL2A1 gene, has been recognized as an essential matrix protein for stiffening cartilaginous, connective and skeletal tissues during embryogenesis and adult life. This gene is localized to chromosome 12q13.11 and two transcripts are identified for that (32). Many consensus of amino acid with a triplet structure including Gly-X-Y, are located the core area of this protein. Gly is highly important and its replacement with another amino acid destroys the protein structure (33). Several studies have assessed the mutations of COL2A1 gene in LCPD patients. The first mutation of COL2A1 gene (p. Gly1170Ser) was described in a study on a Japanese family with LCPD. After a year, this result was confirmed in a research on 42 members from a five generations Chinese pedigree (34, 35). Moreover, another paper on a Chinese pedigree suggested this mutation as a causative agent of osteonecrosis in the femoral head (ONFH) (36). Also, the novel mutations, c.638G>A (G/A) and c.2014G>T (G/C), in this gene were reported in two children who had abnormally developed hips (37). In another study on 45 members from 4 generations of a Chinese family diagnosed with LCPD and avascular necrosis of the femoral head (ANFH), a novel heterozygous mutation (c.1888 G>A, p. Gly630Ser) in exon 29 of COL2A1 was found in the Gly-X-Y domain (38). Also, other studies have shown the strong correlation between the mutations of COL2A1 gene and LCPD(39). However, the results of a research in Ashkenazi Israel population reported lack of any mutations in COL2A1 gene of all patients (22).

3.3. Transcriptional Repressor GATA Binding 1 (TRPS1) Gene

The human TRPS1 gene is localized on the chromosome 8q23.3 and is organized as seven exons. The alternative exon splicing generates twelve different isoforms in which TRPS1-203
is the predominant isoform (40). This gene encodes a 1281 amino acid nuclear protein. This protein is a zinc finger transcription factor that binds to a dynein light chain protein with high affinity and suppresses GATA-regulated genes. This interaction affects binding to GATA consensus sequences and represses its transcriptional activity (41). TRPS1 gene plays a vital role in the control of cell cycle and amplification during the development of different cancers. The silencing of the gene leads to the downregulation of histone deacetylase activity of cell, such as HDAC2 and HDAC4, followed by increased acetylation of histon H4-K16 (42). Thus, the gene expression changes, which influence tumor growth, have been observed in many cancers including breast cancer (43), lung cancer (44) and brain cancer (45). Indeed, TRPS1 gene serves as a key factor in differentiation and growth of normal mammary epithelial cells, and dysfunction contributes to the breast cancer development (46). Other hereditary disorders caused by mutation affecting TRPS1 gene are Tricho-rhino-phalangeal syndromes type I, II, III. These syndromes are a rare malformation complex characterized by some shared clinical features such as skeletal and facial anomalies (47).

The first novel mutation underlying LCPD in TRPS1 gene was discovered in a family with four patients over three generations. In all patients, Perthes disease had been diagnosed in childhood. A new missense mutation in exon 6, c.2726G>A (p.C909Y) of the TRPS1 gene was identified in two cases of these patients (48). Also, a recent study reported LCPD-related mutations in TRPS1 gene in two patients with craniofacial features and various skeletal abnormalities. The first patient had a novel heterozygous mutation that involved the deletion of two base pairs (c.3198-3199delAT) in the TRPS1 gene, which led to a translational frameshift and premature termination codons. The other patient had a deletion of 3.08 million base-pair at 8q23.3, which contains the TRPS1 gene and CSMD3(49).
3.4. Endothelial Nitric Oxide Synthase (eNOS or NOS III)

