**Name of Journal: *World Journal of Orthopedics***

**Manuscript NO: 40536**

**Manuscript Type: Systematic review**

**etiology of** **Legg-Calvé-Perthes disease: a systematic review**

Pavone V *et al.* The etiology of Legg-Calvé-Perthes disease

Vito Pavone, Emanuele Chisari, Andrea Vescio, Claudio Lizzio, Giuseppe Sessa, Gianluca Testa

**Vito Pavone, Emanuele Chisari, Andrea Vescio, Claudio Lizzio, Giuseppe Sessa, Gianluca Testa**, Department of General Surgery and Medical Surgical Specialties, Section of Orthopaedics and Traumatology, University Hospital Policlinico-Vittorio Emanuele, University of Catania, Catania 95100, Italy

**ORCID numbers:** Vito Pavone (0000-0001-5664-8066); Emanuele Chisari (0000-0003-0933-6806); Andrea Vescio (0000-0002-1677-927X); Claudio Lizzio (0000-0003-2870-1995); Giuseppe Sessa (0000-0001-6114-2609); Gianluca Testa (0000-0001-5246-9714).

**Author contributions:** Pavone V, Chisari E and Testa G contributed equally to the work; Chisari E conceptualized and designed the review together with Vescio A and Testa G carried out the analysis; Lizzio C and Sessa G drafted the initial manuscript; all authors reviewed and approved the final manuscript as submitted.

**Conflict-of-interest statement:** All authors have no conflicts of interest to report.

**PRISMA 2009 Checklist statement:** The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

**Manuscript source:** Invited manuscript

**Corresponding author: Vito Pavone, MD, PhD, Associate Professor,** Department of General Surgery and Medical Surgical Specialties, Section of Orthopaedics and Traumatology, University Hospital Policlinico-Vittorio Emanuele, University of Catania, Via Plebiscito 628, Catania 95100, Italy. vpavone@unict.it

**Received:** November 2, 2018

**Peer-review started:** November 2, 2018

**First decision:** November 29, 2018

**Revised:** December 6, 2018

**Accepted:** January 10, 2019

**Article in press:**

**Published online:**

**Abstract**

***BACKGROUND***

Legg-Calvé-Perthes disease (LCPD) is a clinical condition affecting the femoral head of children during their growth. Its prevalence is set to be between 0.4/100000 to 29.0/100000 children less than 15 years of age with a peak of incidence in children aged from 4 to 8 years. LCPD etiology has been widely studied, but it is still poorly understood.

***aim***

to analyse the available literature to document the up-to-date evidence on LCPD etiology.

***METHODS***

A systematic review of the literature was performed regarding LCPD etiology, using the following inclusion criteria: studies of any level of evidence, reporting clinical or preclinical results and dealing with the etiology or pathogenesis of LCPD. Two reviewers searched the PubMed and Science Direct databases from their date of inception to the 20th of May 2018 in accordance with the Preferred Reporting Items for Systemic Reviews and Meta-Analyses guidelines. To achieve the maximum sensitivity of the search strategy, we combined the terms: ‘‘Perthes disease OR LCPD OR children avascular femoral head necrosis” with “pathology OR etiology OR biomechanics OR genetics” as either key words or MeSH terms.

***RESULTS***

We include 64 articles in this review. The avaiable evidence on LCPD etiology is still debated. Several hypotheses have been researched but none of them was found decisive. While emerging evidence showed the role of environmental risk factors and evidence emerging from twin studies do not support a major role for genetic factors, a congenital or acquired predisposition can not be excluded in disease pathogenesis.
One of the most supported theories involve mechanical induced ischemia which then evolves in avascular necrosis of the femoral head in sensible patients.

***CONCLUSION***

The literature available on the etiology of LCPD presents major limitations in terms of great heterogeneity and a lack of high-profile studies. Although a lot of studies focused on the genetic, biomechanical, and radiological background of the disease there is a lack of consensus on one or multiple major actors of the etiopathogenesis. More studies are needed to understand the complex and multifactorial genesis of the avascular necrosis characterizing the disease.

**Key words**: Legg-Calvé-Perthes disease; etiology; pathogenesis; genetics; risk factors

**© The Author(s) 2019.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Legg-Calvé-Perthes disease is a complex disease affecting the epiphysis of the femoral head in the paediatric population. Historically considered as an osteochondrosis, it is now being referred to as an idiopathic avascular necrosis of the femoral head in the paediatric population. Despite the etiology of the disease having been widely researched, it is still not fully understood. The major hypothesis relies on a multifactorial genesis involving mechanical, genetic, and systemic conditions. Further studies are necessary to understand the complex and multifactorial genesis of the avascular necrosis characterizing the disease.

Pavone V, Chisari E, Vescio A, Lizzio C, Sessa G, Testa G. etiology of Legg-Calvé-Perthes disease: a systematic review. *World J Orthop* 2019; In press

**Introduction**

Legg-Calvé-Perthes disease (LCPD) is a complex disease affecting the epiphysis of the femoral head in the paediatric population. Historically considered as an osteocondrosis, it is now being referred to as an idiopathic avascular necrosis of the femoral head in the paediatric population. Among its prevalence there is no general agreement. It is set to be between 0.4/100000 to 29.0/100000 children < 15 years of age with a peak of incidence in children aged from 4 to 8 years and a male/female ratio of 5:1[1,2]. A high profile epidemiological study held in 2017[3] involving 2.1 million individuals attempted to report a more accurate prevalence of this disease. An overall prevalence of 9.3 per 100000 subjects was found. The male/female ratio was 3.1:1. Even though the study was conducted in Sweden from 1973 to 1993 this is one of the most up-to-date sources of evidence of LCPD epidemiology.

Despite the etiology of the disease having been widely researched, it is still not fully understood. While the major hypothesis relies on a multifactorial genesis, several hypotheses involving mechanical, genetic, and systemic condition have been proposed to explain the pathogenesis of the femoral head osteonecrosis. The best-supported theory involves interference with normal blood supply to epiphysis due to repetitive mechanical stress[4,5]. There is no consensus for the optimum treatment. The aim of treatment is to maintain the sphericity of the femoral head and the congruency of the femur-acetabulum relationship to prevent secondary degenerative arthritis, which eventually lead to total hip arthroplasty in 5% of cases[6]. Early diagnosis and management can help prevent the collapse of the femoral head, progressive femoral head deformity, and impingement[7].

Children who have a skeletal age of 6.0 years or less at the onset of the disease do well without treatment[8]. Operative treatment should be considered in children who are six years old or older and have over 50% femoral head necrosis when the diagnosis is made[9].

**MATERIALS AND METHODS**

***Literature search strategy***

We conducted this systematic review according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[10]. A systematic review of two medical electronic databases (PubMed and Science Direct) was performed by two independent authors from their date of inception to May 20, 2018. To achieve the maximum sensitivity of the search strategy, we combined the terms: ‘‘Perthes disease OR LCPD OR children avascular femoral head necrosis” with “pathology OR etiology OR biomechanics OR genetics” as either key words or MeSH terms. The reference lists of all retrieved articles were reviewed for further identification of potentially relevant studies and assessed using the inclusion and exclusion criteria.

***Selection criteria***

Eligible studies for the present systematic review included those dealing with the etiology of LCPD. The initial titles and abstracts screening was made using the following inclusion criteria: studies of any level of evidence, written in English, reporting clinical or preclinical results, published in peer review journals and dealing with the etiology of LCPD. Exclusion criteria were articles written in other languages or studies with a focus on secondary/LCPD-like diseases caused by systemic conditions such as sickle-cell disease, inflammatory disease, the effects of chemotherapy, radiation or prolonged steroid use. We also excluded all the remaining duplicates, articles dealing with other topics, those with poor scientific methodology or without an accessible abstract. Reference lists were also hand-searched for further relevant studies. All publications were limited to *in vivo*, *in vitro*, animal and human studies in the English language. Abstracts, case reports, conference presentations, editorials and expert opinions were excluded.

***Data extraction and criteria appraisal***

All data were extracted from article texts, tables and figures. Two investigators independently reviewed each article. Discrepancies between the two reviewers were resolved by discussion and consensus. The final results and any remaining controversy on the reviewed article were reviewed and discussed with senior investigators.

***Risk of bias assessment***

In this systematic review risk of bias assessment of the *in vitro* studies was not performed as there is no accepted grading scale for such studies. Risk of bias assessment of all *in vivo* selected full-text articles was performed according to the ROBINS-I tool for non-randomized studies[11]. The ROBINS-I tool consist of three stage assessment of the studies included. First stage regards the planning of the systematic review, the second stage is the assessment of the common bias possibly found in these studies and the latter is about the overall risk of bias (Table 1).

The assessments were performed by 3 authors independently. Any discrepancy was discussed with the senior investigator for the final decision. All the rater agreed on the final result of every stages of the assessment.

**Results**

***Included studies***

A total of 1630 articles were found. After the exclusion of duplicates 1078 articles were selected. At the end of the first screening, following the previously described selection criteria, we selected 130 articles eligible for full text reading. Ultimately, after full text reading, and reference list check, we selected n=64 articles following previous written criteria. A PRISMA[10] flowchart of the method of selection and screening is provided (Figure 1). The included articles[11–85] mainly focus on genetic research, epidemiological studies, magnetic resonance imaging analysis and histological histochemical analysis. The main findings of the included articles were summarized (Tables 1-4).

***Smoking***

While other environmental factors may be present, smoking seems to one of the most reported risk factor for developing LCPD[11–16]. In particular, Perry *et al*[12] recently showed how maternal smoking can affect the risk of developing the disease in a case control study. In addition to this, other four studies[13–16] report evidence of the association between environmental smoke and LCPD, both during maternal pregnancy and the childhood of the patient. Lastly, a study involving 852 patient showed how the smoking habit augmented the risk for LCPD by 100% in the examination sample[17].

***Socioeconomical deprivation***

Three different studies[14,18,19] involving an overall 340 LCPD patients, explored the link between the disease and socioeconomic deprivation without revealing a significant association. On the contrary, Kealey *et al*[20], investigating 311 patients, found a higher prevalence among the most deprived rural category. In 2012 another study[21] analysed the incidence of LCPD between 1990 and 2008 in children ages 0-14 years in the United Kingdom. They reported how the incidence was coherently higher within the quintile with the highest degree of deprivation (RR = 1.49, 95%CI: 1.10–2.04) (*P* < 0.01). Five more studies[22–26] held in United Kingdom by the same research group reported similar results.

***Low birth weight***

Metcalfe *et al*[27] reported an increased presence of low birth weight in children affected by LCPD. Similarly to the results provided by Lappin *et al*[28], Sharma *et al*[18] reported an association between low birth weight and LCPD, whereas the weight of the children at the moment of the diagnosis and follow up did not shown significant alteration. This was further supported by other studies with similar results[26]. On the contrary, Bahmanyar *et al*[17] reported a possible association between low birth weight and LCPD, but that was shown to be insignificant after evaluating other risk factors like maternal smoking. Only really low birth weight (< 1500 g) seemed to be independently associated with LCPD. Another nationwide study[29] held in Norway and involving 425 patients reported similar results. Their results supported the presence of environmental or genetic factors but not the low birthweight. Similar results were reporter by other epidemiological studies[12–16].

