



# Osteonecrosis of the femoral head: genetic basis

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## ABSTRACT

**Purpose** Genetic factors and hereditary forms of osteonecrosis of the femoral head (ONFH) have been elucidated through genetic association studies. The significance of these cases is that they suggest an alternative hypothesis to the development of the disease. 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 purpose of this review is to consolidate and categorize genetic linkages according to physiological pathways.

**Methods** A systematic review of literature from PubMed and Google Scholar was undertaken with a focus on genetic linkages and hereditary case studies of the disease. Recent genetic analysis studies published after 2007 were the focus of genetic linkages in non-hereditary cases.

**Results** 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.

**Conclusion** Taken together, these associations may lead to new pathways of bone repair and remodeling while opening new avenues for therapeutic targets. Knowledge of genetic variations could help identify individuals considered to be at higher risk of developing ONFH and prevent the multiple hit effect.

**Keywords** Osteonecrosis of the femoral head · Polymorphisms · Coagulation defects · Steroids · Immunity · Hereditary ONFH · TRPV4

## 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 USA [1]. 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]. This usually results in severe hip pain that is treated with conservative methods such as weight-bearing restriction, the use of

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bisphosphonates and statins, or femoral head decompression at early stage [3]. 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 [4]. Classic clinical presentations are seen in late adolescence and young to middle-aged adults; however, the disease may occur in children between four and 12 years old and is then called Legg-Calvé-Perthes Disease (LCPD), often with early asymptomatic ischemic changes [3]. There is a wide spectrum of aetiological risk factors in non-traumatic ONFH including alcohol consumption and chemotherapy; however, the most common risk factor is the use of glucocorticoids [3]. 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 [5, 6]. Some studies also report a genetic predisposition that explains ethnic and individual differences in ONFH incidence [7]. 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 (Fig. 1), as well as a summary of a recent discovery of a novel mutation in *TRPV4* gene, in a family affected by hereditary ONFH [8].

## 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 [5]. 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 [9].

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) [9].

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 [9]. 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 [10].

Inherited forms of LCPD show a similar association with *COL2A1*, with Miyamoto et al. identifying a Japanese family

with five members affected with LCPD [11]. They identified the same mutation (p. Gly1170Ser) as Liu et al. Su et al. also found the same mutation in a five-generation Chinese family with 16 individuals affected with some degree of ONFH [12]. 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 [8]. The affected siblings showed no signs of known ONFH risk factors. Genetic analysis by exome sequencing showed a NM\_021625.4 c.2480\_2483delCCCCG 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 [8].

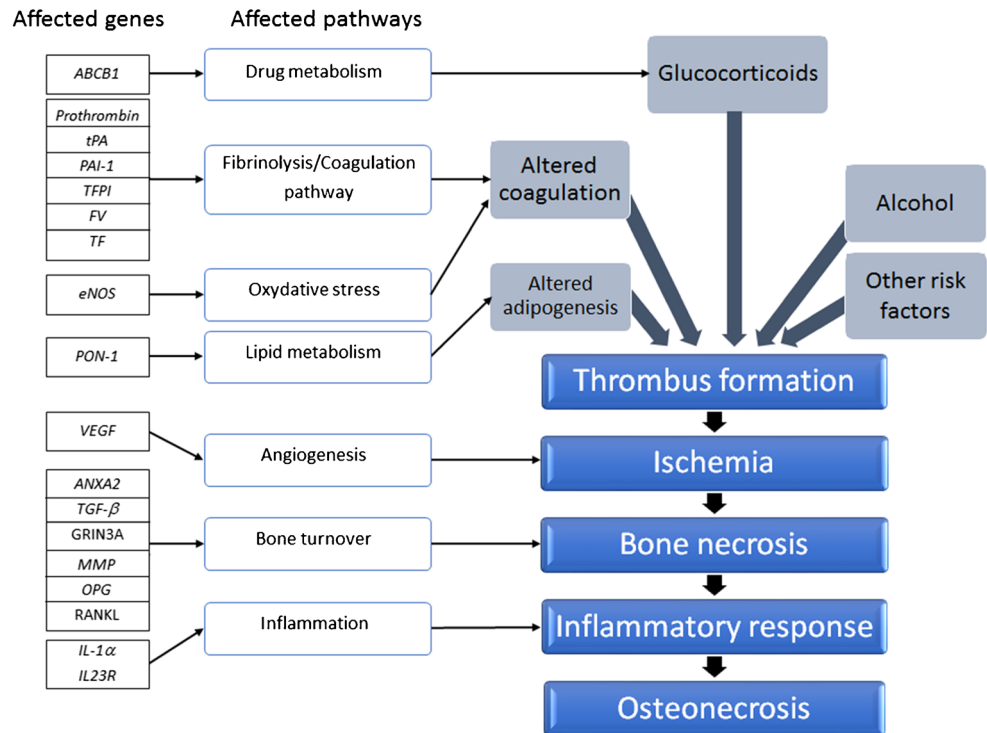
*TRPV4* is a non-selective cation channel involved in a broad range of physiological processes such as pain, thermoregulation, and calcium homeostasis. It is also known to regulate vascular tone and osteoclastic differentiation while also exhibiting mechanosensitive properties, proposed to play a role in the sensing of weight loading essential to bone development and mechanosensation in endothelial cells [13]. 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 [14]. This hypothesis is supported by the association between sickle cell disease (SCD) and other haemoglobinopathies 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. Amongst 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 [7].

