Review Article:
Genetics of Legg-Calvé-Perthes Disease: A Review Study

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ABSTRACT

Background: Legg-Calvé-Perthes Disease (LCPD), a juvenile hip disorder, is caused by impaired blood flow 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.

Objectives: This review attempts to summarize the current knowledge on the role of these genetic variants in the incidence of LCPD.

Methods: We searched for articles published in English using the special related search terms.

Results: The genetic causes of this disease include mutations in the genes of thrombophilia factors, such as FV Leiden and anticardiolipin antibodies. The mutations of *COL2A1*, *TRPS1*, *eNOS* genes are the other causes. 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.

Conclusions: The results indicated that genetic testing may be useful in diagnosing and managing patients with juvenile hip disorders.

1. Context

Legg-Calvé-Perthes Disease (LCPD) or femoral head ischemia (OMIM#150600) is a childhood hip disorder caused by the loss of blood flow to the femoral head, and consequently, the cells within the bone tissue begin to die [1]. 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 have hip pain in early adulthood and develop disabling osteoarthritis before the age of 60 (due to the femoral head deformity). Generally, children older than 6 diagnosed with LCPD are more likely to develop hip problems in adulthood. Diagnosis at a younger age may lead to a better prognosis [3]. The treatment is successful when the femur head is regenerated 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

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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 aged 2 to 14 years. The prevalence rate of the disease is between 0.4 per 100000 to 29.0 per 100000 in children below 15. Also, children between 4 and 8 years 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, poor socioeconomic status, Attention Deficit Hyperactivity Disorder (ADHD), low birth weight, psychological burden, obesity and high plasma level of leptin, familial history and genetics, coagulation disturbance, inflammation markers, and apoptosis factors [8].

2. Evidence Acquisition

To investigate genetic agents and mutations that can cause LCPD [9], we reviewed several articles published up to 2020. 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 articles 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 search with Google Scholar and Science Direct.

3. Results and Discussion

As shown in Table 1, the main findings of these articles are summarized as follows.

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, i.e., hereditary thrombophilia, is caused by mutations in the genes coding for either coagulation factors like FV and prothrombin or anticoagulants like protein C or protein S; also 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 9 to 12 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 the *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 made 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 factors Va and VIIIa 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 Activated Protein C (APC). Then by augmenting APC, they inactivate factors Va and VIIIa [18]. About 60% of total PS circulating in plasma is bound to C4b binding protein, and 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 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 and 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 the Serbian population. There were no significant differences between the intervention and control groups regarding *TLR4* gene

polymorphisms. Moreover, heterozygotes for the *IL-6* variants had a lower chance of developing the disease. Also, complete linkage disequilibrium was observed between these gene polymorphisms [25]. In another study on the Iranian population, the association of *IL-6* polymorphisms (-174G>C and -572G>C) 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 associated 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 research results suggested that two thrombophilia risk factors, the factor-V Leiden mutation, and anticardiolipin 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 odds ratios were not statistically significant, the interplay of passive smoking and this polymorphism was found to influence the incidence of LCPD [30]. The levels of protein C, protein S, and anticardiolipin antibody were 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].

Collagen Type II Alpha 1 Chain (*COL2A1*) gene

Pro-alpha1(II) chain, a component of type II collagen, encoded by the *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 have been identified for that [32]. Many consensuses of amino acids with a triplet structure, including Gly-X-Y, are located in 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 the *COL2A1* gene in LCPD patients. The first mutation of the *COL2A1* gene (p. Gly1170Ser) was described in a study on a Japanese family with LCPD. After a year, this result was confirmed in research on 42 members from five generations of Chinese pedigree [34, 35]. Besides, another paper on a Chinese pedigree suggested this mutation as a causative agent of Osteo-Necrosis 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 a strong correlation between the mutations of the *COL2A1* gene and LCPD [39]. However, the research results in the Ashkenazi Israel population reported no mutations in the *COL2A1* gene of all patients [22].

Transcriptional Repressor GATA Binding 1 (*TRPS1*) gene

The human *TRPS1* gene is localized on chromosome 8q23.3 and is organized as 7 exons. The alternative exon splicing generates 12 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 controlling the cell cycle and amplification during the development of different cancers. The silencing of the gene leads to the downregulation of histone deacetylase activity of the cell, such as HDAC2 and HDAC4, followed by increased acetylation of histone H4-K16 [40]. Thus, the gene expression changes, which influence tumor growth, have been observed in many cancers, including breast cancer [42], lung cancer [43], and brain cancer [44]. Indeed, the *TRPS1* gene serves as a key factor in the differentiation and growth of normal mammary epithelial cells, and its dysfunction contributes to breast cancer development [45]. Other hereditary disorders caused by a mutation affecting the *TRPS1* gene are tricho-rhino-phalangeal syndromes type I, II, III. These syndromes are a rare malformation complex characterized by shared clinical features, such as skeletal and facial anomalies [46].