***attention deficit hyperactivity disorder and psychological burden***

Loder *et al*[30] in 1993 studied the association between attention deficit hyperactivity disorder (ADHD) and LCPD in 24 patients. He reported the presence of one third (33%) of the children with abnormally high scores in profiles associated with ADHD. Perry *et al*[31,32] investigated both general practise registry of comorbidities in LCPD patients and the incidence of behavioural disturbance. ADHD was not associated with Perthes' disease (OR = 1.01, 95%CI: 0.48-2.12) in the first study[31] while in the second one[32], a case control study involving 146 cases of LCPD and 142 hospital controls, was reported the presence of behavioural disturbance.

 In 2015 a prospective study[33] researched in 58 adolescents patients (11 with LCPD) undergoing hip preservation surgery the presence of psychological disturbances. A significant presence of depression and anxiety symptoms was reported. Bergman *et al*[34] investigated ADHD in LCPD patients and in siblings of the child affected. A similar incidence was reported in both the groups. Hailer *et al* investigated the prevalence of the disease in two high profile studies held in 2012[35] and in 2014[36], respectively. The first one involved 2579 LCPD patients and 13,748 controls. It investigated the risk of injury in both groups reporting a higher percentage in the LCPD group. The second one involved 4,057 individuals with LCPD in Sweden during the period 1964-2011 and 40,570 individuals without LCPD randomly selected from the Swedish general population and matched by year of birth, sex, and region. They reported that individuals with LCPD also had a raised hazard ratio (HR) of 1.5 (95%CI: 1.2-1.9) for ADHD.

 Türkmen *et al*[37], instead, did not find a significant difference in ADHD prevalence between LCPD group and control groups.

***Obesity and leptin***

A study held in 2016[38] reported a positive association between LCPD and obesity. It was associated with a more severe clinical presentation and femoral head deformity. Lee *et al*[39] have investigated the levels of leptin in LCPD patients compared to the control subject. A significantly higher value concordantly with the severity of the disease was reported.

Familiarity and genetic role

In the years, several cases of LCPD in the same family have been reported[40,41]. This suggested evidence of a genetic role in the development of the disease. In particular Miyamoto *et al*[42] were the first to report a case of familiar LCPD associated with a mutation of the Collagen type II gene (*COL2A1*). Thus, *COL2A1* genes were proposed as potential pathogenic trigger of LCPD. Several case reports found the same evidence of this association in LCPD patients[43,44]. Further studies investigated the relationship between this gene mutation and LCPD. Su *et al*[45] recruited forty-two members of a 5-generation family and found in sixteen patients a p.Gly1170Ser mutation of *COL2A1* cosegregated with LCPD, precocious hip osteoarthritis or avascular femoral head necrosis not linked with LCPD. Li *et al*[46] in 2014 held a study including a four-generation family reporting the presence of the mutation in six affected family members.

Metcalfe *et al*[27] investigated the presence of genetic factors using the information derived from the Danish Twin Registry. After studying 81 twin pairs (10 monozygotic, 51 dizygotic, and 20 unclassified) concordance with LCPD, they concluded that the absolute risk that a co-twin of an affected individual will develop LCPD is low, even in the case of monozygotic twin pairs. While Metcalfe *et al*[27] did not find an association between LCPD and birth weight, another twin study[28] and a case control study[17] involving an overall of 320 twin patient and 852 patients, respectively, concluded that low birth weight may play a role in developing the disease.

Coagulation disturbance

A meta-analysis[47] held in 2012 investigated factor V Leiden, prothrombin II and methylenetetrahydrofolate reductase (MTHFR) polymorphism as possible genetic etiology of LCPD. They included 12 case–control studies including 824 children in the Perthes group and 2033 children in the control group. Factor V Leiden polymorphism (carrying the minor A allele instead the G allele) was associated with a three times higher incidence of the disease. Prothrombin II polymorphism (A allele instead of G allele) reported giving a 1.5-fold increase risk of disease. There was no association between MTHFR polymorphism and Perthes disease. In 2015 Srzentic *et al*[48] investigating genes expression and variants by quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) reported no difference between LCPD patients and controls for Factor V Leiden, Factor II, MTHFR and Plasminogen activator inhibitor-1 (PAI-1).
 Also a prospective study held in 2002 do not suggest that thrombotic diatheses due to deficiency of protein C, protein S, or antithrombin III or due to factor-V Leiden mutation are major causes of Legg-Perthes disease[49].

Inflammation markers

Liu *et al*[50] analysed age- and sex-matched serum samples from 10 control subjects and 10 patients with LCPD. They reported a higher presence of proteins and factors linked to complement and coagulation cascade. A higher activity of these factors may contribute to LCPD etiology. In 2014 Srzentić *et al*[51] studied the association of frequencies of genetic variants of immune response genes with LCPD. They found significantly over-represented heterozygous subjects with an IL-6 polymorphism (G-174C/G-597A) in the LCPD group. In 2015 another studied reported a significantly increased IL-6 protein level in the synovial fluid of the hips affected by LCPD [52].

Apoptosis factors

Srzentić *et al*[48] investigated the expression of apoptosis genes by the qRT-PCR technique in 37 patients. They reported a higher presence of proapoptotic factor Bcl-2-associated X protein (Bax) along with a significantly higher Bax/Bcl-2 ratio in the patient group. Zhang *et al*[49] found, in a rat model, similar results with an higher expression of apoptotic genes, Casp3, Casp8 and Casp9, elevated in chondrocytes after hypoxia.

**Mechanical stress and ischemia damage:** Ischemia damage was reported starting by the first research document we have on LCPD and its pathology insight[53,54]. In 1976, one of the first studies[55] about the etiology of LCPD reported the presence of areas of infarction in 51 per cent of hips histopathologically examined through fifty-seven cases of femoral head biopsy. Calver *et al*[56] examined radiologically the ischemia areas in the hips of 50 children. The five found with evidence of ischemia areas developed LCPD within six months. The other 45 were healthy at one year follow up. Catteral *et al*[57] and Ponseti *et al*[58], respectively, reported an area of ischemia in two children’s biopsies and morpho-structural alteration possibly associated with ischemic damage in two different historical studies. In addition, several animal studies were conducted. A study held in 1983[59] investigated how joint tamponade in pigs would provoke ischemia of the epiphyseal plate. The necessity of high and prolonged pressure, which is difficult to achieve in normal conditions, was reported. Several research reported evidence of ischemia damage through radiological technique[60–64]. This provides evidence of its role in the enteropathogenesis of the LCPD. Recently, Pinheiro *et al*[65] proposed a biomechanical model which further support a combined role of both ischemic condition, skeletal immaturity and altered biomechanics. Also studies on piglets hip osteonecrosis confirmed this etiology[66–69].

Mechanical stress-induced ischemia was found to be a possible etiology of LCPD also using several animal models[70–72] and experimental analysis model[65,73,74]. In one particular study held by Suehiro *et al*[49] involving Wistar Kyoto rats reported how repetitive mechanical stress on the femoral heads from 5 to 9 weeks of age played an important role in the etiology of osteonecrosis. This theory is historically and currently one of the most supported[54,61,75–77].

**Delayed epiphysial growth and hip geometry alterations:** In 2003, Kitoh *et al*[78] investigated the epiphyseal height (EH) and width (EW) of the unaffected hip in 125 children affected by LCPD. A positive linear correlation (R = 0.87) was observed in the EH:EW ratio in these patients. A smaller EH than expected for EW in our series indicated epiphyseal flattening of the femoral head in LCPD cases supports the hypothesis that a delay in endochondral ossification in the proximal capital femoral epiphysis may be associated with the onset of Perthes' disease.

In 2014, Kocjančič *et al*[79] investigated the resultant hip force and contact hip stress distribution in a population of 135 adult hips of patients who had been treated for LCPD. Contra-lateral hips with no record of disease were taken control. The contact hip stress gradient index was 148% higher (*p* < 0.001); expressing an unfavourable, steep decrease of contact stress at the lateral acetabular rim. On the contrary, Pinheiro *et al*[80] reported how the anatomical variations appear to have only a limited effect on the stress distribution in the femoral epiphysis, even during high impact activities, and even in the presence of a skeletally immature epiphysis. However, the same group recently published a study[65] on a new biomechanical model, which reported a possible involvement of vascular obstruction to the epiphysis that may arise when there is delayed ossification and when articular cartilage has reduced stiffness under compression.

**vascular endothelial growth factor and HIF-1:** In 2004, an animal study[49] involving piglets with avascular necrosis reported an increased vascular endothelial growth factor (VEGF) protein and mRNA expression, respectively, in the epiphyseal cartilage of the infarcted heads compared with the contralateral normal heads. Therefore, VEGF upregulation in the proliferative zone after ischemic damage was proposed as possible stimulator of vascular invasion and granulation tissue formation. Zhang *et al*[81] reported also an higher expression of VEGF and hypoxia-inducible factor (HIF-1) in rat models chondrocytes.

VEGF was found to be low in LCPD patients in Indian population-based study[49]. In 2014 28 LCPD cases (mean age: 8 ± 3.8) and 25 healthy age-matched control subjects investigated VEGF, endothelial progenitor cell and immunoglobulins does not achieve a significantly higher level of correlation (*p* = 0.354). The endothelial progenitor cell count was inversely correlated with serum IgG levels of the LCPD group (*r* = 0.403, *p* = 0.03). The absolute endothelial progenitor cell count was also significantly higher in the fragmentation stage than in the healing stage and they were also greater in bilaterally affected cases than in unilaterally affected patients. These result were found also in pigs model[82] and Sprague-Dawley rats[81]. In a rat model, however, the interruption of vascularization in the proximal femoral growth plate was not followed by a diffuse damage.

**Insulin growth factor 1:** The role of insulin growth factor 1 (IGF-1) role in LCPD etiology was investigated. In particular, a study in spontaneously hypertensive rat (SHR) demonstrates an altered IGF-I expression during early postnatal life and suggests that the altered IGF-I expression may cause the mechanical vulnerability of the femoral epiphysis. Low levels of serum IGF-1 and IGF-1 binding protein 3 have been reported in patients with LCPD[83,84]. However, these results conflict with another two studies, which reported normal IGF-1 binding protein levels[85,86].

**Discussion**

The etiology of LCPD is still mainly unknown. The heterogeneous prevalence reported opens the discussion to the examination of the possible environmental and social factors involved in the etiology of the disease[1–3,29]. This was further supported by three studies[21,26,87], which reported a possible association between the decrease of the incidence and lifestyle changes over the recent years, exposure to environmental risk factors like smoking, delayed epiphyseal ossification, low birthweight, child deprivation and obesity. However, the results are not definitive.

Several studies reported strong evidence supporting the role of social and economic deprivation in the incidence of LCPD in children[14,17,28,18–22,24–26]. This was proposed as a possible consequence of two factors: low birth weight and smoking habits. In addition, maternal smoking is associated with a low birthweight[88,89]. Thus, low birthweight could be often falsely associated with LCPD because of the smoking habit typically present in these families, which is the more probable cause of the higher incidence of LCPD. For these reasons, the role of low birth weight lacks strong evidence[12–17,29] and the smoking habit, being more common in social-economical deprived families, may play a more important role in etiopathogenesis of LCPD. This was further confirmed by studies investigating the association between smoking and LCPD, which reported a significant augmentation of the risk in smokers and smoke-exposed family. The mechanism proposed is the smoke-dependent damage of vessel endothelium, which could ultimately lead to epiphyseal infarction[17].

