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Clinical Research

CORR® ORS Richard A. Brand Award: Disruption in Peroxisome Proliferator-Activated Receptor-γ (*PPARG*) Increases Osteonecrosis Risk Through Genetic Variance and Pharmacologic Modulation

Cody C. Wyles MD, Christopher R. Paradise BA, Matthew T. Houdek MD, Susan L. Slager PhD, Andre Terzic MD, PhD, Atta Behfar MD, PhD, Andre J. van Wijnen PhD, Rafael J. Sierra MD

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Abstract

Background The pathophysiology of osteonecrosis of the femoral head (ONFH) is poorly understood, and the diagnosis is idiopathic in as many as 40% of patients. Genetic and epigenetic etiologies have been postulated, yet no single nucleotide polymorphisms (SNPs) with intuitive biologic implications have been elucidated.

Questions/purposes (1) Do individuals with ONFH share common biologically relevant genetic variants associated with disease development? (2) What is the mechanism by which these SNPs may impact the expression or function of the affected gene or protein?

Methods This retrospective genome-wide association study (GWAS) evaluated participants from the Mayo Clinic

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This investigation was performed at the Mayo Clinic, Rochester, MN, USA.

C. C. Wyles, M. T. Houdek, A. J. v. Wijnen, R. J. Sierra, Mayo Clinic Department of Orthopedic Surgery, Rochester, MN, USA

C. R. Paradise, Mayo Clinic Graduate School of Biomedical Sciences, Rochester, MN, USA

S. L. Slager, Mayo Clinic Division of Biostatistics and Informatics, Rochester, MN, USA

A. Terzic, A. Behfar, Mayo Clinic Division of Cardiovascular Diseases, Rochester, MN, USA

C. C. Wyles, M. T. Houdek, A. Terzic, A. Behfar, A. J. v. Wijnen, R. J. Sierra, Mayo Clinic Center for Regenerative Medicine, Rochester, MN, USA

R. J. Sierra (✉), Mayo Clinic Center for Regenerative Medicine, 200 1st St SW, Rochester, MN, 55905 USA, Email: sierra.rafael@mayo.edu

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Biobank and Mayo Clinic Genome Consortium between August 2009 and March 2017. We included every patient with atraumatic ONFH in each of these respective registries and every control patient in a previous GWAS with an acceptable platform to perform statistical imputation. The study was performed in two phases, with an initial discovery cohort and a subsequent validation cohort. The initial discovery cohort consisted of 102 patients with ONFH and 4125 controls. A logistic regression analysis was used to evaluate associations between SNPs and the risk of ONFH, adjusted for age and sex. Seven SNPs were identified in a gene of biological interest, peroxisome proliferator-activated receptor gamma (*PPARG*), which were then evaluated in a subsequent validation cohort of 38 patients with ONFH and 464 controls. Age, sex, race, and previous steroid exposure were similar between patients with ONFH and controls in both the discovery and validation cohorts. Separate from the two-phase genetic investigation, we performed targeted pharmacosurveillance to evaluate the risk association between the use of antidiabetic thiazolidinediones, a class of *PPARG* agonists, and development of ONFH by referencing 9,638,296 patient records for individuals treated at Mayo Clinic.

Results A combined analysis of the discovery and validation cohorts revealed that seven SNPs were tightly clustered adjacent to the 3' end of *PPARG,* suggesting an association with the risk of ONFH ($p = 1.58 \times 10^{-2}$-5.50×10^{-6}). *PPARG* gene-level significance was achieved ($p = 3.33 \times 10^{-6}$) when all seven SNPs were considered. SNP rs980990 had the strongest association with the risk of ONFH (odds ratio [OR], 1.95; 95% CI, 1.46-2.59; $p = 5.50 \times 10^{-6}$).

The seven identified SNPs were mapped to a region near the *PPARG* gene and fell in a highly conserved region consisting of several critical transcription factor binding sites. Nucleotide polymorphisms at these sites may compromise three-dimensional chromatin organization and alter *PPARG* 3' end interactions with its 5' promoter and transcription start site. Pharmacosurveillance identified that patients who were exposed to thiazolidinediones had an increased relative risk of developing ONFH of 5.6 (95% CI, 4.5-7.1).