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

**Fig. 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



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 [15]. 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 [16]. Another meta-analysis including all published studies (12 studies with 1181 patients and 1961 controls) found no overall association [17]. 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 [18]. 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 fourfold higher risk of ONFH when compared to carriers of the G allele [19]. 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 [20]. *tPA*, the gene product of *PLAT* is a major component of the fibrinolytic system and 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 [21]. Gagala et al. showed higher frequency of the D allele in ONFH patients compared to controls [20].

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 [20] 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 [22]. 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 [23]. Li et al. analyzed two other *PAI-1* SNPs (rs6092 and rs7242) in a population of 106 ONFH patients and 151 healthy controls and found no association between these two SNPs with ONFH. However, their statistical analysis showed that the haplotype G-T may be a protective factor of ONFH [24].

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 [25].

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 [26] and Han Chinese [27, 28] patients. Sterol regulatory element-binding transcription factor 1 (*SREBF1*) is a transcription factor involved in cholesterol and fatty acid metabolism [29]. In a study of Korean ONFH patients and controls, rs4925115, an 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 also involved in lipid metabolism, playing a role in lipid 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 [30].

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 27 bp in intron 4 can impact nitric oxide synthesis. Two alleles have been identified, a larger form with five repeats (4b) and a smaller form with 4 repeats (4a). Subjects with the smaller form (4a) have lower levels of nitric oxide [31]. The *eNOS* 4a/b polymorphism has been previously associated with ONFH [32] with a subsequent examination of 68 patients and 100 controls of Polish descent supporting this finding [20]. Song et al. performed a meta-analysis of five studies (566 cases and 833 controls) and showed the same association between the *eNOS* 4a/b polymorphism and osteonecrosis [33].

Samples from 460 ONFH patients and 300 controls were analyzed using SNP chip array, a microarray chip used to detect SNP. Six candidate genes were found 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 I converting enzyme (*ACE*) [34]. Interestingly, some SNPs in *KDR*, *VEGFC*, and *NRP1* were linked to protection against ONFH (listed in Table 1). The protective effect of *VEGF* was reported by another group that investigated SNPs in the promoter and 5' UTR regions of the gene [38]. Liu et al. performed a meta-analysis of three studies and confirmed that *VEGF* -634G/C SNP was significantly associated with increased risk for ONFH [47].

Sickle-cell disease (SCD) is a group of inherited blood disorders characterized by the presence of hemoglobin S

(HbS), an abnormal haemoglobin caused by a mutation on chromosome 11 that results in an amino acid substitution of valine for glutamic acid at the sixth position of the beta-globin subunit of haemoglobin. This mutation causes the red blood cells to become rigid, sticky and distorted (sickled). These sickled cells can get stuck in small blood vessels, leading the blood flow to slow or stop which explains why osteonecrosis is a common complication of SCD and why several studies have reported a high prevalence (11 to 50%) of ONFH in SCD patients [48].

SCD is an autosomal recessive disease, which means that it requires the inheritance of two sickle cell genes, HbSS. The patients who are homozygous (HBSS), or the association with another abnormal hemoglobin gene, particularly with HbC (HBSC), have a high risk of osteonecrosis. Recently, Daltro et al. studied the clinical features of ON in SCD patients in Bahia, Brazil. They showed that the majority of patients (246/283) have bilateral ON; 74.6% have ON on different sites (hip, shoulder, knee, and ankle). They found no genetic effect (genotype SS vs SC) on clinical features of ON. They recommend that SCD patients should have radiological examination more frequently in order to avoid subsequent complications [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, endothelial cell differentiation, and function in bone remodeling [50]. 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 [51, 52].

