GAVIN PUBLISHERS

Journal of Orthopedic Research and Therapy

Séguin C, et al. J Orthop Ther. JORT-142.

Review Article

Genetic Basis of Osteonecrosis of the Femoral Head: An Updated Review

Tracy Wang[1 **], Bouziane Azeddine[1 **], Wayne Mah[1], Edward J Harvey[1,2], David Rosenblatt[3], Chantal Séguin[1,4*]

[1]Vascular Biology Research lab, Research Institute (RI) McGill University Health Centre, C9 Montreal General Hospital, Canada

[2]Department Surgery, Division Orthopaedic Surgery, McGill University Health Centre, B5 Montreal General Hospital, Canada

[3]Department of Human Genetics, McGill University, Montreal, Quebec, Canada

[4]Department of Medicine, Division of Hematology and Oncology, McGill University Health Centre, Canada

[*]**Corresponding author:** Chantal Séguin, McGill University Health Centre, Glen site, Cedars Cancer Centre, 1001 Décarie Blvd., room D02.7519, Montreal, Quebec, Canada. Tel: +15149341934; Fax: +15149348221; Email: chantal.seguin2@mcgill.ca

[**]TW and BA contributed equally to this work

Citation: Wang T, Azeddine B, Mah W, Harvey EJ, Rosenblatt D, Séguin C (2017) Genetic Basis of Osteonecrosis of the Femoral Head: An Updated Review. J Orthop Ther. JORT-142.

Received Date: 28 June, 2017; **Accepted Date:** 29 July, 2017; **Published Date:** 05 August, 2017

Abstract

Osteonecrosis of the Femoral Head (ONFH) is caused by reduced blood flow to the trabecular bone near the surface, leading to the death of bone tissue and femoral head collapse. Patients usually report groin pain which tends to worsen as the disease progresses. At a later stage, patients often lose the ability to use their joint. There are approximately 20,000 new cases each year in the United States. Although its pathophysiology remains poorly understood, several risk factors have been identified such as glucocorticoid use, alcohol abuse, systemic lupus erythematosus and sickle cell disease. Not all patients exposed to one or more of these risk factors will develop ONFH, indicating the presence of an underlying genetic predisposition.

Although hereditary ONFH has been reported in only a few families, the involvement of genetic factors in the disease and its progression is gaining interest particularly through genetic association studies. This review presents a summary of Single Nucleotide Polymorphisms (SNPs) and other genetic mutation variations found in association with ONFH, including our recent identification of a novel mutation in the Transient Receptor Potential Vanilloid 4 (TRPV4) gene in association with inherited ONFH. The summary of these genetic findings identifies biological processes believed to be involved in the development of ONFH, which include circulation, steroid metabolism, immunity and the regulation of bone formation. Taken together, these associations may lead to new pathways of bone repair and remodeling while opening new avenues for therapeutic targets.

Keywords: Coagulation Defects; Hereditary ONFH; Immunity; Osteonecrosis of the Femoral Head; Polymorphisms; Steroids; TRPV4

Abbreviations:

ABCB1	:	Adenosine Triphosphate-Binding Cassette B1
ACE	:	Angiotensin Converting Enzyme
ANXA2	:	Annexin A2
ApoB	:	Apoliprotein B
AVN	:	Avascular Necrosis
BMP	:	Bone Morphogenetic Protein
CAT	:	Catalase
CBP	:	CREB-Binding Protein
COL2A1	:	Collagen 2 Alpha 1
CTDP1	:	C-Terminal Domain of RNA Polymerase II Subunit A, Phosphatase of Subunit 1

CYP27C1	:	Cytochrome P450, Family 27, Subfamily C, Polypeptide 1
eNOS	:	Endothelial Nitric Oxide Synthase
GRIN3A	:	Glutamate Receptor, Ionotropic, N-Methyl-D-Aspartate 3a
ILGF	:	Insulin-like Growth Factor
IGFBP3	:	Insulin-Like Growth Factor Binding Protein 3
IL	:	Interleukin
KDR	:	Kinase Insert Domain Receptor
LCPD	:	Legg-Calvé-Perthes Disease
MDR1	:	Multidrug Resistance 1
MRI	:	Magnetic Resonance Imaging
MTHFR	:	5, 10-Methylenetetrahydrofolate Reductase
NRP1	:	Neuropilin 1
ONFH	:	Osteonecrosis of the Femoral Head
OPG	:	Osteoprotegerin
PAI-1	:	Plasminogen Activator Inhibitor-1
P-gp	:	P-Glycoprotein
PLAT or TPA	:	Tissue Plasminogen Activator
PON-1	:	Paraoxonase 1
RANKL	:	Receptor Activator of Nuclear Factor Kappa-B Ligand
SNP	:	Single Nucleotide Polymorphism
SREBF1	:	Sterol Regulatory Element-Binding Transcription Factor 1
SREBF2	:	Sterol Regulatory Element-Binding Transcription Factor 2
TF	:	Transferrin
TFPI	:	Tissue Factor Pathway Inhibitor
TGF-β	:	Transforming Growth Factor β
TNF-α	:	Tumor Necrosis Factor α
TRPV4	:	Transient receptor potential vanilloid 4
VDR	:	Vitamin D Receptor
VEGFC	:	Vascular Endothelial Growth Factor C

Introduction

Non-hereditary Osteonecrosis of the Femoral Head (ONFH), also known as Avascular Necrosis (AVN), ischemic necrosis, or aseptic necrosis, is a debilitating disease with 20,000 new cases diagnosed each year in the United States [1]. Hereditary ONFH is rare and has been reported in only a few families. Both hereditary and non-hereditary ONFH meet the criteria of a rare disease, affecting less than 1 in 2000 (<6-9/10,000; ORPHA399158). ONFH is thought to arise from a temporary or permanent loss of blood flow to the femoral head, causing bone necrosis and eventually its collapse [2,3]. This usually results in severe hip pain that is treated with conservative methods such as weight-bearing restriction, the use of bisphosphonates, statins [4] or femoral head decompression at early stage. Invasive surgical procedures such as nonvascularized or vascularized bone grafting of the affected area, or total hip replacement are more often used at advanced stages [5].