Conclusions We found that disruption of *PPARG* regulatory domains is linked to an increased risk of ONFH. Mechanistically, aberrant regulation of *PPARG* compromises musculoskeletal differentiation because this master regulator creates a proadipogenic and antiosteogenic state. Furthermore, *PPARG* alters steroid metabolism and vasculogenesis, processes that are inextricably linked with ONFH. Pharmacologically, predisposition to ONFH was further exposed with thiazolidinedione use, which upregulates the expression of *PPARG* and is known to alter bone metabolism. Collectively, these findings provide a foundation to perform confirmatory studies of our proposed mechanism in preclinical models to develop screening diagnostics and potential therapies in patients with limited options.

Level of Evidence Level III, prognostic study.

Introduction

In the United States, osteonecrosis of the femoral head (ONFH) occurs in an estimated 20,000 new patients every year, predominantly in those younger than 40 years [36,44,45]. ONFH occurs when trabecular bone osteocytes undergo necrosis and fail to regenerate appropriately [13,46]. Severe ONFH is marked by loss of bony architecture, leading to subchondral collapse and progressive degenerative joint changes [22]. The pathophysiology of ONFH remains poorly understood; however, once the femoral head has collapsed, patients often undergo THA for pain relief and improvement in daily function. Importantly, patients with ONFH typically undergo THA at a younger age than is characteristic among patients with primary osteoarthritis, generating interest in improved diagnostics and therapeutics [4,21,27,46].

Risk factors associated with ONFH include alcohol use, coagulopathies, sickle cell disease, HIV, radiation exposure, smoking, pregnancy, and autoimmune conditions [7,8,24]. Although corticosteroid use has been identified as perhaps the strongest primary risk factor, ONFH develops in only a minority of patients using high-dose corticosteroid regimens [44,46]. Despite the multitude of etiologic associations, the disease of up to 40% of patients is eventually classified as idiopathic [46]. This large proportion of patients with idiopathic disease and the fact that ONFH develops in only 6% of patients exposed to steroids indicates that some patients may be genetically predisposed to ONFH.

Previous investigations have shown derangements in pathways potentially related to the development of ONFH. Studies examining the effect of coagulation have noted that up to 82% of patients with ONFH had at least one coagulation abnormality [28,29]. Likewise, polymorphisms in genes regulating blood vessel tone, specifically endothelial nitric oxide synthase [15-18,34], have been associated with ONFH. Genetic polymorphisms have also been described in collagen production and the metabolism of steroids and alcohol, two major risk factors of ONFH development [5,32,39,60]. Although insightful, these early studies were performed with older techniques, were limited by small population sizes, and remain unvalidated.

Rapid advances have occurred in technologies capable of probing the contribution of genetic variation to disease. One such technique is genome-wide association study (GWAS), which identifies single nucleotide polymorphisms (SNPs) in the genome and establishes their relative association to a particular phenotype [40]. Leveraging the power of this

technology for complex diseases can help elucidate pathophysiology and provide a basis for more-targeted and selective study [41,49]. In particular, GWAS has recently been proposed as a critical early-stage investigation to understand the contribution of genetics to the potentiation of musculoskeletal pathology [48]. In multifactorial and largely idiopathic diseases such as ONFH, a GWAS generates an invaluable roadmap to lead investigators in the right direction during the initial study. SNPs often impact the expression levels of the genes they are located adjacent to or within. By identifying SNPs associated with a specific phenotype, researchers are provided with signposts throughout the genome that mark areas and genes of interest for further investigation. SNPs detected via GWAS are germline variations at the DNA level; therefore, they remain consistent throughout every cell within an individual and do not change over time. Thus, GWAS provides several advantages over other global "omics" approaches such as mRNA- or ChIP sequencing because these assays assess metrics that are highly variable between cell types and become less informative when extracted from whole tissues (such as bone). Additionally, GWAS can be conducted on easily accessible, peripheral sources of DNA (such as blood), which lends itself well as a diagnostic tool. Other approaches require that specimens be collected directly from the tissue of interest and may not always be feasible. We believe GWAS can help us narrow our search for an underlying molecular mechanism of ONFH and therefore chose to begin our studies with a GWAS. Using this roadmap, we might subsequently turn our attention to specific locations and genes within the genome to inform the use of other diagnostic tools such as RNA sequencing and ChIP sequencing. Identification of genetic variants associated with the risk of ONFH could provide valuable insight for disease pathophysiology, risk-stratification screening tests, and targeted interventions for at-risk patients.