In addition, recent evidence supports obesity as a major risk factor[38]. Leptin is a hormone that is expressed in adipose tissue and, at lower levels, in gastric epithelium and placenta associated with both obesity and bone metabolism[90,91]. It has been reported that both leptin and obesity are positively associated with the severity of LCPD[38,39]. Most obese humans have very high plasma leptin concentrations, suggesting they are resistant to its anorectic and metabolic effects[92]. Based on these findings, Bartell *et al*[93] conducted a study involving intracerebroventricular (ICV) and subcutaneous (SC) administration of leptin in leptin-deficient ob/ob mice, investigating its effect on bone and muscular tissues. In both experiments, leptin had a key role in decrease of body weight, food intake, and body fat and in increase of muscle mass, bone mineral density, bone mineral content, bone area, marrow adipocyte number, and mineral apposition rate. Thus, leptin administration seemed to be real effective in obese patients bone metabolism. Following this rationale, Zhou *et al*[94] in 2015 tested these effects in a LCPD obese rat model. At six weeks post-surgery induced avascular necrosis of the femoral head, radiologic and histomorphometric assessments were performed. Radiographs showed better preservation of the femoral head architecture in the leptin-treated group. Histology and immunohistochemistry revealed that the leptin group had significantly increased osteoblastic proliferation and vascularity in infarcted femoral heads compared with control groups. The mechanism proposed is linked to both a direct action of leptin on bone metabolism and an indirect action through the upregulation of VEGF[95]. In particular, leptin acts physiologically through MAPK/ERK 1/2 and PI-3K/AKT1 pathway and a series of transcription factors such as HIF-1α[92,96]. Emerging evidence supports how leptin and obesity may play a role in LCPD etiopathogenesis. We strongly encourage further studies on leptin and obesity, associated with their effects of bone metabolism, and in LCPD patients should be conducted both as therapeutic option and as possible actor in the etiology of the disease.

The dissimilarity in incidence between male and female subjects was initially thought to rely on the different etiopathogenesis of the disease. In particular, pioneer studies on these differences reported a more severe presentation and prognosis in female patients compared to male subjects[97]. However, recent high profile epidemiological studies reported evidence of a lack of significant differences in clinical presentation, outcome and prognosis between boys and girls[98,99]. Thus, the LCPD male/female ratio appears not to be associated with different clinical presentation and should not be part of clinical treatment algorithm.

While the reviewed literature provided evidence of cases of LCPD running in families[41–46], and epidemiological studies suggested a genetic role[29], twin studies[27,28] reported evidence of the absence of significant concordance. This was further by another recent study[48] involving 37 patients and 100 controls which reported no difference in genotype variants and expression of coagulation and inflammatory factors. However, Zheng *et al*[100] investigated the relationship between global DNA methylation involving 82 children with LCPD and 120 matched controls. They reported significant differences in the global methylation of peripheral blood DNA between patients with LCPD and matched controls. Further high-profile epigenetic study in key tissues should be conducted providing new protagonist of the LCPD etiology. *COL2A1* alterations were also investigated. Several case reports and studies were reported in our review[42–46]. Their association with the disease was found to be weak in a case study published by Kenet *et al*[101] involving 119 LCPD affected children and 276 controls, further supported by a review of the literature. In the same study the absence of significant association with Gaucher's disease and Factor V Leiden alterations was also reported. On the contrary, further histological and ultrastructural studies found how p.Gly1170Ser mutation of *COL2A1* is involved in the pathogenesis of a type II collagenopathy[55]. This alteration leads to an amino acid change that perturbs a Gly-X-Y triple-helix repeat, which is a fundamental structure in type II collagen function[102]. Thus, *COL2A1* alterations should be further studied to clarify their role in pathogenesis and etiology of the LCPD.

Besides factor V Leiden and Prothrombin II polymorphisms being associated with a three times higher likelihood of disease and a 1.5-fold increase of risk, the results were not statistically significant[47]. Thus, even though the topic was extensively studied, recent evidence of hypercoagulability is inconclusive. Baltzer *et al*[103] reported a case of a child affected by Kienbock’s disease and factor V thrombophilia who developed LCPD. The patient’s hypercoagulability state may link with the bilateral manifestation of Perthes disease. After a further review of the literature, the authors also found two other cases of multifocal osteonecrosis and hypercoagulable disorder in adult patients. We therefore encourage further molecular and clinical studies on hypercoagulative states and LCPD.

Among the studied molecules, inflammatory cytokine involved in immune responses provide a crucial prospective of research. In particular, a study [51] found how a polymorphism of IL-6, proinflammatory cytokine, involved in chronic inflammation and immune response[104], was found to be associated with LCPD. In addition, another study[52] reported a high synovial level of IL-6 in LCPD patients. This suggests that IL-6 is a possible future protagonist of new therapeutic approaches and translational research. We strongly encourage further studies on the IL-6 pathway. Another molecule studied is SIRT1, the mammalian ortholog of the yeast SIR2 (Silencing Information Regulator) and a member of the Sirtuin family. It was reported that it can inhibit the NF-kB transactivational activity by deacetylating Lys310 of the RelA/p65 subunits. Thus, SIRT1 inhibits both its transcriptional activity and the release of inflammatory cytokines mediated by NF-kB[105]. What emerged in a recent research project held in 2017 is that treatment with IFN-β increased SIRT1 expression and inhibited secretion of IL-6 in avascular femoral head necrosis mouse model. IFN-β activated SIRT1 in the RAW 264.7 cell and bone marrow-derived osteoclasts decreasing IL-6 secretion. What we can assume from these papers is that IL-6, NF-kB, SIRT1 and other molecules involved in the autoimmune response could be linked with LCPD etiology and should be further investigated both as a possible cause of the disease and as a new potential therapeutic target.

A study of 2003[78] found how also retarded epiphyseal ossification could be linked to LCPD. However there is lack of consensus on the role of these anatomical alteration[65,79,80]. During development, the epiphyseal blood supply is almost exclusively provided by the deep branch of the medial femoral circumflex artery (MFCA)[75]. Both imaging and histological studies have shown a partial or complete loss of blood flow[61,77] and the development of ischemic necrosis [54] of the femoral head in LCPD. Mechanical-induced ischemia is one of the most supported hypotheses for the etiology of LCPD[54,61,75–77]. Emerging evidence is supporting a role of the repetitive mechanical stress to the epiphysis and de subsequent ischemia, as stated in different experimental model[65–69,73,74]. In addition, the association with ADHD[30–34,36] and hyperactive modification of behaviour could be easily explained with repetitive activity-induced mechanical stress. This was further explained with an higher risk of injury[35] in LCPD patients. The recent evidence strongly supports the role of mechanical stress in the etiology of LCPD. We encourage high profile clinical studies to investigate more this etiopathogenesis.

Our study has some strength. We extensively searched and identified all relevant genetic association studies, the most common comorbid and the possible etiologic hypothesis. Therefore, risk of bias assessment showed moderate overall risk which could influence our analysis.

The literature available on the etiology of LCPD presents major limitations in terms of great heterogeneity and lack of high-profile studies. Although a lot of studies focused on the genetic, biomechanical, and radiological background of the disease, there is lack of consensus on one or multiple major actors of the etiopathogenesis. While obesity, smoking exposure and child deprivation seems to be associated with LCPD etiology, more studies are needed to understand the complex and multifactorial genesis of the avascular necrosis characterizing the disease.

**Article Highlights**

***Research background***

Legg-Calvé-Perthes disease (LCPD) is a complex disease with a multifactorial etiology. The etiopathogenesis of the disease was widely investigated in the last 20 years but it is still unknown.

***Research motivation***

Numerous studies tried to explain the major actors in LCPD etiology but there is a lack of synthesis of the evidence.

***Research objectives***

The purpose of the study was to summarize the current evidence on the etiology of LCPD.

***Research methods***

Two databases (Pubmed and Science Direct) were systematically searched for relevant article by two independent reviewers from their date of inception to the 20th of May 2018. Every step of the review was done according to PRISMA guidelines. Due to article heterogeneity and the topic after data analysis, a descriptive (synthesis) analysis was performed.

***Research results***

Sixty four articles were included in this systematic review after applying our inclusions and exclusion criteria. Available evidence on LCPD etiology is still inconclusive. Several hypotheses have been researched but none of them was found decisive.

***Research conclusions***

After our systematic review of the available evidence we conclude that LCPD etiology rely on a multifactorial basis where environment in genetical predisposed patients participate in the pathogenesis of the disease.

***Research perspectives***

Further clinical and preclinical studies are strongly encouraged to understand better the mechanical and vascular basis of the etiopathogenesis of the disease. Interesting perspective from studies on Leptin, obesity, and mechanical trauma were found and should be further investigated.

**References**

1 **Loder RT**, Skopelja EN. The epidemiology and demographics of legg-calvé-perthes' disease. *ISRN Orthop* 2011; **2011**: 504393 [PMID: 24977062 DOI: 10.5402/2011/504393]

2 **Perry DC**, Hall AJ. The epidemiology and etiology of Perthes disease. *Orthop Clin North Am* 2011; **42**: 279-283, v [PMID: 21742139 DOI: 10.1016/j.ocl.2011.03.002]

3 **Johansson T**, Lindblad M, Bladh M, Josefsson A, Sydsjö G. Incidence of Perthes' disease in children born between 1973 and 1993. *Acta Orthop* 2017; **88**: 96-100 [PMID: 27587239 DOI: 10.1080/17453674.2016.1227055]

4 **Guerado E**, Caso E. The physiopathology of avascular necrosis of the femoral head: an update. *Injury* 2016; **47** Suppl 6: S16-S26 [PMID: 28040082 DOI: 10.1016/S0020-1383(16)30835-X]

5 **Douglas G**, Rang M. The role of trauma in the pathogenesis of the osteochondroses. *Clin Orthop Relat Res* 1981; **(158)**: 28-32 [PMID: 7273524 DOI: 10.1097/00003086-198107000-00005]

6 **Larson AN**, Sucato DJ, Herring JA, Adolfsen SE, Kelly DM, Martus JE, Lovejoy JF, Browne R, Delarocha A. A prospective multicenter study of Legg-Calvé-Perthes disease: functional and radiographic outcomes of nonoperative treatment at a mean follow-up of twenty years. *J Bone Joint Surg Am* 2012; **94**: 584-592 [PMID: 22488614 DOI: 10.2106/JBJS.J.01073]

7 **Shah H**. Perthes disease: evaluation and management. *Orthop Clin North Am* 2014; **45**: 87-97 [PMID: 24267210 DOI: 10.1016/j.ocl.2013.08.005]

8 **Herring JA**, Kim HT, Browne R. Legg-Calve-Perthes disease. Part II: Prospective multicenter study of the effect of treatment on outcome. *J Bone Joint Surg Am* 2004; **86-A**: 2121-2134 [PMID: 15466720 DOI: 10.1097/BPO.0000000000000157]

9 **Wiig O**, Terjesen T, Svenningsen S. Prognostic factors and outcome of treatment in Perthes' disease: a prospective study of 368 patients with five-year follow-up. *J Bone Joint Surg Br* 2008; **90**: 1364-1371 [PMID: 18827249 DOI: 10.1302/0301-620X.90B10.20649]

10 **Moher D**, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009; **6**: e1000097 [PMID: 19621072 DOI: 10.1371/journal.pmed.1000097]