Classic clinical presentations are seen in late adolescence and young to middle-aged adults, however, the disease may occur in children between 4 and 12 years old and is then called Legg-Calvé-Perthes Disease (LCPD), often with early asymptomatic ischemic changes [6]. There is a wide spectrum of etiological risk factors in non-traumatic ONFH including alcohol consumption and chemotherapy, however the most common risk factor is the use of glucocorticoids [6]. While most cases of non-traumatic ONFH are secondary, several studies have shown involvement of genetic factors, with hereditary ONFH found in Asian and Caucasian families [7,8]. Some studies also report a genetic predisposition that explains ethnic and individual differences in ONFH incidence [9]. Here, we present a summary of recent genetic studies and review articles on ONFH pathogenesis, including 17 case-control and 7 meta-analyses which identify genetic variations thought to play a pivotal role in ONFH development (Figure 1), as well as a summary of a recent discovery of a novel mutation in TRPV4 gene, in a family affected by hereditary ONFH [10].

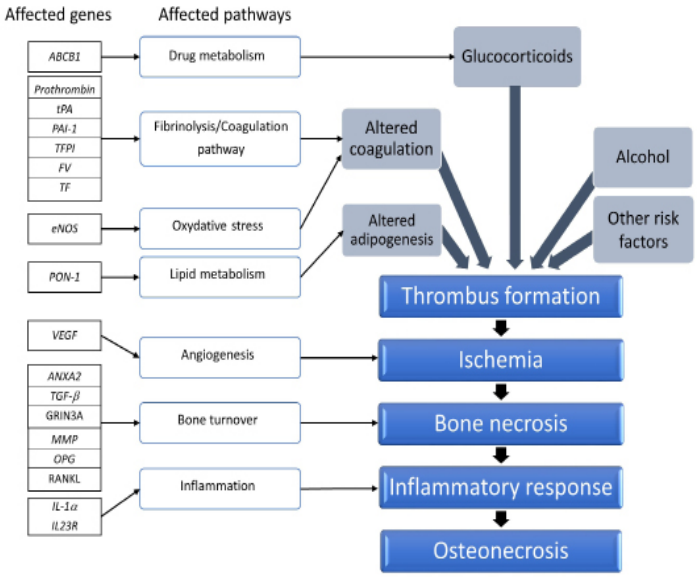

Figure 1: Schematic diagram showing on the right the common pathway leading to ONFH development. In the middle are listed the systems affected on each step and on the left, genes playing a role on these systems and where polymorphisms were identified.

Genetic Methods Used For ONFH

Identifying genes causing or associated with complex diseases is challenging due to the involvement of several factors. Therefore, for complex diseases with multiple aetiologies such as osteonecrosis, we aim to determine if there is an underlying genetic susceptibility to the disease. Several techniques have been developed to perform genetic association studies, focusing predominantly on Single Nucleotide Polymorphisms (SNPs), the most common DNA sequence variations. These techniques, such as Genome Wide Association Studies, have led to major advances in this field. However, they require a larger sample size in order to obtain statistically significant results [11]. For orphan diseases, such as osteonecrosis, most researchers use the candidate gene approach which is the investigation of a genetic association between the disease and a list of genes that are pre-selected by analysing the phenotype and biological functions involved in the disease.

Recently, the discovery of the next-generation sequencing technology has led to the development of new innovative ways of genetic analysis. The facts that sequencing costs have drastically decrease and the ability to produce a large volume of data allowed this technique to become a powerful tool for genetic research. Exome sequencing is the most widely used technique for targeted sequencing. It allows to sequence all the expressed genes on the human genome which contains most of the known genetic variations responsible for many diseases.

Hereditary ONFH

COL2A1 was the first gene to be linked to hereditary ONFH. Chen et al. identified two four-generation Taiwanese families which showed an autosomal dominant mode of inheritance [7]. A genome wide scan for linkage analysis isolated candidate genes to a region on chromosome 12q13. Specifically, they proposed that COL2A1 and VDR could be putative causal genes. In the following year, an additional Taiwanese family with ONFH was reported with the same autosomal dominant heredity [12].

Genetic analyses were completed comparing all three families with hereditary ONFH, idiopathic cases of ONFH (sporadic cases) and wild type individuals. Candidate genes were selected following haplotype analysis of the families. Thirty-nine microsatellites repeat markers were analyzed in DNA from leukocytes and the causal gene was mapped to the same region that had been previously identified (12q13) [12]. Liu et al. identified COL2A1 mutations (NM_001844: c.3655G>A; p. Gly1170Ser and c.2149G A; p. Gly717Ser) that were not seen in sporadic cases and controls. None of the individuals with inherited ONFH had predisposing risk factors, suggesting that the COL2A1 mutations are causal for the disease [12]. Kannu et al. identified a missense mutation of COL2A1 gene (NM_001844.3: c. 4148 C>T; p. Thr1383Met) located on the conserved C-propeptide region which is required for collagen chain trimerization [13].

Inherited forms of LCPD show a similar association with COL2A1. Miyamoto et al. identified a Japanese family with five members affected with LCPD harboring the c.3655G>A mutation [14]. Su et al. also found the same mutation in a five-generation Chinese family with 16 individuals affected with some degree of ONFH [15]. Although these findings are interesting from a clinical perspective, these mutations were not characterized functionally and therefore the exact mechanism and their clinical impact remain poorly understood. Recently, our group has identified a Canadian family of Greek origin where four of six siblings were diagnosed by x-ray and/or MR Imaging to have advanced bilateral osteonecrosis [10]. The affected siblings showed no signs of known ONFH risk factors. Genetic analysis by whole exome sequencing showed a NM_021625.4 c.2480_2483delCCCG frameshift deletion followed by a c.2486T>A substitution of TRPV4 (transient receptor potential vanilloid 4 cation channel, subfamily V) gene. These mutations result in amino acid changes p.829V>W and p.830V>N followed by a stop codon at position 831 resulting in premature truncation of a highly conserved region [10].

TRPV4 is a non-selective cation channel involved in a broad range of physiological processes such as pain, thermoregulation and calcium homeostasis. It is also known to regulate vascular tone and osteoclastic differentiation while also exhibiting mecha-

nosensitive properties, proposed to play a role in the sensing of weight loading essential to bone development and mechanosensation in endothelial cells [16]. Considering that our functional studies showed impaired closure of the calcium channels, identifying specific TRPV4 pathways could have the potential to develop new targeted therapies in ONFH.

Polymorphisms in Genes Affecting Blood Circulation

The leading hypothesis for the development of ONFH is that vascular obstruction prevents blood flow to the femoral head, leading to the deterioration and collapse of the bone [17]. This hypothesis is supported by the association between sickle cell anemia and other hemoglobinopathies with ONFH. To help elucidate the pathogenesis and delineate the biological changes of the disease, several studies have focused on serological levels of factors participating in the coagulation pathway (extrinsic and intrinsic), including fibrinolysis, while others have searched for genetic variations in genes related to coagulation defects. Among genes involved in this process, factor V, prothrombin, and 5, 10-methylenetetrahydrofolate reductase (MTHFR) are of most interest, being known as thrombophilic risk factors. Some well-known variations in these genes have been associated with ONFH [9].