The first novel mutation underlying LCPD in the *TRPS1* gene was discovered in a family with four patients over

Table 1. The main results of the role of genetic variants in LCPD

Populations	Gene	Variant	Disease	Association	Year	Reference
Germany and Ashkenazi Jews	Factor V Leiden		LCPD	Yes	2001-2008	20-22
	Prothrombin		LCPD	No	2001-2008	20-22
	MTHFR		LCPD	No	2001-2008	20-22
Iranian and Serbian	*MTHFR*	677C>T ,1298A>C	LCPD	No	2015-2018	23,24
	TNF-α	-308G>A ,-238G>A	LCPD	No	2015-2018	23,24
	PAI-1		LCPD	No	2015-2018	23,24
	IL-3		LCPD	No	2015-2018	23,24
Serbian	*TLR4*	Asp299Gly,Thr399Ile	LCPD	No	2014	25
	IL-6	-174G>C, -597G>A	LCPD	Yes	2014	25
Iranian	*IL-6*	-174G>C	LCPD	Yes	2019	26
		-572G>C	LCPD	No	2019	26
Brazilian	Factor V Leiden	G1691A	LCPD	Yes	1999	27
	Prothrombin II	G21210A	LCPD	No	1999	27
	Beta fibrinogen	G-455-A	LCPD	No	1999	27
Japanese	*COL2A1*	p. Gly1170Ser	LCPD	Yes	2007	34
Chinese	*COL2A1*	p. Gly1170Ser	LCPD	Yes	2008	35
Chinese	*COL2A1*	p. Gly1170Ser	ONFH	Yes	2014	36
	COL2A1	c.638G>A	abnormally developed hips	Yes	2011	37
	COL2A1	c.2014G>T	LCPD	Yes	2011	37
Chinese	*COL2A1*	c.1888 G>A	ANFH	Yes	2014	38
Ashkenazi Jews	*COL2A1*		LCPD	Yes	2013	39
Germany	*TRPS1*	c.2726G>A	LCPD	Yes	2015	48
	TRPS1	c.3198-3199delAT		Yes	2017	49
	TRPS1	Deletion of 3.08 million base-pair at 8q23.3		Yes	2017	49
	eNOS	27-bp VNTR in intron 4		Yes	2016	58
		G894T		Yes	2016	58
Iranian		894G>T		Yes	2019	59
		-786T>C		Yes	2019	59
		27-bp VNTR in intron 4		No	2019	59

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ANFH: Avascular Necrosis of the Femoral Head; ONFH: Osteonecrosis in the Femoral Head; LCPD: Legg-Calvé-Perthes Disease.

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 [47]. Also, a recent study reported LCPD-related mutations in the *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* [48].

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, flavin adenine dinucleotide, flavin mononucleotide, and (6R-)5,6,7,8-tetrahydrobiopterin [49]. NO is an important signaling molecule involved in neural development, angiogenesis, insulin secretion, and other mechanisms [50]. There are three different isoforms of this enzyme (nNOS, iNOS, and eNOS) 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 the *NOS1* gene located on the large arm of chromosome 12. Thus, many neurodegenerative problems, such as multiple sclerosis, stroke, Parkinson disease, and Alzheimer are caused by abnormal NO signaling due to nNOS disturbance [51]. The inducible NOS (iNOS) is produced in the immune system by the *NOS2* gene, which is positioned in an area on the large arm of chromosome 17 [52]. 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 [53].

The endothelial NOS (eNOS), known as eNOS or cNOS, is produced by the *NOS3* gene in the endothelium. This gene is located on chromosome7q36 with a total size of 21 kb and 26 exons [54]. As a vasodilator, this enzyme contributes to controlling blood pressure level, vasodilation, and vasoprotection and prevents atherosclerosis [55]. All NOSs interact with Ca^{2+}-activated calmodulin, but iNOS is always active and does not need regulation for intracellular Ca^{2+} concentration [50]. Recently, some studies have explored the role of *eNOS* variants and the 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 group. Also, the prevalence of heterozygous genotype GT was higher in the case group than in the control group [56]. 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 [57].

Gaucher's Disease (GD)

Gaucher's Disease (GD), inherited in an autosomal recessive pattern, contains many clinical features [58]. 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 crucial 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 [59]. 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 [60]. One 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 [61]. Nevertheless, another study by the same research team rejected this finding. When retested in a larger sample of a case-control study group, they could not confirm their previous results. Thus, a genetic association between these diseases was rejected [22].

4. Conclusion

Legg-Calvé-Perthes Disease (LCPD) is a juvenile hip disorder caused by the loss of blood flow to the bony epiphysis, which leads to 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 mutations can cause disease but the level of PC and PS have no effect on the pathogenesis process. 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* genes, 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.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

Conceptualization and supervision: Mohammadreza Sobhan; Methodology: Samira Asadollahi; Investigation, Writing – original draft, and Writing – review & editing: All authors; Data collection: Samira Asadollahi and Hosein Neamatzadeh; Data analysis: Nasim Namiranian.

Conflicts of interest

All authors declared no conflict of interest.

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