11 **Sterne JA**, Hernán MA, Reeves BC, Savović J, Berkman ND, Viswanathan M, Henry D, Altman DG, Ansari MT, Boutron I, Carpenter JR, Chan AW, Churchill R, Deeks JJ, Hróbjartsson A, Kirkham J, Jüni P, Loke YK, Pigott TD, Ramsay CR, Regidor D, Rothstein HR, Sandhu L, Santaguida PL, Schünemann HJ, Shea B, Shrier I, Tugwell P, Turner L, Valentine JC, Waddington H, Waters E, Wells GA, Whiting PF, Higgins JP. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. *BMJ* 2016; **355**: i4919 [PMID: 27733354 DOI: 10.1136/bmj.i4919]

12 **Perry DC**, Thomson C, Pope D, Bruce CE, Platt MJ. A case control study to determine the association between Perthes' disease and the recalled use of tobacco during pregnancy, and biological markers of current tobacco smoke exposure. *Bone Joint J* 2017; **99-B**: 1102-1108 [PMID: 28768789 DOI: 10.1302/0301-620X.99B8.BJJ-2016-1282.R1]

13 **Daniel AB**, Shah H, Kamath A, Guddettu V, Joseph B. Environmental tobacco and wood smoke increase the risk of Legg-Calvé-Perthes disease. *Clin Orthop Relat Res* 2012; **470**: 2369-2375 [PMID: 22090357 DOI: 10.1007/s11999-011-2180-8]

14 **Gordon JE**, Schoenecker PL, Osland JD, Dobbs MB, Szymanski DA, Luhmann SJ. Smoking and socio-economic status in the etiology and severity of Legg-Calvé-Perthes' disease. *J Pediatr Orthop B* 2004; **13**: 367-370 [PMID: 15599226 DOI: 10.1097/01202412-200411000-00003]

15 **García Mata S**, Ardanaz Aicua E, Hidalgo Ovejero A, Martinez Grande M. Legg-Calvé-Perthes disease and passive smoking. *J Pediatr Orthop* 2000; **20**: 326-330 [PMID: 10823599 DOI: 10.1097/01241398-200005000-00011]

16 **Glueck CJ**, Freiberg RA, Crawford A, Gruppo R, Roy D, Tracy T, Sieve-Smith L, Wang P. Secondhand smoke, hypofibrinolysis, and Legg-Perthes disease. *Clin Orthop Relat Res* 1998; **(352)**: 159-167 [PMID: 9678044 DOI: 10.1097/00003086-199807000-00019]

17 **Bahmanyar S**, Montgomery SM, Weiss RJ, Ekbom A. Maternal smoking during pregnancy, other prenatal and perinatal factors, and the risk of Legg-Calvé-Perthes disease. *Pediatrics* 2008; **122**: e459-e464 [PMID: 18625663 DOI: 10.1542/peds.2008-0307]

18 **Sharma S**, Sibinski M, Sherlock DA. A profile of Perthes' disease in Greater Glasgow: is there an association with deprivation? *J Bone Joint Surg Br* 2005; **87**: 1536-1540 [PMID: 16260675 DOI: 10.1302/0301-620X.87B11.16608]

19 **Pillai A**, Atiya S, Costigan PS. The incidence of Perthes' disease in Southwest Scotland. *J Bone Joint Surg Br* 2005; **87**: 1531-1535 [PMID: 16260674 DOI: 10.1302/0301-620X.87B11.16744]

20 **Kealey WD**, Moore AJ, Cook S, Cosgrove AP. Deprivation, urbanisation and Perthes' disease in Northern Ireland. *J Bone Joint Surg Br* 2000; **82**: 167-171 [PMID: 10755420 DOI: 10.1302/0301-620X.82B2.10033]

21 **Perry DC**, Bruce CE, Pope D, Dangerfield P, Platt MJ, Hall AJ. Legg-Calvé-Perthes disease in the UK: geographic and temporal trends in incidence reflecting differences in degree of deprivation in childhood. *Arthritis Rheum* 2012; **64**: 1673-1679 [PMID: 22143958 DOI: 10.1002/art.34316]

22 **Perry DC**, Bruce CE, Pope D, Dangerfield P, Platt MJ, Hall AJ. Perthes' disease of the hip: socioeconomic inequalities and the urban environment. *Arch Dis Child* 2012; **97**: 1053-1057 [PMID: 23104772 DOI: 10.1136/archdischild-2012-302143]

23 **Perry DC**, Bruce CE, Pope D, Dangerfield P, Platt MJ, Hall AJ. Perthes' disease: deprivation and decline. *Arch Dis Child* 2011; **96**: 1124-1128 [PMID: 22080458 DOI: 10.1136/archdischild-2011-300413]

24 **Hall AJ**, Barker DJ. Perthes' disease in yorkshire. *J Bone Joint Surg Br* 1989; **71**: 229-233 [PMID: 2925740]

25 **Hall AJ**, Barker DJ, Dangerfield PH, Taylor JF. Perthes' disease of the hip in Liverpool. *Br Med J (Clin Res Ed)* 1983; **287**: 1757-1759 [PMID: 6416578 DOI: 10.1136/BMJ.287.6407.1757]

26 **Margetts BM**, Perry CA, Taylor JF, Dangerfield PH. The incidence and distribution of Legg-Calvé-Perthes' disease in Liverpool, 1982-95. *Arch Dis Child* 2001; **84**: 351-354 [PMID: 11259241 DOI: 10.1136/adc.84.4.351]

27 **Metcalfe D**, Van Dijck S, Parsons N, Christensen K, Perry DC. A Twin Study of Perthes Disease. *Pediatrics* 2016; **137**: e20153542 [PMID: 26908702 DOI: 10.1542/peds.2015-3542]

28 **Lappin K**, Kealey D, Cosgrove A, Graham K. Does low birthweight predispose to Perthes' disease? Perthes' disease in twins. *J Pediatr Orthop B* 2003; **12**: 307-310 [PMID: 12973037 DOI: 10.1097/01.bpb.0000079203.23239.2d]

29 **Wiig O**, Terjesen T, Svenningsen S, Lie SA. The epidemiology and aetiology of Perthes' disease in Norway. A nationwide study of 425 patients. *J Bone Joint Surg Br* 2006; **88**: 1217-1223 [PMID: 16943476 DOI: 10.1302/0301-620X.88B9.17400]

30 **Loder RT**, Schwartz EM, Hensinger RN. Behavioral characteristics of children with Legg-Calvé-Perthes disease. *J Pediatr Orthop* 1993; **13**: 598-601 [PMID: 8376559 DOI: 10.1097/01241398-199313050-00008]

31 **Perry DC**, Bruce CE, Pope D, Dangerfield P, Platt MJ, Hall AJ. Comorbidities in Perthes' disease: a case control study using the General Practice Research database. *J Bone Joint Surg Br* 2012; **94**: 1684-1689 [PMID: 23188912 DOI: 10.1302/0301-620X.94B12.29974]

32 **Perry DC**, Pope D, Bruce CE, Dangerfield P, Hall AJ, Platt MJ. Hyperactivity and the psychological burden of Perthes disease: a case-control study. *J Pediatr Orthop* 2013; **33**: 644-649 [PMID: 23812131 DOI: 10.1097/BPO.0b013e31829494c0]

33 **Podeszwa DA**, Richard HM, Nguyen DC, De La Rocha A, Shapiro EL. Preoperative psychological findings in adolescents undergoing hip preservation surgery. *J Pediatr Orthop* 2015; **35**: 253-257 [PMID: 24992348 DOI: 10.1097/BPO.0000000000000243]

34 **Berman J**, Aran A, Berenstein-Weyel T, Lebel E. Exploring the Association between Legg-Calvé-Perthes Disease and Attention Deficit Hyperactivity Disorder in Children. *Isr Med Assoc J* 2016; **18**: 652-654 [PMID: 28466612]

35 **Hailer YD**, Montgomery S, Ekbom A, Nilsson O, Bahmanyar S. Legg-Calvé-Perthes disease and the risk of injuries requiring hospitalization: a register study involving 2579 patients. *Acta Orthop* 2012; **83**: 572-576 [PMID: 23043293 DOI: 10.3109/17453674.2012.736167]

36 **Hailer YD**, Nilsson O. Legg-Calvé-Perthes disease and the risk of ADHD, depression, and mortality. *Acta Orthop* 2014; **85**: 501-505 [PMID: 25036717 DOI: 10.3109/17453674.2014.939015]

37 **Türkmen I**, Poyanlı O, Unay K, Kemah B, Akan K, Ulusan S. Association between attention deficit and hyperactivity disorder and Perthes disease. *Acta Orthop Traumatol Turc* 2014; **48**: 67-72 [PMID: 24643103 DOI: 10.3944/AOTT.2014.3248]

38 **Neal DC**, Alford TH, Moualeu A, Jo CH, Herring JA, Kim HK. Prevalence of Obesity in Patients With Legg-Calvé-Perthes Disease. *J Am Acad Orthop Surg* 2016; **24**: 660-665 [PMID: 27471901 DOI: 10.5435/JAAOS-D-16-00120]

39 **Lee JH**, Zhou L, Kwon KS, Lee D, Park BH, Kim JR. Role of leptin in Legg-Calvé-Perthes disease. *J Orthop Res* 2013; **31**: 1605-1610 [PMID: 23832827 DOI: 10.1002/jor.22415]

40 **O'Sullivan M**, O'Rourke SK, MacAuley P. Legg-Calvé-Perthes disease in a family: genetic or environmental. *Clin Orthop Relat Res* 1985; **(199)**: 179-181 [PMID: 4042476 DOI: 10.1007/BF00435451]

41 **Livesey JP**, Hay SM, Bell MJ. Perthes disease affecting three female first-degree relatives. *J Pediatr Orthop B* 1998; **7**: 230-231 [PMID: 9702675 DOI: 10.1097/01202412-199807000-00010]

42 **Miyamoto Y**, Matsuda T, Kitoh H, Haga N, Ohashi H, Nishimura G, Ikegawa S. A recurrent mutation in type II collagen gene causes Legg-Calvé-Perthes disease in a Japanese family. *Hum Genet* 2007; **121**: 625-629 [PMID: 17394019 DOI: 10.1007/s00439-007-0354-y]

43 **Al-Omran AK**, Sadat-Ali M. Legg-Calve-Perthes disease in two generations of male family members: a case report. *J Orthop Surg (Hong Kong)* 2013; **21**: 258-261 [PMID: 24014797 DOI: 10.1177/230949901302100230]

44 **Kannu P**, Irving M, Aftimos S, Savarirayan R. Two novel COL2A1 mutations associated with a Legg-Calvé-Perthes disease-like presentation. *Clin Orthop Relat Res* 2011; **469**: 1785-1790 [PMID: 21442341 DOI: 10.1007/s11999-011-1850-x]

45 **Su P**, Li R, Liu S, Zhou Y, Wang X, Patil N, Mow CS, Mason JC, Huang D, Wang Y. Age at onset-dependent presentations of premature hip osteoarthritis, avascular necrosis of the femoral head, or Legg-Calvé-Perthes disease in a single family, consequent upon a p.Gly1170Ser mutation of COL2A1. *Arthritis Rheum* 2008; **58**: 1701-1706 [PMID: 18512791 DOI: 10.1002/art.23491]