MTHFR gene encodes for an enzyme involved in the process of converting homocysteine to methionine. Several polymorphisms of the MTHFR gene affect its activity and leads to an elevated plasma homocysteine (hyperhomocysteinemia), a condition that has been associated with the development of vascular disease, including stroke, acute myocardial infarction, peripheral artery disease and venous thrombosis especially in homozygous SNP findings [18]. The most studied polymorphisms of this gene (rs1801133 also known as c.677C T) leads to reduced enzyme activity, with several studies exploring association of this SNP with ONFH, and showing inconsistent results. Shang et al performed a meta-analysis including data from eight studies for a total of 778 patients with ONFH and 1162 controls and found an association in non-Asian populations, but there was no clear evidence of this association across worldwide populations [19].

Another meta-analysis including all published studies (12 studies with 1181 patients and 1961 controls) found no overall association [20]. This difference is explained by differences in the design, inclusion criteria and characteristics of the patients (age, gender, etiology) enrolled in these studies.

Factor V is a coagulation factor that binds to activated platelets and is inactivated by activated protein C. This inactivation is prevented by a change in amino acid (p. Arg506Gln, c. 1691G>A (rs6025) that is associated with venous thrombosis and known as Factor V Leiden [21]. Shang et al. performed a meta-analysis of 7 studies with 481 patients and 867 controls and concluded that patients with the A allele have a 4-fold higher risk of ONFH when compared to carriers of the G allele [22]. While Gagala et al. failed to find this association in 68 patients and 100 controls in Poland, they found a statistically significant association with a genetic variation (rs1880669) in the tissue Plasminogen Activator (PLAT) gene [23]. tPA, the gene product of PLAT is a major component of the fibrinolytic system. The rs1880669 (TPA 25 I/D) polymorphism is an Insertion/Deletion of an Alu sequence on the intron 8. It affects the release of tPA after endothelial cell activation with the D allele being the slowest [24]. Gagala et al. showed higher frequency of the D allele in ONFH patients compared to controls [23].

Plasminogen Activator Inhibitor-1 (PAI-1) is a key regulator of the coagulation-fibrinolysis pathway, a process affected in ONFH. Several studies have shown an upregulation of the PAI-1 serum levels in ONFH patients compared to controls [25] which may be caused by a genetic difference. In fact, the PAI-1 4G/5G SNP (rs1799889) is associated with elevated PAI-1 plasma levels. 4G/4G carriers have higher PAI-1 plasma levels compared to 4G/5G which is higher than 5G/5G [26]. A meta-analysis of PAI-1 4G/5G polymorphisms (five studies with 419 cases of ONFH and 969 controls) showed that the 4G/4G genotype is a significant risk factor for predicting ONFH [27]. Tissue factor pathway inhibitor (TFPI) is an important regulator of the tissue-factor mediated blood coagulation pathway. 37339T>A, a novel SNP located in the 3'-UTR and 24999A>G (rs8176592) are related to both alcohol-induced and idiopathic ONFH in Korean individuals [28].

Blood flow may also be impeded by hyperlipidemia. PON-1 is a member of the paraoxonase gene family and is involved in lipid metabolism. The rs662 SNP affects PON-1 catalytic efficiency and has been shown to be associated with steroid-induced ONFH in Greek [29] and Han Chinese [30] patients. Sterol regulatory element-binding transcription factor 1 (SREBF1) is a transcription factor involved in cholesterol and fatty acid metabolism [31]. In a study of Korean ONFH patients and controls, rs4925115, a SNP located on intron 7 of SREBP1 gene, was linked to ONFH, particularly amongst male ONFH patients. When the research participants were classified into subgroups based on etiological risk factors, the alcohol-induced group was associated with this SNP.

SREBP-2 is closely related to SREBF1 and is involved in lipid metabolism, and homeostasis by stimulating gene expression of cholesterol biosynthetic pathways. The rs2267439 and rs2267443 SREBP-2 SNPs have an increased frequency in a study of 49 ONFH patients and 42 controls [32].

Another factor to consider in genes that impact blood flow is oxygen and oxidative dynamics. Nitric oxide is synthesized by endothelial Nitric Oxide Synthase (eNOS) and has been implicated in many biological processes affected in ONFH including bone angiogenesis, thrombosis, and cellular turnover. An eNOS gene polymorphism that consists of a variable number of tandem repeats of 27bp in intron 4 can impact nitric oxide synthesis. Two alleles have been identified, a larger form with 5 repeats (4b) and

a smaller form with 4 repeats (4a). Subjects with the smaller form (4a) have lower levels of nitric oxide [33]. The eNOS 4a/b polymorphism has been previously associated with ONFH [34]. Results from Gagala et al. support this finding by analyzing 68 patients and 100 controls of Polish descent [23]. Song et al. performed a meta-analysis of 5 studies (566 cases and 833 controls) and showed the same association between the eNOS 4a/b polymorphism and osteonecrosis [35].

By analyzing samples from 460 ONFH patients and 300 controls using SNP chip array (a microarray chip used to detect SNPs), Hong et al. found six candidate genes to be associated with ONFH: Transferrin (TF), Kinase Insert Domain Receptor (KDR), Vascular Endothelial Growth Factor C (VEGFC), Insulin-Like Growth Factor Binding Protein 3 (IGFBP3), Neuropilin 1 (NRP1), and Angiotensin Converting Enzyme (ACE) [36]. Interestingly, some SNPs in KDR, VEGFC and NRP1 were linked to protection against ONFH (Table 1).