Nitric Oxide Synthases (NOSs) are an enzymatic family that catalyzes the production of L-citrulline and frees radical Nitric Oxide (NO) from L-arginine as a substrate. These enzymes need different cofactors such as nicotinamide-adenine-dinucleotide phosphate (NADPH), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and (6R-5,6,7,8-tetrahydrobiopterin (BH4) (50). NO is an important signaling molecule involved in neural development, angiogenesis, insulin secretion and many other mechanisms(51). There are three different isoforms of this enzyme (nNOS, iNOS, and eNOS) that are classified and nominated according to their expression location. They are coded by a separate gene. The neuronal NOS (nNOS) is mainly generated in the neural tissue (central and peripheral neurons) by NOS1 gene located on the large arm of chromosome 12. Thus, many neurodegenerative problems such as multiple sclerosis (MS) stroke, Parkinson's diseases and Alzheimer's are caused by abnormal NO signaling due to nNOS disturbance(52). The inducible NOS (iNOS) is produced in the immune system by NOS2 gene, which is positioned in an area on the large arm of chromosome 17(53). iNOSs are produced in several cells in response to lipopolysaccharide, cytokines, and other agents. They synthesize high-level NO for the cytotoxicity of the pathogenic target cells. It is highly effective in the pathophysiology of inflammatory disease and septic shock (54).

The endothelial NOS (eNOS), known as eNOS or cNOS, is produced by NOS3 gene in the endothelium. This gene is located on chromosome7q36 with a total size of 21 kb and 26 exons(55). As a vasodilator, this enzyme contributes to the control of blood pressure level, vasodilation and vasoprotection and prevents atherosclerosis (56). All NOSs interact with Ca²⁺-activated calmodulin, but iNOS is always active and does not need regulation for intracellular Ca²⁺ concentration (57). Recently, some studies have explored the role of eNOS variants and incidence of LCPD. One study established that 27-bp VNTR in intron 4 and
G894T polymorphism in exon 7 of this gene may be a risk factor for Perthes disease. The frequency of VNTR was significantly higher in the case group than in the control. Also, the prevalence of heterozygous genotype GT was higher in case groups than in controls (58). However, another study in a group of Iranian children reported different results. Although eNOS 894G>T and -786T>C polymorphisms were meaningfully related to a higher risk of LCPD, no significant relationship was observed between eNOS 27-bp VNTR polymorphism and LCPD risk (59).

3.5. Gaucher’s Disease

Gaucher’s disease, inherited in an autosomal recessive pattern, contains a wide range of clinical features (60). There are three main types (GD1, GD2, and GD3) and two subtypes (perinatal-lethal and cardiovascular) of this disease, which the detection of them is highly important in disease management. GD1 embraces a series of clinical and radiographic documents related to bone abnormalities like osteopenia, focal lytic or sclerotic lesions, and osteonecrosis (61). As mentioned earlier, some of the most significant symptoms of Gaucher’s disease are similar to LCPD signs. Therefore, the clinical symptoms of avascular necrosis may be indiscernible in patients with Gaucher’s disease or LCPD and the differential diagnosis is a challenge (62). A study explored the most common variants of Gaucher’s disease such as 1226G>A (N370S) and distinguished these diseases in a group of Ashkenazi Jews patients. According to results, its mutation was three-fold higher in patients with LCPD (63). Nevertheless, another study by the same research team repudiated this finding. When retested in a larger sample of case-control study group, they could not confirm their previous results. Thus, a genetic association between these diseases was rejected (22).

4. Discussion:

Legg-Calvé-Perthes disease (LCPD) is a juvenile hip disorder caused by the loss of blood flow to the bony epiphysis, which leads to the osteonecrosis of the femoral head. The purpose
of this review was to determine the genetic agents involved in LCPD. Studies on thrombophilia factors have reported contradictory results. As mentioned, FV is directly associated and PC and PS are reversely relationship with disease. Accordingly, a correlation between other thrombophilia factors and the disease was ruled out. Moreover, some variants of IL6 and anticardiolipin antibody may increase the risk of disease. TRPS1 and COL2A1 gene as well as some eNOS gene variations are directly related to the onset of the disease. However, no association was found between Gaucher’s disease causing genes and LCPD.

Table 1- the main findings of the role of genetic variants in LCPD

<table>
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<th>Population</th>
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<th>Association</th>
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**Competing interests**

All authors declare that they have no conflict of interest.

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**Authors’ contributions**

S.A: performing the main steps of essay, acquisition of data and writing the manuscript.

H.N: Study concept and design

N.N: manuscript editing and critical revision

M.R.S: Head of team and monitoring and fixing technical errors during all steps of the study.
References