46 **Li N**, Yu J, Cao X, Wu QY, Li WW, Li TF, Zhang C, Cui YX, Li XJ, Yin ZM, Xia XY. A novel p. Gly630Ser mutation of COL2A1 in a Chinese family with presentations of Legg-Calvé-Perthes disease or avascular necrosis of the femoral head. *PLoS One* 2014; **9**: e100505 [PMID: 24949742 DOI: 10.1371/journal.pone.0100505]

47 **Woratanarat P**, Thaveeratitharm C, Woratanarat T, Angsanuntsukh C, Attia J, Thakkinstian A. Meta-analysis of hypercoagulability genetic polymorphisms in Perthes disease. *J Orthop Res* 2014; **32**: 1-7 [PMID: 23983171 DOI: 10.1002/jor.22473]

48 **Srzentić S**, Nikčević G, Spasovski D, Baščarević Z, Živković Z, Terzic-Šupić Z, Matanović D, Djordjević V, Pavlović S, Spasovski V. Predictive genetic markers of coagulation, inflammation and apoptosis in Perthes disease—Serbian experience. *Eur J Pediatr* 2015; **174**: 1085-1092 [PMID: 25754626 DOI: 10.1007/s00431-015-2510-z]

49 **Suehiro M**, Hirano T, Shindo H. Osteonecrosis induced by standing in growing Wistar Kyoto rats. *J Orthop Sci* 2005; **10**: 501-507 [PMID: 16193363 DOI: 10.1007/s00776-005-0927-3]

50 **Liu R**, Fan L, Yin L, Wang K, Miao W, Song Q, Dang X, Gao H, Bai C. Comparative study of serum proteomes in Legg-Calve-Perthes disease. *BMC Musculoskelet Disord* 2015; **16**: 281 [PMID: 26438379 DOI: 10.1186/s12891-015-0730-z]

51 **Srzentić S**, Spasovski V, Spasovski D, Zivković Z, Matanović D, Bascarević Z, Supić ZT, Stojiljković M, Karan-Djurasević T, Stanković B, Pavlović S, Nikcević G, Vukasinović Z. Association of gene variants in TLR4 and IL-6 genes with Perthes disease. *Srp Arh Celok Lek* 2014; **142**: 450-456 [PMID: 25233690 DOI: 10.2298/SARH1408450S]

52 **Kamiya N**, Yamaguchi R, Adapala NS, Chen E, Neal D, Jack O, Thoveson A, Gudmundsson P, Brabham C, Aruwajoye O, Drissi H, Kim HK. Legg-Calvé-Perthes disease produces chronic hip synovitis and elevation of interleukin-6 in the synovial fluid. *J Bone Miner Res* 2015; **30**: 1009-1013 [PMID: 25556551 DOI: 10.1002/jbmr.2435]

53 **Catterall A**, Pringle J, Byers PD, Fulford GE, Kemp HB, Dolman CL, Bell HM, McKibbin B, Rális Z, Jensen OM, Lauritzen J, Ponseti IV, Ogden J. A review of the morphology of Perthes' disease. *J Bone Joint Surg Br* 1982; **64**: 269-275 [PMID: 6807991]

54 **jonsater S**. Coxa plana; a histo-pathologic and arthrografic study. *Acta Orthop Scand Suppl* 1953; **12**: 5-98 [PMID: 13065067 DOI: 10.3109/ort.1953.24.suppl-12.01]

55 **Su P**, Zhang L, Peng Y, Liang A, Du K, Huang D. A histological and ultrastructural study of femoral head cartilage in a new type II collagenopathy. *Int Orthop* 2010; **34**: 1333-1339 [PMID: 20204389 DOI: 10.1007/s00264-010-0985-9]

56 **Calver R**, Venugopal V, Dorgan J, Bentley G, Gimlette T. Radionuclide scanning in the early diagnosis of Perthes' disease. *J Bone Joint Surg Br* 1981; **63-B**: 379-382 [PMID: 7263749 DOI: 10.1007/BF00267846]

57 **Catterall A**, Pringle J, Byers PD, Fulford GE, Kemp HB. Perthes' disease: is the epiphysial infarction complete? *J Bone Joint Surg Br* 1982; **64**: 276-281 [PMID: 7096391]

58 **Ponseti IV**, Maynard JA, Weinstein SL, Ippolito EG, Pous JG. Legg-Calvé-Perthes disease. Histochemical and ultrastructural observations of the epiphyseal cartilage and physis. *J Bone Joint Surg Am* 1983; **65**: 797-807 [PMID: 6863362 DOI: 10.2106/00004623-198365060-00011]

59 **Gershuni DH**, Hargens AR, Lee YF, Greenberg EN, Zapf R, Akeson WH. The questionable significance of hip joint tamponade in producing osteonecrosis in Legg-Calvé-Perthes syndrome. *J Pediatr Orthop* 1983; **3**: 280-286 [PMID: 6874923 DOI: 10.1097/01241398-198307000-00002]

60 **Royle SG**, Galasko CS. The irritable hip. Scintigraphy in 192 children. *Acta Orthop Scand* 1992; **63**: 25-28 [PMID: 1738964 DOI: 10.3109/17453679209154843]

61 **Lamer S**, Dorgeret S, Khairouni A, Mazda K, Brillet PY, Bacheville E, Bloch J, Penneçot GF, Hassan M, Sebag GH. Femoral head vascularisation in Legg-Calvé-Perthes disease: comparison of dynamic gadolinium-enhanced subtraction MRI with bone scintigraphy. *Pediatr Radiol* 2002; **32**: 580-585 [PMID: 12136349 DOI: 10.1007/s00247-002-0732-5]

62 **Atsumi T**, Yamano K, Muraki M, Yoshihara S, Kajihara T. The blood supply of the lateral epiphyseal arteries in Perthes' disease. *J Bone Joint Surg Br* 2000; **82**: 392-398 [PMID: 10813176 DOI: 10.1302/0301-620X.82B3.10193]

63 **de Camargo FP**, de Godoy RM Jr, Tovo R. Angiography in Perthes' disease. *Clin Orthop Relat Res* 1984; **(191)**: 216-220 [PMID: 6499314]

64 **Théron J**. Angiography in Legg-Calvé-Perthes disease. *Radiology* 1980; **135**: 81-92 [PMID: 7360984 DOI: 10.1148/radiology.135.1.7360984]

65 **Pinheiro M**, Dobson CA, Perry D, Fagan MJ. New insights into the biomechanics of Legg-Calvé-Perthes' disease: The Role of Epiphyseal Skeletal Immaturity in Vascular Obstruction. *Bone Joint Res* 2018; **7**: 148-156 [PMID: 29437587 DOI: 10.1302/2046-3758.72.BJR-2017-0191.R1]

66 **Cheon JE**, Yoo WJ, Kim IO, Kim WS, Choi YH. Effect of arterial deprivation on growing femoral epiphysis: quantitative magnetic resonance imaging using a piglet model. *Korean J Radiol* 2015; **16**: 617-625 [PMID: 25995692 DOI: 10.3348/kjr.2015.16.3.617]

67 **Li X**, Hu J, Zhen H, Tang L, Xu A. Early reversible ischemia of femoral head epiphysis in piglets on gadolinium-enhanced MRI: an experimental study. *J Huazhong Univ Sci Technolog Med Sci* 2006; **26**: 482-484 [PMID: 17120755 DOI: 10.1007/s11596-006-0428-4]

68 **Babyn PS**, Kim HK, Gahunia HK, Lemaire C, Salter RB, Fornasier V, Pritzker KP. MRI of the cartilaginous epiphysis of the femoral head in the piglet hip after ischemic damage. *J Magn Reson Imaging* 1998; **8**: 717-723 [PMID: 9626892 DOI: 10.1002/jmri.1880080331]

69 **Li X**, Qi J, Xia L, Li H, Hu J, Yu C, Pen W, Guan J, Hu D. Diffusion MRI in ischemic epiphysis of the femoral head: an experimental study. *J Magn Reson Imaging* 2008; **28**: 471-477 [PMID: 18666196 DOI: 10.1002/jmri.21458]

70 **Levin D**, Norman D, Zinman C, Misselevich I, Reis DN, Boss JH. Osteoarthritis-like disorder in rats with vascular deprivation-induced necrosis of the femoral head. *Pathol Res Pract* 1999; **195**: 637-647 [PMID: 10507084 DOI: 10.1016/S0344-0338(99)80129-0]

71 **Kandzierski G**. Remarks on the etiology and pathogenesis of Perthes' disease: an experiment-based hypothesis. *Ortop Traumatol Rehabil* 2004; **6**: 553-560 [PMID: 17618202]

72 **Suehiro M**, Hirano T, Mihara K, Shindo H. Etiologic factors in femoral head osteonecrosis in growing rats. *J Orthop Sci* 2000; **5**: 52-56 [PMID: 10664439 DOI: 10.1007/s007760050008]

73 **Vaverka M**, Návrat TS, Vrbka M, Florian Z, Fuis V. Stress and strain analysis of the hip joint using FEM. *Technol Health Care* 2006; **14**: 271-279 [PMID: 17065750]

74 **Naito M**, Schoenecker PL, Owen JH, Sugioka Y. Acute effect of traction, compression, and hip joint tamponade on blood flow of the femoral head: an experimental model. *J Orthop Res* 1992; **10**: 800-806 [PMID: 1403293 DOI: 10.1002/jor.1100100608]

75 **Trueta J**. The normal vascular anatomy of the human femoral head during growth. *J Bone Joint Surg Br* 1957; **39-B**: 358-394 [PMID: 13438980 DOI: 10.1007/978-1-4471-5451-8\_103]

76 **Perry DC**, Green DJ, Bruce CE, Pope D, Dangerfield P, Platt MJ, Hall AJ, Jones H. Abnormalities of vascular structure and function in children with Perthes disease. *Pediatrics* 2012; **130**: e126-e131 [PMID: 22665417 DOI: 10.1542/peds.2011-3269]

77 **Kim HK**, Kaste S, Dempsey M, Wilkes D. A comparison of non-contrast and contrast-enhanced MRI in the initial stage of Legg-Calvé-Perthes disease. *Pediatr Radiol* 2013; **43**: 1166-1173 [PMID: 23478799 DOI: 10.1007/s00247-013-2664-7]

78 **Kitoh H**, Kitakoji T, Katoh M, Takamine Y. Delayed ossification of the proximal capital femoral epiphysis in Legg-Calvé-Perthes' disease. *J Bone Joint Surg Br* 2003; **85**: 121-124 [PMID: 12585590 DOI: 10.1302/0301-620X.85B1.13426]

79 **Kocjančič B**, Moličnik A, Antolič V, Mavčič B, Kralj-Iglič V, Vengust R. Unfavorable hip stress distribution after Legg-Calvé-Perthes syndrome: a 25-year follow-up of 135 hips. *J Orthop Res* 2014; **32**: 8-16 [PMID: 24038236 DOI: 10.1002/jor.22479]

80 **Pinheiro MDS**, Dobson C, Clarke NM, Fagan M. The potential role of variations in juvenile hip geometry on the development of Legg-Calvé-Perthes disease: a biomechanical investigation. *Comput Methods Biomech Biomed Engin* 2018; **21**: 194-200 [PMID: 29419321 DOI: 10.1080/10255842.2018.1437151]