Gene	SNP	Risk Association	Patient population	Presence of Other Risk Factors	Reference
Hereditary ONFH					
COL2A1	3655G>A		Four-generation Taiwanese families with inherited ONFH Patients described in case reports	None	[12] [13] [37] [14]
	2149G>A				
	4148 C>T				
	c.2014G>T				
	c.638G>A				
TRPV4	c.2480_2483delCCCG		Family of Greek origin	None	[10]
Coagulation Defects					
PON-1	rs662	+ (p=0.022 in over-dominant model)	Chinese Han 94 patients (40M, 54F), 106 controls	Glucocorticoids (64M, 42F GC users without ONFH)	[30]
Factor V Leiden	rs6025	-	Korean patients 71 patients (53M, 18F), 200 controls (128M, 72F) Polish patients 68 patients (58M, 12F), 100 controls	Alcohol (n=51) diopathic (n=18), Glucocorticoids (n=1) Dysbaric (n=1) Idiopathic (n=45) Glucocorticoids (n=11) Chemotherapy (n=7) Alcohol (n=4) Renal transplantation (n=1)	[38] [23]
MTHFR	rs1801133	+ Twofold increase (95% CI, 1.05-3.81)			
	rs1801131	-			
TFPI	-50984A>G (T-287C)	-	Korean patients 474 patients (346M, 128F), 349 controls (299M, 50F) Polish patients 68 patients (58M, 12F), 100 controls	Idiopathic (n=140) Glucocorticoids (n=129) Alcohol (n=205)	[28]
	+24999A>G (Int7 -33T>C)	-			
	+37339T>A	-			
eNOSa	-786 T>C rs2070744	+ (p=0.026)	Polish patients 68 patients (58M, 12F), 100 controls	Idiopathic (n=45) Glucocorticoids (n=11) Chemotherapy (n=7) Alcohol (n=4) Renal transplantation (n=1)	[39]

PLAT	TPA25 I/D rs1880669	+ (p=0.0049- 0.0448, OR E291.27-1.69)	Korean population 460 patients (377M, 83F), 300 controls (210M, 90F)	Idiopathic (n=45) Glucocorticoids (n=11) Chemotherapy (n=7) Alcohol (n=4) Renal transplantation (n=1)	
TF	rs2692695	+ (p=0.0049- 0.0448, OR 1.27-1.69) + (p=0.0042- 0.0107, OR1.33-1.67)	Korean population 460 patients (377M, 83F), 300 controls (210M, 90F) orean population 423 patients (342M, 81F), 348 controls (298M, 50F)	Alcohol induced (n=215) Idiopathic (n=186) Glucocorticoids (n=59)	[36]
	rs2718806				
	rs1485766				
VEGFC	rs3775203	+ D31(p=0.0042-0.0107, OR1.33-1.67) P (p=0.0087 or 0.55)		Alcohol induced (n=215) Idiopathic (n=186) Glucocorticoids (n=59)	
	rs2333496				
	rs2453839	+ (p=0.0061, OR F307.74)			
ACE	rs4344	+ (p=0.0044- 0.00367, OR > 1.34-1.63) P (p=0.0357, OR=0.67)			
	rs4461142				
	rs6837735				
KDR	1870377	Protective (p=0.0488, OR 0.67)			
	rs12573218	Protective (p=0.0019-0.0423, OR 0.55-0.75)			
NRP1	rs12358370	Protective (p=0.0019-0.0423, OR 0.55-0.75) -			
	rs2269091				
	rs12601420				
SREBF1	rs9925115	-	Korean population 423 patients (342M, 81F), 348 controls (298M, 50F) 18 patients (14M, 4F)	Glucocorticoids (n=77) Alcohol (n=77) Idiopathic (n=140)	[31]
	rs12601420				
VEGF	rs1570360	+ Lower frequency in steroid (7.4% vs. 18.1%; OR=0.363)	160 patients (108M, 52F), 160 controls Indian patients 150 patients (90M, 60F), 154 controls	Idiopathic (n=86) Glucocorticoids (n=74)	[40]
	rs2010963	-			
	rs2010963	Lower frequency in steroid (74.3% vs. 84.4$, OR=0.492)			
	rs7170178	+ (p<0.001)			
Bone structure					
ANXA2	rs73435133	-	Indian patients 150 patients (90M, 60F), 154 controls 443 patients (366M, 77F), 273 controls (206M, 67F)	All patients have sickle cell; 3 patient groups Gr. 1 (n=60): sickle homozygous Gr. 2 (n=75): β-thalassemia Gr. 3 (n=15) sickle cell D	[41]
	rs7348020	-			
	rs72746635	-			
	rs73418025	-			
	rs4655686	+			

GRIN3A	rs10989692	+ P=3.59x10-7	1275 caucasian 139 black 601 hispanic 48 asian 222 others	All patient have Acute Lymphoblastic Leukemia and received high doses of GC: 250 have ONFH and 2035 controls	[42]
MMP3	rs650108	+	Chinese male patients:300 Alcohol induced ONFH 308 healthy control	Associated with decreased risk for ONFH	
MMP8	rs2012390 rs11225394	+		Associated with increased risk for ONFH	
MMP9	rs2274755	+ P=0.025	Chinese patients: 285 GC induced ONFH 507 healthy control	SNP associated with decreased risk for ONFH	[44]
OPG	rs1032128	+	Chinese patients: 335 Alcohol induced ONFH 335 healthy control		[45]
	rs11573828	+			
RANKL	rs2200287				
Immunity					
IL23R	rs1569922	+	443 patients (366M, 77F), 273 controls (206M, 67F)	Glucocorticoids (n=56) Alcohol (n=206) Idiopathic (n=181)	` [46]
	rs7539625	+			
Steroid Metabolism					
ABCB1	rs1045642 (C3435T)	+	Meta-analysis of a total of 336 patients and 712 controls	All glucocorticoids	
	rs2032582 (C7623T)	+			
ApoB	rs3751845	+	Japanese population 34 patients, 123 controls	Renal transplantation (n=34)	[47]
CBP	rs3751845				
Miscellaneous/ Other					
CAT	rs7943316	Protective	443 patients (366M, 77F), 273 controls (206M, 67F)		
	rs1049982	Protective	443 patients (366M, 77F), 273 controls (206M, 67F)	Glucocorticoids (n=56) Alcohol (n=206) Idiopathic (n=181)	[48]
	rs525938	Protective			
	rs3758730	+			
	rs769217	+			
	rs2284365	+			

Table 1: Summary of genetic variations in genes linked to osteonecrosis pathogenesis. This table shows a list of genes where polymorphisms have been identified along with name of the SNP (position or rs number if known), if there is an association with the disease or not, type of population studied, presence or not of other risk factors and reference number.

The protective effect of VEGF was reported by another group that investigated SNPs in the promoter and 5' UTR regions of the gene [40]. Liu et al. performed a meta-analysis of 3 studies and confirmed that VEGF -634G/C SNP was significantly associated with increased risk for ONFH [49]. From a clinical and vascular bed perspective, osteonecrosis is a microvascular disease and thrombophilic markers involving the fibrinolytic pathway are probably the most relevant. Clinically, markers that usually involve macrovessels (thrombosis of lower extremities and pulmonary bed) such as Factor V Leiden and Prothrombin 20210A are probably less important in terms of risk factors for microvascular disease such as ONFH. Conversely, VEGF is more likely to have a role due to its function in microvascular disease, regeneration of blood vessels and function in bone remodeling. VEGF remains a

promising target in the clinical setting with some in vitro and in vivo studies having looked at the expression of VEGF in glucocorticoid-induced ONFH [50,51].