### 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 [53, 54]. 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

**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

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	[9]
	2149G>A				[10]
	4148 C>T				[35]
	c.2014G>T				[11]
	c.638G>A				
TRPV4	c.2480_2483delCC-CG		Family of Greek origin	None	[8]
Coagulation defects					
PON-1	rs662	+ ( $p = 0.022$ in overdominant model)	Chinese Han 94 patients (40M, 54F), 106 controls	Glucocorticoids (64M, 42F GC users without ONFH)	[27]
Factor V Leiden	rs6025	–	Korean patients 71 patients (53M, 18F), 200 controls (128M, 72F)	Alcohol ( $n = 51$ ) Idiopathic ( $n = 18$ ), Glucocorticoids ( $n = 1$ )	[36]
Prothrombin	rs1799963	–	Polish patients 68 patients (58M, 12F), 100 controls	Dysbaric ( $n = 1$ ) Idiopathic ( $n = 45$ )	
MTHFR	rs1801133	+ Twofold increase (95% CI, 1.05–3.81)		Glucocorticoids ( $n = 11$ ) Chemotherapy ( $n = 7$ )	
	rs1801131	–		Alcohol ( $n = 4$ ) Renal transplantation ( $n = 1$ )	[20]
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 ( $n = 205$ )	[25]
	+24999A>G (Int7-33T>C)	–			
	+37339T>A Intron 4 genotype (4a/a, 4a/b, 4b/b)	+ Higher frequency of 4a allele and 4a/b genotype in patients			
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 ( $n = 1$ )	[37]
PLAT	TPA25 I/D rs1880669	+ ( $p = 0.0049–0.0448$ , OR 1.27–1.69)	Korean population 460 patients (377M, 83F), 300 controls (210M, 90F)	Idiopathic ( $n = 45$ ) Glucocorticoids ( $n = 11$ ) Chemotherapy ( $n = 7$ ) Alcohol ( $n = 4$ ) Renal transplantation ( $n = 1$ )	
PAI-1	4G/5G	+	419 cases of ONFH and 969 controls		[23]
	rs1799889	–	106 ONFH patients and 151 healthy controls	Haplotype G-T may be a protective factor of ONFH	[24]
	rs6092 rs7242	–			
TF	rs2692695	+	Korean population 460 patients (377M, 83F), 300 controls (210M, 90F) Korean population 423 patients (342M, 81F), 348 controls (298M, 50F)	Alcohol induced ( $n = 215$ ) Idiopathic ( $n = 186$ ) Glucocorticoids ( $n = 59$ )	[34]
	rs2718806	+ ( $p = 0.0049–0.0448$ , OR 1.27–1.69)			
	rs1485766	+ ( $p = 0.0042–0.0107$ , OR 1.33–1.67)			
VEGFC	rs3775203	+			
	rs2333496	+ ( $p = 0.0042–0.0107$ , OR 1.33–1.67)			
	rs2453839	+ P ( $p = 0.0087$ or 0.55)			