81 **Zhang W**, Yuan Z, Pei X, Ma R. In vivo and in vitro characteristic of HIF-1α and relative genes in ischemic femoral head necrosis. *Int J Clin Exp Pathol* 2015; **8**: 7210-7216 [PMID: 26261616]

82 **Kim HK**, Bian H, Aya-ay J, Garces A, Morgan EF, Gilbert SR. Hypoxia and HIF-1alpha expression in the epiphyseal cartilage following ischemic injury to the immature femoral head. *Bone* 2009; **45**: 280-288 [PMID: 19345751 DOI: 10.1016/j.bone.2009.03.665]

83 **Neidel J**, Zander D, Hackenbroch MH. Low plasma levels of insulin-like growth factor I in Perthes' disease. A controlled study of 59 consecutive children. *Acta Orthop Scand* 1992; **63**: 393-398 [PMID: 1529687 DOI: 10.3109/17453679209154752]

84 **Matsumoto T**, Enomoto H, Takahashi K, Motokawa S. Decreased levels of IGF binding protein-3 in serum from children with Perthes' disease. *Acta Orthop Scand* 1998; **69**: 125-128 [PMID: 9602767 DOI: 10.3109/17453679809117611]

85 **Grasemann H**, Nicolai RD, Hauffa BP, Reinhardt W, Nicolai H, Hövel M. Skeletal immaturity, IGF-I and IGFBP-3 serum concentrations in Legg-Calvé-Perthes disease (skeletal immaturity, IGF-I and IGFBP-3 in LCPD). *Klin Padiatr* 1996; **208**: 339-343 [PMID: 8962421 DOI: 10.1055/s-2008-1046494]

86 **Neidel J**, Schönau E, Zander D, Rütt J, Hackenbroch MH. Normal plasma levels of IGF binding protein in Perthes' disease. Follow-up of previous report. *Acta Orthop Scand* 1993; **64**: 540-542 [PMID: 7694440 DOI: 10.3109/17453679308993688]

87 **Mullan CJ**, Thompson LJ, Cosgrove AP. The Declining Incidence of Legg-Calve-Perthes' Disease in Northern Ireland: An Epidemiological Study. *J Pediatr Orthop* 2017; **37**: e178-e182 [PMID: 27328117 DOI: 10.1097/BPO.0000000000000819]

88 **Vielwerth SE**, Jensen RB, Larsen T, Greisen G. The impact of maternal smoking on fetal and infant growth. *Early Hum Dev* 2007; **83**: 491-495 [PMID: 17079098 DOI: 10.1016/j.earlhumdev.2006.09.010]

89 **Jaddoe VW**, Verburg BO, de Ridder MA, Hofman A, Mackenbach JP, Moll HA, Steegers EA, Witteman JC. Maternal smoking and fetal growth characteristics in different periods of pregnancy: the generation R study. *Am J Epidemiol* 2007; **165**: 1207-1215 [PMID: 17329715 DOI: 10.1093/aje/kwm014]

90 **Friedman JM**, Halaas JL. Leptin and the regulation of body weight in mammals. *Nature* 1998; **395**: 763-770 [PMID: 9796811 DOI: 10.1038/27376]

91 **Chen XX**, Yang T. Roles of leptin in bone metabolism and bone diseases. *J Bone Miner Metab* 2015; **33**: 474-485 [PMID: 25777984 DOI: 10.1007/s00774-014-0569-7]

92 **Garonna E**, Botham KM, Birdsey GM, Randi AM, Gonzalez-Perez RR, Wheeler-Jones CP. Vascular endothelial growth factor receptor-2 couples cyclo-oxygenase-2 with pro-angiogenic actions of leptin on human endothelial cells. *PLoS One* 2011; **6**: e18823 [PMID: 21533119 DOI: 10.1371/journal.pone.0018823]

93 **Gimble JM**. Leptin's balancing act between bone and fat. *J Bone Miner Res* 2011; **26**: 1694-1697 [PMID: 21698664 DOI: 10.1002/jbmr.406]

94 **Zhou L**, Jang KY, Moon YJ, Wagle S, Kim KM, Lee KB, Park BH, Kim JR. Leptin ameliorates ischemic necrosis of the femoral head in rats with obesity induced by a high-fat diet. *Sci Rep* 2015; **5**: 9397 [PMID: 25797953 DOI: 10.1038/srep09397]

95 **Ma HZ**, Zeng BF, Li XL. Upregulation of VEGF in subchondral bone of necrotic femoral heads in rabbits with use of extracorporeal shock waves. *Calcif Tissue Int* 2007; **81**: 124-131 [PMID: 17629736 DOI: 10.1007/s00223-007-9046-9]

96 **Ruiz M**, Pettaway C, Song R, Stoeltzing O, Ellis L, Bar-Eli M. Activator protein 2alpha inhibits tumorigenicity and represses vascular endothelial growth factor transcription in prostate cancer cells. *Cancer Res* 2004; **64**: 631-638 [PMID: 14744778 DOI: 10.1158/0008-5472.CAN-03-2751]

97 **Catterall A**. The natural history of Perthes' disease. *J Bone Joint Surg Br* 1971; **53**: 37-53 [PMID: 5578764 DOI: 10.1302/0301-620X.53B1.37]

98 **Guille JT**, Lipton GE, Szöke G, Bowen JR, Harcke HT, Glutting JJ. Legg-Calvé-Perthes disease in girls. A comparison of the results with those seen in boys. *J Bone Joint Surg Am* 1998; **80**: 1256-1263 [PMID: 9759808]

99 **Georgiadis AG**, Seeley MA, Yellin JL, Sankar WN. The presentation of Legg-Calvé-Perthes disease in females. *J Child Orthop* 2015; **9**: 243-247 [PMID: 26210773 DOI: 10.1007/s11832-015-0671-y]

100 **Zheng P**, Yang T, Ju L, Jiang B, Lou Y. Epigenetics in Legg-Calvé-Perthes disease: A study of global DNA methylation. *J Int Med Res* 2015; **43**: 758-764 [PMID: 26443715 DOI: 10.1177/0300060515591062]

101 **Kenet G**, Ezra E, Wientroub S, Steinberg DM, Rosenberg N, Waldman D, Hayek S. Perthes' disease and the search for genetic associations: collagen mutations, Gaucher's disease and thrombophilia. *J Bone Joint Surg Br* 2008; **90**: 1507-1511 [PMID: 18978274 DOI: 10.1302/0301-620X.90B11.20318]

102 **Pouya F**, Kerachian MA. Avascular Necrosis of the Femoral Head: Are Any Genes Involved? *Arch Bone Jt Surg* 2015; **3**: 149-155 [PMID: 26213697 DOI: 10.1001/jama.1977.03280010029013]

103 **Baltzer HL**, Riester S, Moran SL. Bilateral Legg-Calve-Perthes Disease and Kienbock's Disease in a Child With Factor V Leiden Thrombophilia: A Case Report. *Hand (N Y)* 2016; **11**: NP16-NP19 [PMID: 27698645 DOI: 10.1177/1558944715627274]

104 **Tanaka T**, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol* 2014; **6**: a016295 [PMID: 25190079 DOI: 10.1101/cshperspect.a016295]

105 **Yeung F**, Hoberg JE, Ramsey CS, Keller MD, Jones DR, Frye RA, Mayo MW. Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. *EMBO J* 2004; **23**: 2369-2380 [PMID: 15152190 DOI: 10.1038/sj.emboj.7600244]

**P-Reviewer:** Elgafy H, Slomiany BL **S-Editor:** Ma YJ **L-Editor:** **E-Editor:**

**Specialty type:** Orthopedics

**Country of origin:** Italy

**Peer-review report classification**

Grade A (Excellent): A

Grade B (Very good): B

Grade C (Good): 0

Grade D (Fair): 0

Grade E (Poor): 0



**Figure 1 PRISMA (preferred reporting items for systematic reviews and meta-analysis) flowchart of the systematic literature review.**