Steroid Metabolism and ONFH: A Spotlight on ABCB1

Glucocorticoids are a major risk factor in the development of ONFH and are often administered at high dose for treatment of diverse disorders which may lead to the development of ONFH. Variation in the susceptibility of patients receiving high doses of glucocorticoids to ONFH strongly suggests a genetic variation that potentially involves the presence of SNPs [52,53]. Several SNPs in Adenosine Triphosphate-Binding Cassette B1 (ABCB1), also known as MDR1, have been linked to ONFH. ABCB1 encodes the transport protein P-glycoprotein (P-gp) that plays an important role in absorption and distribution of a broad range of therapeutic compounds [54]. Higher P-gp activity was shown to be protective from ONFH development [55]. There are over 50 known SNPs in ABCB1 which may account for the variation in individual sensitivity to steroids [56]. The two most studied SNPs of this gene, rs1045642 and rs2032582, impact its activity. Studies investigating the association of these two SNPs with ONFH gave conflicting results prompting Zhou et al. to perform a meta-analysis of 7 studies and concluded that these SNPs are associated with low risk of glucocorticoid-induced ONFH [57]. Zhang et al. showed the same results for the rs1045642 SNP in a meta-analysis of 5 studies [58].

Lastly, an analysis of combinations of SNPs between genes involved in steroid metabolism show that ABCB1 and CBP, which encodes an important transcriptional co-regulator of glucocorticoid receptors, may interact with each other. Thirty-four patients diagnosed with glucocorticoid-induced ONFH and 123 controls (who were exposed to glucocorticoids but did not develop ONFH) were screened for SNPs in ABCB1 (rs1045642), apoliprotein B (ApoB; c.7623C T), and cAMP-response element binding protein (CBP; rs3751845). A synergistic index >1.00 (1.99) was observed between ABCB1 and CBP, and the odds ratio of the presence of both SNPs in ONFH was very high (22.91). This suggests that both genes are involved in the pathology of ONFH through steroid metabolism [47]. These findings are significant because screening for the combination of SNPs in ABCB1 and CBP in patients undergoing high dose glucocorticoids could provide the identification of susceptible individuals at higher risk of developing ONFH. Susceptible individuals undergoing glucocorticoid treatment would then require close monitoring of joint symptoms and frequent radiological evaluations of their hip joints.

Immunity and ONFH

Several genes related to immunity have been associated with ONFH. Interleukin (IL)-1 is a proinflammatory cytokine that stimulates the expression of genes related to inflammation and immunity. The rs1800587 SNP of IL-1 has been associated with increased risk of ONFH likely because of the stimulating role IL-1 plays in bone resorption [59]. In this study of 112 ONFH affected individuals and 438 healthy controls, the authors also found an association of ONFH with IL-10, which can inhibit the synthesis of other pro-inflammatory cytokines [59].

The specific SNPs with the haplotype rs1800896 G, rs1800871C and rs1800872C are listed in (Table 1). IL23 and IL-33 are two other interleukin genes shown to be predictive for increased risk of ONFH. The proinflammatory cytokine IL23 regulates the activity of an immune response by promoting inflammation through the IL23 Receptor (IL23R). The two cytokines are primarily expressed in cells that are closely related to the immune system such as T cells, macrophages, and dendritic cells [60,61]. The rs4655686, rs1569922 and rs7539625 SNPs on IL23R gene were found to be associated with an increased risk of ONFH in a Korean study that involved 443 ONFH patients and 272 controls [57]. However, Wang et al. showed another SNP on this gene (rs6693831) to be protective for ONFH [62]. Interleukin 33 (IL-33) is also implicated in immune responses in a similar fashion to IL23R, inducing helper T cells, mast cells, eosinophils, and basophils to produce type 2 cytokines. IL-33 is also known to be released from necrotic cells and is constitutively expressed in osteoblasts. IL-33 serum levels were significantly higher in two studies with a total of 165 ONFH patients compared to controls, with no significant differences seen across glucocorticoid-induced, alcohol-induced, or idiopathic cases [63,64]. Zheng et al. proposed that instead of using MRI scans that are expensive and have limited availability in some countries, measuring IL-33 plasma levels could be a cost-effective and efficient method for diagnosing the early stages of ONFH [63]. While this study did not investigate specific genetic anomalies, i.e. SNPs or mutations, these findings emphasize the substantial clinical applicability of genetic research that aims to investigate the biological contributions of candidate genes to ONFH. However, before considering the use of IL-33 plasma levels as a potential biomarker of early ONFH, further clinical studies are needed to support a positive correlation of increased IL-33 plasma levels with ONFH development on MRI scans over time.

Tumor Necrosis Factor (TNF)-α is a potent inflammatory cytokine released by macrophages to regulate an immune response by promoting the expression of other cytokine molecules [59]. As such, TNF-α is suggested to act upon osteoblasts or bone marrow stromal cells to release cytokines that are associated with osteoclast proliferation and maturation [65]. The GA genotype of the rs361525 SNP site is associated with an increase in TNF-α expression, altering the osteoblast-osteoclast compositional balance [59]. This polymorphism is associated with an increased risk for ONFH [59,66]. As well, in a study investigating genetic factors that contribute to the occurrence and progression of SARS-CoV infection, the rs1799964 (c.1031CT CC) and rs1800630 (c.863A C) genotypes in TNF-α were found to be associated with ONFH in discharged SARS patients [67].