**Table 1** (continued)

Gene	SNP	Risk association	Patient population	Presence of other risk factors	Reference
IGFBP3	rs4309	( $p = 0.0061$ , OR 7.74) + ( $p = 0.0044$ – $0.00367$ , OR > 1.34–1.63)			
ACE	rs4344 rs4461142 rs6837735	+ ( $p = 0.0044$ – $0.00367$ , OR > 1.34–1.63) P ( $p = 0.0357$ , OR = 0.67)			
KDR	1870377 rs12573218	Protective ( $p = 0.0488$ , OR 0.67) Protective ( $p = 0.0019$ – $0.0423$ , OR 0.55–0.75)			
NRP1	rs12358370 rs2269091 rs12601420	Protective ( $p = 0.0019$ – $0.0423$ , OR 0.55–0.75) –			
SREBF1	rs9925115 rs12601420	+ (AOR = 6.88, 95% CI; 3.74–12.67) –	Korean population 423 patients (342M, 81F), 348 controls (298M, 50F) 18 patients (14 M, 4F)	Glucocorticoids ( $n = 77$ ) Alcohol ( $n = 77$ ) Idiopathic ( $n = 140$ )	[29]
VEGF	rs1570360 rs2010963 rs2010963 rs7170178	+ Lower frequency in steroid (7.4% vs. 18.1%; OR = 0.363) – Lower frequency in steroid (74.3% vs. 84.4%, OR = 0.492) + ( $p < 0.001$ )	160 patients (108M, 52F), 160 controls Indian patients 150 patients (90M, 60F), 154 controls	Idiopathic ( $n = 86$ ) Glucocorticoids ( $n = 74$ )	[38]
Bone structure					
ANXA2	rs73435133 rs7348020 rs72746635 rs73418025 rs4655686	– – – – +	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 ( $n = 75$ ): $\beta$ -thalassemia Gr. 3 ( $n = 15$ ): sickle cell D	[39]
GRIN3A	rs10989692	+ $p = 3.59 \times 10^{-7}$	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	[40]
MMP3	rs650108	+	Chinese male patients: 300 Alcohol induced ONFH 308 healthy control	Associated with decreased risk for ONFH	[41]
MMP8	rs2012390 rs11225394	+ +	Chinese patients: 285 GC induced ONFH 507 healthy control	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	[42]
OPG	rs1032128 rs11573828	+ +	Chinese patients: 335 Alcohol induced ONFH 335 healthy control		[43]
RANKL	rs2200287				
Immunity					
IL23R	rs1569922 rs7539625	+ +	443 patients (366M, 77F), 273 controls (206M, 67F)	Glucocorticoids ( $n = 56$ ) Alcohol ( $n = 206$ ) Idiopathic ( $n = 181$ )	[44]

**Table 1** (continued)

Gene	SNP	Risk association	Patient population	Presence of other risk factors	Reference
Steroid Metabolism					
ABCB1	rs1045642 (C3435T)	+	Meta-analysis of a total of 336 patients and 712 controls	All glucocorticoids	
	rs2032582 (C7623T)	+			
ApoB	rs3751845	+	Japanese population	Renal transplantation ( <i>n</i> = 34)	[45]
CBP	rs3751845		34 patients, 123 controls		
Miscellaneous/Other					
CAT	rs7943316	Protective	443 patients (366M, 77F), 273 controls (206M, 67F)		[46]
	rs1049982	Protective	443 patients (366M, 77F), 273 controls (206M, 67F)	Glucocorticoids ( <i>n</i> = 56)	
	rs525938	Protective		Alcohol ( <i>n</i> = 206)	
	rs3758730	+	Idiopathic ( <i>n</i> = 181)		
	rs769217	+			
	rs2284365	+			

In risk association column: (–) no association; (+) presence of association; protective indicates protection against ONFH

Abbreviations: *ABCB1* adenosine triphosphate-binding cassette B1; *ACE* Angiotensin I converting enzyme; *ANXA2* annexin A2; *ApoB* Apolipoprotein B; *CAT* Catalase; *CBP* CREB-binding protein; *CTDPI* 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; *FLJ40296*; *GRIN3A* Glutamate Receptor, Ionotropic, N-Methyl-D-Aspartate 3A; *IGFBP-3* Insulin-like growth factors binding protein; *KDR* Kinase insert domain receptor; *MMP* metalloproteinase; *MTHFR* 5, 10-methylenetetrahydrofolate reductase; *NRP1* Neuropilin 1; *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; *SREBF1* Sterol regulatory element-binding transcription factor 1; *TF* Transferrin; *TFPI* Tissue factor pathway inhibitor; *VEGFC* Vascular endothelial growth factor C

compounds [55]. Higher P-gp activity was shown to be protective from ONFH development [56]. There are over 50 known SNPs in *ABCB1* which may account for the variation in individual sensitivity to steroids [57]. 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 [58]. Zhang et al., showed the same results for the rs1045642 SNP in a meta-analysis of 5 studies [59].

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* (C3435T), apolipoprotein 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 [45]. 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*)- $1\alpha$  is a proinflammatory cytokine that stimulates the expression of genes related to inflammation and immunity [60]. The rs1800587 SNP of *IL-1\alpha* has been associated with increased risk of ONFH likely because of the stimulating role *IL-1\alpha* plays in bone resorption [60]. In this study of 112 ONFH affected individuals and 438 healthy controls, the author also found an association of ONFH with IL-10, which can inhibit the synthesis of other proinflammatory cytokines [60]. 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 [61, 62]. 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 [44]. However, Wang et al. showed another SNP on this gene (rs6693831) to be protective for ONFH [63].