**Table 1 The main findings of included case-control studies are reported**

|  |  |  |  |
| --- | --- | --- | --- |
| **Ref.** | **Subjects** | **Association/molecule studied** | **Results** |
| Perry *et al*[12] (2017) | A hospital case-control study (*n* = 149/146) | Tobacco smoke exposure during pregnancy | The odds of Perthes' disease significantly increased with reported in utero exposure after adjustment for socioeconomic deprivation (maternal smoking OR = 2.06, 95%CI: 1.17-3.63; paternal smoking OR = 2.09, 95%CI: 1.26-3.46). |
| Daniel *et al*[13] (2012) | 128 children with LCPD and 384 children attending the hospital for other orthopaedic complaints | environmental tobacco smoke, firewood smoke, and socioeconomic status and the risk of LCPD | The main risk factors for LCPD were indoor use of a wood stove (adjusted odds ratio [OR], 2.56) and having a family member who smoked indoors (adjusted OR, 2.07). |
| García Mata *et al*[15] (2000) | 90 patients with LCPD and 183 normal children, as controls, selected at random to determine whether the condition of passive smoking is related to the disease | LCPD and passive smoking | The association between LCPD and passive smoking, after controlling for age and gender, became significant (*p* = 0.0000). Thus the risk of LCPD in passive smoking children is more than five times higher than in children who are not exposed to smoke. |
| Bahmanyar *et al*[17] (2008) | The Swedish Inpatient Register identified 852 individuals with a diagnosis of LCPD from 1983 to 2005, individually matched by year of birth, age, sex, and region of residence with 4432 randomly selected control subjects. | Mathernal smoking during pregnancy and LCPD | Maternal smoking during pregnancy was associated with an increased LCPD risk, and heavy smoking was associated with a risk increase of almost 100%. Very low birth weight and cesarean section were independently associated with approximately 240% and 36% increases in the risk of LCPD, respectively. |
| Wiig *et al*[29] (2006) | 402 patients with a matched control group of non-affected children (*n* = 1025952) from the Norwegian Medical Birth Registry | Epidemiology and possible etiology of LCPD | Applying Sartwell's log-normal model of incubation periods to the distribution of age at onset of Perthes' disease showed a good fit to the log-normal curve. Our findings point toward a single cause, either genetic or environmental, acting prenatally in the aetiology of Perthes' disease. |
| Perry *et al*[32] (2013) | 146 cases of LCPD and 142 hospital controls, frequency matched by age and sex | LCPD and hyperactivity | Significant associations (*P* < 0.05) existed with the majority of the psychological domains captured by the Strength and Difficulties Questionnaire [odds ratio (OR) for "high" level of difficulties-Emotion OR 3.2, Conduct OR 2.1, Inattention-Hyperactivity OR 2.7, Prosocial Behavior OR 1.9]. Hyperactivity was especially marked among individuals within 2 yr of diagnosis (OR = 8.6; *P* < 0.001), but not so among individuals over 4 years from diagnosis. |
| Berman *et al*[34] (2016) | Sixteen children with LCPD (age 9.1 ± 3.3, 75% males) were compared with their closest-aged siblings (age 9.3 ± 2.6, 30% males). | LCPD and ADHD | Our findings in a small cohort of children with LCPD and their comparably aged siblings do not support an association between LCPD and ADHD |
| Hailer *et al*[35] (2012) | 2579 patients with LCPD in Sweden during the period 1964-2005. 13748 individuals without LCPD were randomly selected from the Swedish general population | LCPD and risk of injury | Patients with LCPD are vulnerable to injuries which could be interpreted as a marker of hyperactive behavior. |
| Hailer *et al*[36] (2014) | 4,057 individuals with LCPD in Sweden during the period 1964-2011. 40,570 individuals without LCPD were randomly selected from the Swedish general population | LCPD and ADHD | Compared to the control group, individuals with LCPD had a raised hazard ratio (HR) of 1.5 (95%CI: 1.2-1.9) for ADHD. |
| Türkmen *et al*[37] (2014) | The study included 3 groups of patients: Perthes patients, trauma patients, and orthopaedic patients without Perthes disease or history of trauma. Each group was comprised of 56 males and 4 females. | LCPD and ADHD | Attention deficit and hyperactivity disorder was diagnosed in 7 patients in the Perthes group. The findings are not significant |
| Lee *et al*[39] (2013) | 38 male and 3 female patients with LCPD, and an equal number of age-(range was 4-12) and sex-matched control patients with healthy fractures. | LCPD and leptin | Leptin, disease severity, and treatment outcomes were associated. This correlation suggests that leptin might play an important role in LCPD pathogenesis. |
| Srzentić *et al*[51] (2014) | 37 patients with Perthes disease and 50 healthy controls | LCPD and IL-6 | Our study revealed that heterozygote subjects for the IL-6 G-174C/G-597A polymorphisms were significantly overrepresented in the control group than in the Perthes patient group. |
| Kamiya *et al*[52] (2015) | 28 patients with matched controls | LCPD and IL-6 | In the synovial fluid of the affected hips, IL-6 protein levels were significantly increased (LCPD: 509 ± 519 pg/mL, non-LCPD: 19 ± 22 pg/mL; *p* = 0.0005) on the multi-cytokine assay. |
| Perry *et al*[76] (2012) | 149 cases and 146 controls | Vascular abnormalities in LCPD patients | Children with Perthes disease exhibit small artery caliber and reduced function, which is independent of body composition. These data imply that that Perthes disease may reflect a wider vascular phenomenon that could have long-term implications for the vascular health of affected individuals. |
| Kitoh *et al*[78] (2003) | 125 children (105 boys, 20 girls) with unilateral LCPD | Delayed ossification in LCPD | Our findings support the hypothesis that a delay in endochondral ossification in the proximal capital femoral epiphysis may be associated with the onset of Perthes' disease. |
| Kocjančič *et al*[79] (2014) | 135 adult hips of patients who had been treated for Perthes disease in childhood with matched controls | Hip stress distribution in LCPD | No differences were found in resultant hip force and in peak contact hip stress between the hips that were in childhood subject to Perthes disease and the control population, but a considerable (148%) and significant (*p* < 0.001) difference was found in the contact hip stress gradient index, expressing an unfavorable, steep decrease of contact stress at the lateral acetabular rim. |
| Neidel *et al*[83] (1992) | 59 consecutive children with Perthes' disease and 59 matched controls | IGF-1 and LCPD | Our data may reflect an impaired synthesis or release of IGF I relative to age in Perthes' disease, or changes in the affinity or metabolism of IGF binding proteins. The observed changes seem to be of a temporary nature. |
| Kim *et al*[82] (2009) | 56 immature pigs | HIF-1α and LCPD | Acute ischemic injury to the immature femoral head induced severe hypoxia and cell death in the bony epiphysis and the deep layer of the epiphyseal cartilage. Viable chondrocytes in the superficial layer of the epiphyseal cartilage showed HIF-1alpha activation and VEGF upregulation with subsequent revascularization occurring in the cartilage. |
| Matsumoto *et al*[84] (1998) | 27 children with Perthes' disease and 10 age-matched control subjects | IGF binding protein -3 and LCPD | The bone age was delayed, 2 years or more compared with the chronological age in 7 of 18 patients, and all of them, except 1, showed decreased levels of IGFBP-3 on WLB. |
| Graseman *et al*[85] (1996) | 23 children with unilateral LCPD and in 23 sex and age matched controls | IGF binding protein -3 and LCPD | ur data confirm that most children with LCPD are skeletally immature. However, IGF-I measured with IGF-II-blocked IGFBP binding sites, and IGFBP-3 serum concentrations analysed with respect to bone age show no evidence for a disturbance of the hypothalamo-pituitary-somatomedin axis in these children. |
| Neidel *et al*[86] (1993) | 55 children with Perthes' disease and 55 age- and sex-matched controls | IGF and LCPD | Our findings indicate that low levels of circulating IGF I in Perthes' disease, as we have reported previously, are caused neither by altered concentrations of the principal IGF-binding protein, IGFBP-3, nor by an underlying growth hormone deficiency. |

LCPD: Legg-Calvé-Perthes disease; ADHD: attention deficit hyperactivity disorder; IGF: insulin growth factor; HIF: hypoxia-inducible factor.

**Table 2 The main findings of included cohort studies are reported**

|  |  |  |  |
| --- | --- | --- | --- |
| **Ref.** | **Subjects** | **Association/molecule studied** | **Results** |
| Gordon *et al*[14] (2004) | 60 patients with LCPD | Smoking and socio-economic status and the severity of LCPD | A significant association was noted between living with a smoker and LCPD as well as between increasing smoke exposure and increased risk of developing LCPD. No significant association was noted between lower income and LCPD. There was no association between increased smoke exposure and increased severity of LCPD as measured by the lateral pillar classification. |
| Glueck *et al*[16] (1998) | 39 children with Legg-Perthes disease | secondhand smoke exposure | Secondhand smoke exposure had no significant effects on other measures of coagulation. Secondhand smoke exposure while in utero and during childhood appears to lower stimulated tissue plasminogen activator activity and additionally may depress heritable low stimulated tissue plasminogen activator activity, leading to hypofibrinolysis. Hypofibrinolysis may facilitate thrombotic venous occlusion in the head of the femur, leading to venous hypertension and hypoxic bone death, Legg-Perthes disease. |
| Sharma *et al*[18] (2005) | 240 children (263 hips) who presented with Perthes' disease in Greater Glasgow | Socio economic deprivation and LCPD | There was no significant evidence of a preponderance of Perthes' disease in the most deprived groups. |
| Pillai *et al*[19] (2005) | 40 LCPD patients and the Southwest Scotland registry | The incidence of LCPD in Southwest Scotland | The incidence of LCPD increases with deprivation and poor living standards. |
| Kealey *et al*[20] (2000) | 313 children with LCPD and Northern Ireland registry | Socio economic deprivation and LCPD | While the incidence of Perthes' disease was found to be associated with indicators of the level of deprivation for areas, there was no evidence to suggest that there was an increased risk in urban areas; the highest rate was found in the most deprived rural category |
| Perry *et al*[21] (2012)  | The General Practice Research database was analyzed to identify incident cases between 1990 and 2008 in children ages 0-14 yr | LCPDincidence in United Kingdom  | The incidence was declining in the study period. The declining incidence, along with the geographic variation, suggests that a major etiologic determinant in LCPD is environmental and closely linked to childhood deprivation. |
| Perry *et al*[22] (2012) | Scottish Morbidity Record, based in Scotland, United Kingdom using data from 2000-2010. A total of 443 LCPD patients | Socio economic deprivation and LCPD | The occurrence of Perthes' disease within urban environments is high, yet this appears to be a reflection of higher socioeconomic deprivation exposure. Disease rates appear equivalent in similarly deprived urban and non-urban areas, suggesting that the determinant is not a consequence of the urban environment. |
| Perry *et al*[23] (2011) | 1082 children with Perthes' disease (682 from a geographically defined area). Regional disease register in Merseyside, United Kingdom, 1976-2009 | Social deprivation and the declining incidence of LCPD | There was a marked decline in disease incidence over the study period, particularly in more deprived areas. The magnitude of the association with deprivation, and the changing incidence, strongly suggest that environmental factor(s) are a major aetiological determinant in Perthes' disease. |
| Hall and Barker[24] (1989) | Yorkshire region registry | Perthes incidence over the region | There were large geographical differences in incidence which could not be explained by urban-rural or social class differences. |
| Hall *et al*[25] (1983) | Case registry in Liverpool and adjacent parts of Knowsley and Sefton during 1976-81 | Incidence of LCPD in the region | The inner city of Liverpool, which has been shown to be underprivileged, had the highest yearly incidence of the disease ever reported--21.1 cases/100000 children aged 14 years and under. The associations with poverty support the hypothesis that undernutrition is a causative factor in the disease. |
| Margetts *et al*[26] (2001) | Registry of Liverpool (1982-1995) | Incidence and distribution of LCPD in Liverpool | We suggest that environmental influences may come into play some years before a child presents with pain in the hip. There may be a genetic predisposition to the disease. |
| Metcalfe *et al*[27] (2016) | All twin pairs from the Danish Twin Registry (DTR) in which at least 1 individual had LCPD (81 twin pair) | Twin study of LCPD | This study found evidence of familial clustering in LCPD but did not show a genetic component. The absolute risk that a co-twin of an affected individual will develop LCPD is low, even in the case of monozygotic twin pairs. |
| Lappin *et al*[28] (2003) | 320 patients on the Northern Ireland Perthes' database | Birthweight and LCPD | We observed that the low birthweight twin in each case was the affected child. It is proposed that environmental factors associated with low birthweight are involved in the aetiology of Perthes' disease. |
| Loder *et al*[30] (1993) | 24 LCPD patients | LCPD and ADHD | One third (33%) of the children had abnormally high scores in profiles associated with attention deficit hyperactivity disorder (ADHD) (impulsive, hyperactive, and psychosomatic categories), much higher than the 3%-5% incidence of ADHD in the general population. |
| Perry *et al*[31] (2012) | General Practise Research database in United Kingdom | LCPD comorbidities  | The risk of Perthes' disease was significantly increased with the presence of congenital anomalies of the genitourinary and inguinal region, such as hypospadias (OR = 4.04, 95%CI: 1.41-11.58), undescended testis (OR = 1.83, 95%CI: 1.12-3.00) and inguinal herniae (OR = 1.79, 95%CI: 1.02-3.16). Attention deficit hyperactivity disorder was not associated with Perthes' disease (OR = 1.01, 95%CI: 0.48-2.12), although a generalised behavioural disorder was (OR = 1.55, 95%CI: 1.10-2.17). Asthma significantly increased the risk of Perthes' disease (OR = 1.44, 95%CI: 1.17-1.76), which remained after adjusting for oral/parenteral steroid use. |
| Podeszwa *et al*[33] (2015) | 11 LCPD patients | Psycological finding in patients undergoing surgery | A significant presence of depression and anxiety symptoms was reported. |
| Neal *et al*[38] (2016) | 150 patients (172 hips) with LCPD | LCPD and obesity | Obesity is common in patients with LCPD and is associated with a later stage of disease presentation. |
| Srzentić *et al*[48] (2015) | 37 LCPD patients | Markers of coagulation, inflammation and apoptosis in LCPD | The results presented indicate that apoptosis could be one of the factors contributing to the lack of balanced bone remodeling process in Perthes patients. |
| Calver *et al*[56] (1981) | 50 children with “irritable hip” | Radionuclide scanning in LCPD | Five of the 50 children seen during the one year had areas of ischaemia in the capital femoral epiphysis demonstrated on the scan. all five developed radiological signs of Perthes' disease within 6 mo. The remaining 45 had radiographically normal hips at one year. |
| Royle and Galasko[60] (1992) | 192 patients with a typical transient synovitis syndrome | Scintigraphy in LCPD patients | Fifteen patients had evidence of ischemia of the femoral head, but only 4 patients went on to develop the typical radiographic features of Perthes' disease. The other 11 patients are thought to represent a minor, radiographically silent form of Perthes' disease. |
| Lamer *et al*[61] (2002) | Twenty-six DGS MRI and bone scintigraphies of 25 hips in 23 children | Blood supply in LCPD | DGS MRI allows early detection of epiphyseal ischaemia and accurate analysis of the different revascularisation patterns. These changes are directly related to the prognosis of LCPD |
| Atsumi *et al*[62] (2000) | 28 hips in 25 patients with LCPD | Blood supply in LCPD | We suggest that in Perthes' disease the blood supply of the LEAs is impaired at their origin and that revascularisation occurs from this site by ingrowth of small vessels into the femoral epiphysis. This process may be the result of recurrent ischaemic episodes. |
| de Camargo *et al*[63] (1984) | 30 patients, including 26 aortographies and six selective angiographies | Blood supply in LCPD | The major angiographic alterations were: general decrease of blood flow in the affected hip, lack of a patent medial circumflex artery, an atrophic medial circumflex artery or obstruction of its branches, distended vessels in subluxations of the hip joint, and almost complete absence of the obturator artery |
| Theron *et al*[64] (1980) | Eleven cases of LCPD | Blood supply in LCPD | The balance between the respective vascular territories of the dilated superior and inferior capsular arteries is variable and seems to affect the position of the sequestrum and the centering of the femoral head. |
| Kitoh *et al*[78] (2003) | 125 children (105 boys, 20 girls) with unilateral LCPD | Delayed ossification in LCPD | Our findings support the hypothesis that a delay in endochondral ossification in the proximal capital femoral epiphysis may be associated with the onset of Perthes' disease. |