Genes Involved in the Regulation of Bone Formation

Although the exact pathogenesis of ONFH is unknown, the genes mentioned to this point support that ONFH results from disrupted blood flow to the femoral head. Impeded circulation would lead to a failure to provide nutrients and immune cells necessary for the maintenance of the bone tissue, leading to its eventual collapse. However, a hypothesis for ONFH relating to the bone physiology of the hip itself is also a possible explanation, as compromised bone physiology at the hip, possibly exacerbated by the weight bearing nature of the joint, could lead to a similar outcome. Therefore, genes involved in bone formation and bone structure are important to consider in the onset and progression of ONFH. This section summarizes the genes that influence processes regulating osteoblasts or osteoclasts. Bone necrosis is a frequent manifestation in sickle cell disease; a case study of Indian patients with sickle cell anemia revealed a linkage in the rs7170178 SNP site of the Annexin A2 (ANXA2) gene [41]. Genes in the annexin family play a role in regulating cellular growth, with ANXA2 encoding an autocrine factor that heightens osteoclast formation and bone resorption. In the study, patients and control subjects were screened for five SNPs of ANXA2, of which only the rs7170718 was seen amongst the study population. The rs7170718 A/G and A/A genotypes were statistically more frequent in the patient groups indicating that detection of this SNP could be a screening tool for sickle cell patients [41]. Another study of sickle cell patients revealed a significant association of the rs3812163 SNP in Bone Morphogenetic Protein 6 (BMP6) with ONFH [68]. Members of the BMP family induce endochondral bone formation when implanted in ectopic sites. Insulin-like Growth Factors (ILGFs) are the most abundant growth factors stored in bones and produced by osteoblasts [69]. IGFBP3 is a member of the IGF binding protein family and helps regulate growth and metabolism, especially bone cell metabolism [32]. The TT and TC genotypes of IGFBP-3 rs2453839 were found to be more frequent in ONFH patients than in the control group [32]. SNPs associated with a protective effect against ONFH have also been identified at the bone level. Transforming growth factor (TGF)-β regulates the proliferation and differentiation of various cells and functions in cell growth, differentiation, apoptosis, cell migration, immune cell function, and extracellular matrix production [59]. A higher frequency of the homozygous C allele at the 25th codon (rs1800471) has been associated with a decreased level of TGF-β and linked to a protective effect [59]. The authors hypothesize that this results in the induction of BMPs, promoting bone regeneration. These findings support the hypothesis that deteriorated bone structure plays a major role in the disease and that SNPs in genes related to bone regulation provide valuable insight into the cellular mechanisms that lead to ONFH.

Karol et al. performed a genome-wide association study of 2285 patients with acute lymphoblastic leukemia treated with high doses of glucocorticoids [42]. 250 of these patients developed ONFH, identifying a SNP near the Glutamate Receptor, Ionotropic, N-Methyl-D-Aspartate 3A (GRIN3A) gene (rs10989692) in association with glucocorticoid-induced ONFH. Glutamate signaling has been shown to regulate bone formation induced by mechanical loading which induces glutamate release from osteocytes. The released glutamate induces osteoblast receptor through activation of calcium channels [42,70], which could implicate TRPV4 in this process, and thus provide a novel additional insight into the study of glucocorticoid-induced ONFH.

Conclusion

ONFH is a multifactorial disease representing a complex interplay of genetic anomalies and environmental factors. Knowledge of genetic variations thought to be involved in the disease could be useful in determining individuals considered at higher risk to develop ONFH with the ultimate goal of preventing the multiple hit effect. COL2A1 mutations remain one of the strongest association with hereditary ONFH while ABCB1 and GRIN3A (glutamate pathway) are gaining importance in glucocorticoid-induced ONFH. Since SNPs are identified within specific pathways, they could account for differences in disease susceptibility and responses to drug therapies, therefore screening an individual at risk for ONFH for the presence of genetic risk factors may be beneficial in evaluating treatment options. As our society is moving towards a more preventive and personalized medicine, genetic studies will likely become of greater value and ONFH is a superb example of the potential applicability of translational research from basic science to patient care.

Acknowledgements

The authors gratefully acknowledge Terry Y. Chow PhD and Michael Churchill-Smith MD for manuscript revision.

References

1. Babis GC, Sakellariou V, Parvizi J, Soucacos P (2011) Osteonecrosis of the femoral head. Orthopedics 34: 39-48.
2. Kerachian MA, Harvey EJ, Cournoyer D, Chow TY, Séguin C (2006) Avascular necrosis of the femoral head: vascular hypotheses. Endothelium 13: 237-244.
3. Orban BH, Cristescu H, Dragusanu M (2009) Avascular necrosis of the femoral head. Maedica (Buchar) 2009: 4.
4. Mont MA, Jones LC, Hungerford DS (2006) Nontraumatic osteonecrosis of the femoral head: ten years later. J Bone Joint Surg Am 88: 1117-1132.
5. Zalavras CG, Lieberman JR (2014) Osteonecrosis of the femoral head: evaluation and treatment. J Am Acad Orthop Surg 22: 455-464.
6. Assouline-Dayan Y, Chang C, Greenspan A, Shoenfeld Y, Gershwin ME (2002) Pathogenesis and natural history of osteonecrosis. Semin Arthritis Rheum 32: 94-124.
7. Chen WM, Liu YF, Lin MW, Chen IC, Lin PY, et al. (2004) Autosomal dominant avascular necrosis of femoral head in two Taiwanese pedigrees and linkage to chromosome 12q13. Am J Hum Genet 75:

310-317.

8. Su P, Li R, Liu S, Zhou Y, Wang X, et al. (2008) Age at onset-dependent presentations of premature hip osteoarthritis, avascular necrosis of the femoral head, or Legg-Calve-Perthes disease in a single family, consequent upon a p. Gly1170Ser mutation of COL2A1. Arthritis Rheum 58: 1701-1706.

9. Hadjigeorgiou G, Dardiotis E, Dardioti M, Karantanas A, Dimitroulias A, et al. (2008) Genetic association studies in osteonecrosis of the femoral head: mini review of the literature. Skeletal Radiol 37: 1-7.

10. Mah W, Sonkusare SK, Wang T, Azeddine B, Pupavac M, et al. (2016) Gain-of-function mutation in TRPV4 identified in patients with osteonecrosis of the femoral head. J Med Genet 53: 705-709 .

11. Hong EP and Park JW (2012) Sample size and statistical power calculation in genetic association studies. Genomics Inform 10: 117-122.

12. Liu YF, Chen WM, Lin YF, Yang RC, Lin MW, et al. (2005) Type II collagen gene variants and inherited osteonecrosis of the femoral head. N Engl J Med 352: 2294-2301.

13. Kannu P, O'Rielly DD, Hyland JC, Kokko LA (2011) Avascular necrosis of the femoral head due to a novel C propeptide mutation in COL2A1. Am J Med Genet A 155: 1759-1762.

14. Miyamoto Y, Matsuda T, Kitoh H, Haga N, Ohashi H, et al. (2007) A recurrent mutation in type II collagen gene causes Legg-Calve-Perthes disease in a Japanese family. Hum Genet 121: 625-629.

15. Su P, Zhang L, Peng Y, Liang A, Du K, et al. (2010) A histological and ultrastructural study of femoral head cartilage in a new type II collagenopathy. Int Orthop 34: 1333-1339.