We performed a large discovery cohort screening GWAS that identified several SNPs related to the gene peroxisome proliferator-activated receptor-γ (*PPARG*). This gene was a potentially attractive target, given its known roles in the differentiation of musculoskeletal tissue, metabolism of steroids and lipids, and vasculogenesis. It is also a pharmacologic target for a class of diabetes drugs known as the thiazolidinediones (TZD), which have been linked to an increased proclivity for fractures. Given the potential biological implications of this gene in relation to the pathophysiology of ONFH, we performed a second-phase targeted validation analysis of SNPs related to *PPARG*. Herein, we report this process and subsequent analyses used to determine the mechanistic relationship of *PPARG* to ONFH.

In this study, we used GWAS to ask: (1) Do individuals with ONFH share common biologically relevant genetic variants associated with disease development? (2) What is the mechanism by which these SNPs may impact the expression or function of the affected gene or protein?

Methods

This institutional review board-approved study was a two-stage retrospective GWAS using participants enrolled in the Mayo Clinic Biobank and Mayo Clinic Genome Consortium from August 2009 to March 2017. The Mayo Clinic Biobank is a tissue database of more than 50,000 volunteers or patients prescheduled for medical examination in the divisions of community internal medicine, family medicine, or general internal medicine [47]. The Mayo Clinic Genome Consortium is a database of approximately 10,000 patients who were part of a historical GWAS at Mayo Clinic [3]. A power analysis was not performed for this study. We included every patient with atraumatic ONFH in each of these respective registries and every control patient in a previous GWAS performed on an acceptable platform, to perform imputation as detailed below.

The study was performed in two phases with an initial discovery cohort and a subsequent validation cohort (Fig. 1). The discovery cohort was established in a stepwise fashion. First, the Mayo Clinic Biobank was queried using ICD-9 code 733.4 for patients with a history of ONFH. The medical records of identified patients were reviewed to determine the anatomical location of ONFH as well as previous corticosteroid exposure and trauma history. In this study, we did not assess each patient's ONFH stage as a variable against genetic association. Eighty-eight patients with atraumatic ONFH were identified. Fifty of these 88 patients had a history of high-dose corticosteroid use ($\geq$ 20 mg/day x $\geq$ 1 month) before ONFH was diagnosed. Controls were also selected from the Mayo Clinic Biobank and were 2:1 frequency matched (176 patients) to patients based on age ($\pm$ 5 years), sex, BMI ($\pm$3 kg/m^2), the patient's self-reported race (exact match), and previous steroid exposure without subsequent development of documented ONFH. Cryopreserved white blood cells from these patients were then genotyped with the Illumina Omni 5.0 platform (Illumina, San Diego, CA).

The discovery cohort's data were then enriched with institutional historical GWAS data from patients in the Mayo Clinic Genome Consortium to increase the sample size. Patients in this registry were identified in a similar fashion to those from the Mayo Clinic Biobank. Fourteen patients with atraumatic ONFH were identified. Seven of those 14 patients had a history of high-dose corticosteroid use before ONFH was diagnosed. Instead of matching controls as we did with the Mayo Clinic Biobank patients, we included all available control patients from the Mayo Clinic Genome Consortium in a GWAS performed on platforms suitable to perform imputation. This constituted