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 [64, 65]. 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 [64]. 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*)- $\alpha$  is a potent inflammatory cytokine released by macrophages to regulate an immune response by promoting the expression of other cytokine molecules [60]. As such, *TNF*- $\alpha$  is suggested to act upon osteoblasts or bone marrow stromal cells to release cytokines that are associated with osteoclast proliferation and maturation [66].

*TNF*- $\alpha$  gene contains a large number of polymorphisms. The most important ones are located on the gene promoter which gives them the ability to alter the *TNF*- $\alpha$  gene expression and subsequently alter its physiological roles [67]. For example, the GA genotype of the -238(G/A) (rs361525) SNP site is associated with an increase in *TNF*- $\alpha$  expression, altering the osteoblast-osteoclast compositional balance [60, 68]. Peng et al. have performed a meta-analysis of 5 studies to confirm conflicting results concerning the association of 2 *TNF*- $\alpha$  SNPs (rs361525 and rs1800629) with ONFH susceptibility. Their results showed that the 2 SNPs are associated with ONFH but for the SNP rs1800629, this association is significant only in Asians [69].

## 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.

As mentioned above, bone necrosis is a frequent manifestation in sickle cell disease. A case study of Indian patients with SCD revealed a linkage in the rs7170178 SNP site of the annexin A2 (*ANXA2*) gene [39]. 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 rs7170178 was seen amongst the study population. The rs7170178 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 [39]. Another study of sickle cell patients revealed a significant association of the rs3812163 SNP in bone morphogenetic protein 6 (*BMP6*) with ONFH [70]. Members of the BMP family induce endochondral bone formation when implanted in ectopic sites.

Insulin-like growth factors (IGFs) are the most abundant growth factors stored in bones and produced by osteoblasts [71]. *IGFBP-3* is a member of the IGF binding protein family and helps regulate growth and metabolism, especially bone cell metabolism [30]. The TT and TC genotypes of *IGFBP-3* rs2453839 were found to be more frequent in ONFH patients than in the control group [30].

SNPs associated with a protective effect against ONFH have also been identified at the bone level. Transforming growth factor (*TGF*)- $\beta$  regulates the proliferation and differentiation of various cells and functions in cell growth, differentiation, apoptosis, cell migration, immune cell function, and extracellular matrix production [60]. A higher frequency of the homozygous C allele at the 25th codon (rs1800471) has been associated with a decreased level of *TGF*- $\beta$  and linked to a protective effect [60]. 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.

Joint-preserving surgical procedures are presently the most commonly used approach to prevent femoral head collapse [72]. Core decompression (CD) is the most widely accepted



procedure and involves drilling a single 8–10-mm core into the necrotic lesion that could provide pain relief and reduce intraosseous pressure. Since the clinical success rate of CD was not satisfactory, CD was combined with different techniques where bone regeneration was stimulated using some bone substitutes, stem cells, or cellular growth factors (such as TGF- $\beta$ , BMP, FGF-2, VEGF, and IGF) [73, 74]. Andriolo et al. have performed a meta-analysis of 41 studies where regenerative techniques were used for the treatment of ONFH. They have concluded that combination of CD with regenerative techniques increases significantly survivorship of ONFH patients compared to CD alone [75].

Karol et al. performed a genome-wide association study of 2285 patient with acute lymphoblastic leukemia treated with high doses of glucocorticoids [40]. Two hundred fifty 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 [40, 76], 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.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Abbreviations** ABCB1, adenosine triphosphate-binding cassette B1; ACE, angiotensin I converting enzyme; ANXA2, annexin A2; ApoB, apolipoprotein B; AVN, avascular necrosis; BMP, Bone morphogenetic protein; CAT, catalase; CBP, CREB-binding protein; COL2A1, Collagen 2 alpha 1; CTDPI, 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; IGF, Insulin-like growth factors; IGFBP3, Insulin-like growth factor binding protein 3; IGFBP-3, Insulin-like growth factors binding protein; 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; PAI-1, Plasminogen activator inhibitor-1; P-gp, P-glycoprotein; PLAT or TPA, tissue plasminogen activator; PON-1, paraoxonase 1; 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- $\beta$ , Transforming growth factor; TNF- $\alpha$ , Tumor necrosis factor; TRPV4, Transient receptor potential vanilloid 4; VDR, vitamin D receptor; VEGFC, Vascular endothelial growth factor C

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