16. W G Darby, M S Grace, S Baratchi, P McIntyre (2016) Modulation of TRPV4 by diverse mechanisms. Int J Biochem Cell Biol 78: 217-228.

17. Urbaniak JR and Harvey EJ (1998) Revascularization of the femoral head in osteonecrosis. J Am Acad Orthop Surg 6: 44-54.

18. Séguin C1, Abid MR, Spokes KC, Schoots IG, Brkovic A, et al. (2008) Priming effect of homocysteine on inducible vascular cell adhesion molecule-1 expression in endothelial cells. Biomed Pharmacother 62: 395-400.

19. Shang XF, Su H, Chang WW, Wang CC, Han Q, et al. (2012) Association between MTHFR C677T polymorphism and osteonecrosis of the femoral head: a meta-analysis. Mol Biol Rep 39: 7089-7094.

20. Chai W, Zhang Z, Ni M, Geng P, Lian Z, et al. (2015) Genetic Association between Methylenetetrahydrofolate Reductase Gene Polymorphism and Risk of Osteonecrosis of the Femoral Head. Biomed Res Int 2015: 196495.

21. Bertina RM1, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, et al. (1994) Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 369: 64-67.

22. Shang X, Luo Z, Li X, Hu F, Zhao Q, et al. (2013) Meta-analysis of Factor V Leiden G1691A polymorphism and osteonecrosis of femoral head susceptibility. Biomed Rep 1: 594-598.

23. Gagala J, Buraczynska M, Mazurkiewicz T, Ksiazek A (2013) Prevalence of genetic risk factors related with thrombophilia and hypofibrinolysis in patients with osteonecrosis of the femoral head in Poland. BMC Musculoskelet Disord 14: 264.

24. Jern C, Ladenvall P, Wall U, Jern S (1999) Gene polymorphism of t-PA is associated with forearm vascular release rate of t-PA. Arterioscler Thromb Vasc Biol 19: 454-459.

25. Dawson S, Hamsten A, Wiman B, Henney A, Humphries S (1991) Genetic variation at the plasminogen activator inhibitor-1 locus is associated with altered levels of plasma plasminogen activator inhibitor-1 activity. Arterioscler Thromb 11: 183-90.

26. Eriksson P, Kallin B, van 't Hooft FM, Båvenholm P, Hamsten A (1995) Allele-specific increase in basal transcription of the plasminogen-activator inhibitor 1 gene is associated with myocardial infarction. Proc Natl Acad Sci USA 92: 1851-1855.

27. Liang XN, Xie L, Cheng JW, Tan Z, Yao J, et al. (2013) Association between PAI-1 4G/5G Polymorphisms and osteonecrosis of femoral head: a meta-analysis. Thromb Res 132: 158-163.

28. Dai XL1, Hong JM, Oh B, Cho YS, Lee JY, et al. (2008) Association analysis of tissue factor pathway inhibitor polymorphisms and haplotypes with osteonecrosis of the femoral head in the Korean population. Mol Cells 26: 490-495.

29. Hadjigeorgiou GM, Malizos K, Dardiotis E, Aggelakis K, Dardioti M, et al. (2007) Paraoxonase 1 gene polymorphisms in patients with osteonecrosis of the femoral head with and without cerebral white matter lesions. J Orthop Res 25: 1087-1093.

30. Wang Z, Zhang Y, Kong X, Li S, Hu Y, et al. (2013) Association of a polymorphism in PON-1 gene with steroid-induced osteonecrosis of femoral head in Chinese Han population. Diagn Pathol 8: 186.

31. Lee HJ1, Choi SJ, Hong JM, Lee WK, Baek JI, et al. (2009) Association of a polymorphism in the intron 7 of the SREBF1 gene with osteonecrosis of the femoral head in Koreans. Ann Hum Genet 73: 34-41.

32. Song Y, Du ZW, Li QJ, Zhang GZ, Wang LL, et al. (2012) Association of sterol regulatory element binding protein 2 and insulin-like growth factor binding protein 3 genetic polymorphisms with avascular necrosis of the femoral head in the Chinese population. Chin Med J (Engl) 125: 4037-4043.

33. Tsukada T1, Yokoyama K, Arai T, Takemoto F, Hara S, et al. (1998) Evidence of association of the ecNOS gene polymorphism with plasma NO metabolite levels in humans. Biochem Biophys Res Commun 245: 190-193.

34. Koo KH1, Lee JS, Lee YJ, Kim KJ, Yoo JJ, et al. (2006) Endothelial nitric oxide synthase gene polymorphisms in patients with nontraumatic femoral head osteonecrosis. J Orthop Res 24: 1722-1728.

35. Song GG and Lee YH (2017) Association of eNOS polymorphisms with susceptibility to osteonecrosis of the femur head: A meta-analysis. Z Rheumatol 76: 267-273.

36. Hong JM, Kim TH, Kim HJ, Park EK, Yang EK, et al. (2010) Genetic association of angiogenesis- and hypoxia-related gene polymorphisms with osteonecrosis of the femoral head. Exp Mol Med 42: 376-385.

37. Kannu P1, Irving M, Aftimos S, Savarirayan R (2011) Two novel COL2A1 mutations associated with a Legg-Calve-Perthes disease-like presentation. Clin Orthop Relat Res 469: 1785-1790.

38. Chang JD, Hur M, Lee SS, Yoo JH, Lee KM (2008) Genetic background of nontraumatic osteonecrosis of the femoral head in the Korean population. Clin Orthop Relat Res 466: 1041-1046.

39. Gagala J, Buraczynska M, Mazurkiewicz T, Ksiazek A (2013) Endothelial nitric oxide synthase gene intron 4 polymorphism in non-traumatic

osteonecrosis of the femoral head. Int Orthop 37: 1381-1385.

40. Lee YJ, Lee JS, Kang EH, Lee YK, Kim SY, et al. (2012) Vascular endothelial growth factor polymorphisms in patients with steroid-induced femoral head osteonecrosis. J Orthop Res 30: 21-27.

41. Pandey S, Ranjan R, Pandey S, Mishra RM, Seth T, et al. (2012) Effect of ANXA2 gene single nucleotide polymorphism (SNP) on the development of osteonecrosis in Indian sickle cell patient: a PCR-RFLP approach. Indian J Exp Biol 50: 455-458.

42. Karol SE, Yang W, Van Driest SL, Chang TY, Kaste S, et al. (2015) Genetics of glucocorticoid-associated osteonecrosis in children with acute lymphoblastic leukemia. Blood 126: 1770-1776.