LCPD: Legg-Calvé-Perthes disease; ADHD: Attention deficit hyperactivity disorder; MRI: magnetic resonance imaging.

**Table 3 The main findings of included animal studies are reported**

|  |  |  |  |
| --- | --- | --- | --- |
| **Ref.** | **Subjects** | **Association/molecule studied** | **Results** |
| Suehiro *et al*[49] (2005) | Mice model | Osteonecrosis in rat model | Repetitive mechanical stress on the femoral heads from 5 to 9 wk of age played an important role in the etiology of osteonecrosis |
| Gershuni *et al*[59] (1983) | Hip of the immature pig | Joint tamponade in LCPD animal model | The data from this experiment do not support the theory that tamponade of the femoral capital epiphysis is the cause of osteonecrosis in Legg-Calvé-Perthes syndrome |
| Cheon *et al*[66] (2015) | 10 piglets | Quantitive MRI and in piglet model of LCPD | The epiphyseal ADC values of the ischemic hip decreased immediately (1 hour) after embolization. However, they increased rapidly at 1 wk after embolization and remained elevated until 4 wk after embolization. Perfusion MRI of ischemic hips showed decreased epiphyseal perfusion with decreased Kep immediately after embolization. |
| Li*et al*[67] (2006) | 20 femoral heads of 10 piglets | MRI in piglet model of LCPD | Gadolinium-enhanced MRI can identify early ischemia and its reversal of the capital femoral epiphysis induced by hip hyper-abduction |
| Babyn *et al*[68] (1998) | Piglet model | MRI in piglet model of LCPD | High resolution MRI can demonstrate changes in the CE associated with ischemic injury and may have a role in the assessment of the CE and its development after ischemic injury. |
| Li *et al*[69] (2008) | 25 piglets models | Diffusive MRI in a model of LCPD | Histological study revealed necrosis of chondrocytes and osteocytes and abnormal thickening of the epiphyseal cartilage in the ischemic femoral head. |
| Levin *et al*[70] (1999) | Rat model | Epiphysis studies in a rat model | Thickening and condensation of the subchondral bone, leading to increased stiffness of the subchondral zone, result in the osteoarthritis-like disorder. Mimicking the well-known phases of human osteonecrosis, the model readily allows for preclinical studies of therapeutic regimens. |
| Kandzierski *et al*[71] (2004) | Calf femurs | Calf femur experimental study | The author concludes that impaired blood flow within the growth layers additionally weakens the immature bone tissue of the femoral head and neck, which may lead to mechanical damage of the bone tissue itself, as well as to the epiphyseal blood vessels entering bony epiphysis. |
| Suehiro *et al*[72] (2000) | Twenty femora from 10 Wistar Kyoto (WKY) rats | Standing and induction of OA | Repetitive mechanical stress on the femoral heads from 5 to 9 wk of age played an important role in the etiology of osteonecrosis |
| Naito *et al*[74] (1992) | Canine femoral head | Acute effect of traction, compression, and hip joint tamponade on blood flow of the femoral head | These experimental data may have important implications for the pathogenesis of iatrogenic avascular necrosis in the treatment of congenitally dislocated hip, Legg-Perthes disease, and avascular necrosis following nondisplaced femoral neck fracture |
| Kim *et al*[77] (2013) | 56 immature pigs | MRI in the initial stage of LCPD | Acute ischemic injury to the immature femoral head induced severe hypoxia and cell death in the bony epiphysis and the deep layer of the epiphyseal cartilage. Viable chondrocytes in the superficial layer of the epiphyseal cartilage showed HIF-1alpha activation and VEGF upregulation with subsequent revascularization occurring in the cartilage. |
| Zhang *et al*[81] (2015) | 6-wk-old Sprague Dawley rats | HIF-1α and LCPD | Hypoxia might be an etiological factor for femoral head necrosis, HIF-1α, VEGF as well as apoptotic genes participated the pathophysiological process of ischemic osteonecrosis. |
| Kim *et al*[83] (2009) | 56 immature pigs | HIF-1α and LCPD | Acute ischemic injury to the immature femoral head induced severe hypoxia and cell death in the bony epiphysis and the deep layer of the epiphyseal cartilage. Viable chondrocytes in the superficial layer of the epiphyseal cartilage showed HIF-1alpha activation and VEGF upregulation with subsequent revascularization occurring in the cartilage. |

LCPD: Legg-Calvé-Perthes disease; HIF-1: Hypoxia-inducible factor; VEGF: Vascular endothelial growth factor; MRI: magnetic resonance imaging.

**Table 4 The main findings of the included *in vitro* human studies are reported**

|  |  |  |  |
| --- | --- | --- | --- |
| **Ref.** | **Subjects** | **Association/molecule studied** | **Results** |
| O’Sullivan *et al*[40] (1985) | A family in which Legg-Calvé-Perthes disease (LCPD) occurred in four members | Genetic factors and LCPD | This unusually high incidence in one family raises questions about the genetic versus the environmental factors in the etiology of LCPD. |
| Livesey *et al*[41] (1998) | Case report of three family with three female first-degree relatives affected by LCPD | Genetic factors and LCPD | First case of three first-degree relative affected |
| Miyamoto *et al*[42] (2007) | A Japanese family with an autosomal dominant hip disorder manifesting as LCPD | LCPD and *COL2A1* | This is the first report of a mutation in hereditary LCPD. *COL2A1* mutations may be more common in LCPD patients than currently thought, particularly in familial and/or bilateral cases. |
| Al-Omran and Sadat-Ali[43] (2013) | 2 generations of 4 male family members with LCPD-like features and mutation of the *COL2A1* gene of the 12q13 chromosome | LCPD and *COL2A1* | If LCPD occurs in any family member, we recommend genetic analysis and counselling as well as early radiological screening of related children. |
| Kannu *et al*[44] (2011) | Two children who presented with abnormal development of both hips and in whom novel mutations in the *COL2A1* gene were found | LCPD and *COL2A1* | The purpose of our report is to alert clinicians to the possibility that children who present with bilateral Perthes-like disease of the hip might have an underlying mutation in the gene encoding type II collagen. |
| Su *et al*[45] (2008) | Forty-two members of a 5-generation family | LCPD and *COL2A1* | The p.Gly1170Ser mutation of *COL2A1* in the family described is responsible for pathology confined to the hip joint, which presents as isolated precocious hip OA, AVN of the femoral head, or Legg-Calvé-Perthes disease. |
| Li *et al*[46] (2014) | Forty-five members of a four-generation family | LCPD and *COL2A1* | In our research, we identify a heterozygous mutation (c.1888 G>A, p. Gly630Ser) in exon 29 of *COL2A1* in the Gly-X-Y domain, in a Chinese family affected by LCPD and ANFH |
| Woratanarat *et al*[47] (2014) | Twelve case–control studies met inclusion criteria and had sufficient data for extraction | Hypercoagulability and LCPD | The factor V Leiden mutation is significantly related to Perthes disease, and its screening in at‐risk children might be useful in the future. |
| Srzentić *et al*[48] (2015) | 37 LCPD patients | Markers of coagulation, inflammation and apoptosis in LCPD | The results presented indicate that apoptosis could be one of the factors contributing to the lack of balanced bone remodeling process in Perthes patients. |
| Liu *et al*[50] (2015) | Age- and sex-matched serum samples from 10 control subjects and 10 patients with LCPD were compared using the isobaric tags for relative and absolute quantification (iTRAQ) technique. | Serum proteomes in LCPD | The complement and coagulation cascades, and abnormal lipid metabolism may be involved in the pathogenesis of LCPD. |
| Srzentić *et al*[51] (2014) | 37 patients with Perthes disease and 50 healthy controls | LCPD and IL-6 | Our study revealed that heterozygote subjects for the IL-6 G-174C/G-597A polymorphisms were significantly overrepresented in the control group than in the Perthes patient group. |
| Kamiya *et al*[52] (2015) | 28 patients with matched controls | LCPD and IL-6 | In the synovial fluid of the affected hips, IL-6 protein levels were significantly increased (LCPD: 509 ± 519 pg/mL, non-LCPD: 19 ± 22 pg/mL; *p* = 0.0005) on the multi-cytokine assay. |
| Su *et al*[55] (2010) | a five-generation family with 42 members with a new type II collagenopathy | LCPD and *COL2A1* | Our study demonstrated that the p.Gly1170Ser mutation of *COL2A1* caused significant structural alterations in articular cartilage, which are responsible for the new type II collagenopathy. |
| Matsumoto *et al*[84] (1998) | 27 children with Perthes' disease and 10 age-matched control subjects | IGF binding protein -3 and LCPD | The bone age was delayed, 2 years or more compared with the chronological age in 7 of 18 patients, and all of them, except 1, showed decreased levels of IGFBP-3 on WLB. |
| Graseman *et al*[85] (1996) | 23 children with unilateral LCPD and in 23 sex and age matched controls | IGF binding protein -3 and LCPD | ur data confirm that most children with LCPD are skeletally immature. However, IGF-I measured with IGF-II-blocked IGFBP binding sites, and IGFBP-3 serum concentrations analysed with respect to bone age show no evidence for a disturbance of the hypothalamo-pituitary-somatomedin axis in these children. |
| Neidel *et al*[86] (1993) | 55 children with Perthes' disease and 55 age- and sex-matched controls | IGF and LCPD | Our findings indicate that low levels of circulating IGF I in Perthes' disease, as we have reported previously, are caused neither by altered concentrations of the principal IGF-binding protein, IGFBP-3, nor by an underlying growth hormone deficiency. |

LCPD: Legg-Calvé-Perthes disease; COL2A1: Collagen type II gene; IGF: insulin growth factor.