43. Chen J, Liu W, Cao Y, Zhang X, Guo Y (2017) MMP-3 and MMP-8 single-nucleotide polymorphisms are related to alcohol-induced osteonecrosis of the femoral head in Chinese males. Oncotarget 8: 25177-25188.

44. Du J, Liu W, Jin T, Zhao Z, Bai R, et al. (2016) A single-nucleotide polymorphism in MMP9 is associated with decreased risk of steroid-induced osteonecrosis of the femoral head. Oncotarget 7: 68434-68441.

45. Li Y, Wang Y, Guo Y, Wang Q, Ouyang Y, et al. (2016) OPG and RANKL polymorphisms are associated with alcohol-induced osteonecrosis of the femoral head in the north area of China population in men. Medicine (Baltimore) 95: 3981.

46. Kim TH, Hong JM, Oh B, Cho YS, Lee JY, et al. (2008) Association of polymorphisms in the Interleukin 23 receptor gene with osteonecrosis of femoral head in Korean population. Exp Mol Med 40: 418-426.

47. Kuribayashi M1, Fujioka M, Takahashi KA, Arai Y, Hirata T, et al. (2008) Combination analysis of three polymorphisms for predicting the risk for steroid-induced osteonecrosis of the femoral head. J Orthop Sci 13: 297-303.

48. Kim TH, Hong JM, Oh B, Cho YS, Lee JY, et al. (2008) Genetic association study of polymorphisms in the catalase gene with the risk of osteonecrosis of the femoral head in the Korean population. Osteoarthritis Cartilage 16: 1060-1066.

49. Liu Y, Zhang Z, Liu S, Su X, Zhou S (2015) Association between VEGF -634G/C polymorphism and osteonecrosis of the femoral head susceptibility: a meta-analysis. Int J Clin Exp Med 8: 10979-10985.

50. Kabata T, Matsumoto T, Yagishita S, Wakayama T, Iseki S, et al. (2008) Vascular endothelial growth factor in rabbits during development of corticosteroid-induced osteonecrosis: a controlled experiment. J Rheumatol 35: 2383-2390.

51. Varoga D, Drescher W, Pufe M, Groth G, Pufe T (2009) Differential expression of vascular endothelial growth factor in glucocorticoid-related osteonecrosis of the femoral head. Clin Orthop Relat Res 467: 3273-3282.

52. Asano T, Takahashi KA, Fujioka M, Inoue S, Satomi Y, et al. (2003) Genetic analysis of steroid-induced osteonecrosis of the femoral head. J Orthop Sci 8: 329-333.

53. He N, Li S, Liu H (2009) High-throughput SNP detection based on PCR amplification on magnetic nanoparticles using dual-color hybridization. Methods Mol Biol 578: 393-402.

54. Zhang Y, Kong X, Wang R, Li S, Niu Y, et al. (2014) Genetic association of the P-glycoprotein gene ABCB1 polymorphisms with the risk for steroid-induced osteonecrosis of the femoral head in Chinese population. Mol Biol Rep 41: 3135-3146.

55. Han N, Yan Z, Guo CA, Shen F, Liu J, et al. (2010) Effects of p-glycoprotein on steroid-induced osteonecrosis of the femoral head. Calcif Tissue Int 87: 246-253.

56. Krupoves A, Mack D, Seidman E, Deslandres C, Amre D (2011) Associations between variants in the ABCB1 (MDR1) gene and corticosteroid dependence in children with Crohn's disease. Inflammatory Bowel Diseases 17: 2308-2317.

57. Zhou Z, Hua Y, Liu J, Zuo D, Wang H, et al. (2015) Association of ABCB1/MDR1 polymorphisms in patients with glucocorticoid-induced osteonecrosis of the femoral head: Evidence for a meta-analysis. Gene 569: 34-40.

58. Zhang Y, Xie H, Zhao D, Wang B, Yang L, et al. (2017) Association of ABCB1 C3435T polymorphism with the susceptibility to osteonecrosis of the femoral head: A meta-analysis. Medicine (Baltimore) 96: 6049.

59. Samara S, Kollia P, Dailiana Z, Chassanidis C, Papatheodorou L, et al. (2012) Predictive role of cytokine gene polymorphisms for the development of femoral head osteonecrosis. Dis Markers 33: 215-221.

60. Oppmann B, Lesley R, Blom B, Timans JC, Xu Y, et al. (2000) Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. Immunity 13: 715-725.

61. Parham C, Chirica M, Timans J, Vaisberg E, Travis M, et al. (2002) A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rbeta1 and a novel cytokine receptor subunit, IL-23R. J Immunol 168: 5699-5708.

62. Wang Y, Yang X, Shi J, Zhao Y, Pan L, et al. (2017) Combination analysis of NOS3, ABCB1 and IL23R polymorphisms with alcohol-induced osteonecrosis of the femoral head risk in Chinese males. Oncotarget 8: 33770-33778.

63. Zheng L, Wang W, Ni J, Li Z, Xiao T, et al. (2014) Plasma interleukin 33 level in patients with osteonecrosis of femoral head: an alarmin for osteonecrosis of the femoral head? J Investig Med 62: 635-637.

64. Ma J, Guo W, Li Z, Wang B, Li S, et al. (2017) Hip Osteonecrosis Is Associated with Increased Plasma IL-33 Level. Mediators Inflamm 2017: 732638.

65. Dai JC, He P, Chen X, Greenfield EM (2006) TNFalpha and PTH utilize distinct mechanisms to induce IL-6 and RANKL expression with markedly different kinetics. Bone 38: 509-520.

66. Wei BF, Feng Z, Wei W, Chen X (2017) Associations of TNF-alpha -238 A/G and IL-10 -1082 G/A genetic polymorphisms with the risk of NONFH in the Chinese population. J Cell Biochem 2017.

67. Wang S, Wei M, Han Y, Zhang K, He L, et al. (2008) Roles of TNF-alpha gene polymorphisms in the occurrence and progress of SARS-Cov infection: a case-control study. BMC Infect Dis 8: 27.

68. Ulug P, Vasavda N, Awogbade M, Cunningham J, Menzel S, et al. (2009) Association of sickle avascular necrosis with bone morphogenic protein 6. Ann Hematol 88: 803-805.

69. Dar AA, Majid S, Nosrati M, de Semir D, Federman S, et al. (2010) Functional modulation of IGF-binding protein-3 expression in melanoma. J Invest Dermatol 130: 2071-2079.

70. Brakspear KS and Mason DJ (2012) Glutamate signaling in bone. Front Endocrinol (Lausanne) 3: 97.