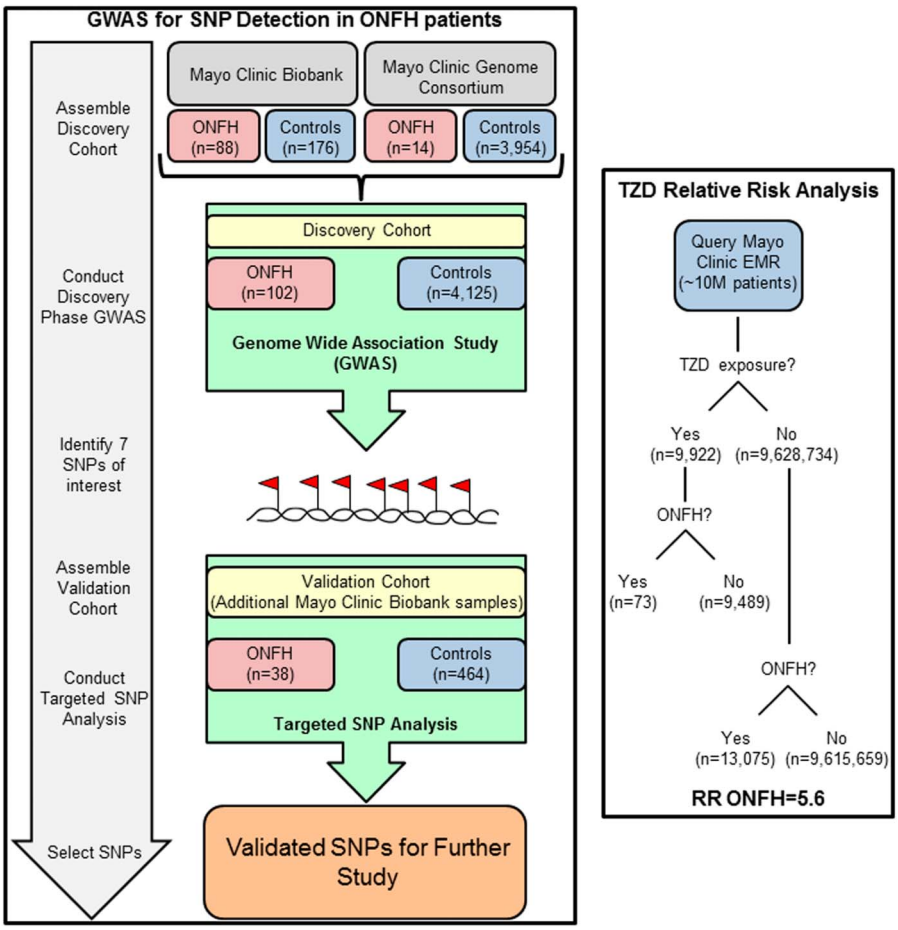

Fig. 1 This schematic diagram outlines the phases of investigation including establishment of the discovery and validation cohorts for genetic evaluation as well as the complementary pharmacologic impact data from patient record review.

3954 patients, including 909 with a history of oral or intravenous corticosteroid use and no subsequent ONFH. All patients with ONFH and controls identified in the Mayo Clinic Genome Consortium were then combined with the Mayo Clinic Biobank patients to arrive at the final cohort for the discovery analysis, which included the following: 102 patients with ONFH (53 with a history of high-dose corticosteroid use) and 4125 controls (1001 with a history of corticosteroid use). The median age was 57 years, 56% were women, and > 99% were white (Table 1).

Imputation was used to combine and compare data from patient samples in the discovery cohort. Each GWAS platform was assessed for standard quality-control metrics; only platforms meeting thresholds for high-fidelity imputation were included. We imputed each GWAS platform separately using IMPUTE2 and the 1000 Genomes Project version 3 (March 2012 release) reference panel.

The discovery cohort GWAS revealed that seven SNPs were related to *PPARG*. Given the biological interest in this gene, a cluster of seven SNPs became the focus of a targeted validation cohort and subsequent investigations of potential mechanistic involvement with ONFH (Fig. 1). For the validation cohort, we queried the Mayo Clinic Biobank again for new patients with ONFH and controls because the database had grown between the discovery phase and the validation analysis. We identified 38 new patients with atraumatic ONFH who had a history of steroid use and 464 controls with a history of steroid use but who did not have symptomatic ONFH. DNA from validation Mayo Clinic Biobank samples were isolated from cryopreserved white blood cells and genotyped using a Sequenom custom-designed panel to assess the seven SNPs of interest that had been identified during the discovery phase. In the validation cohort, the median age was 64 years, 52% were women, and > 99% were white (Table 1).

The evolutionary conservation of the newly identified SNPs was assessed by viewing the 100 Vertebrate Conservation Track using the Track Data Hubs Feature [53] on the Human GRCh37/hg19 Assembly in the UCSC Genome

Table 1. Demographics of the discovery and validation cohorts

Patient characteristic	Discovery osteonecrosis (n = 102)	Discovery control (n = 4125)	p value	Validation osteonecrosis (n = 38)	Validation control (n = 464)	p value
Age, years*						
Median	65	57	< 0.001	62	64	0.063
Q1, Q3	56, 72	45, 67		45, 70	53, 74	
Sex						
Female	55 (54%)	2316 (56%)	0.655	15 (40%)	242 (52%)	0.133
Male	47 (46%)	1809 (44%)		23 (60%)	222 (48%)	
Steroid use†						
No	49 (48%)	3124 (76%)	< 0.001	0 (0%)	0 (0%)	
Yes	53 (52%)	1001 (24%)		38 (100%)	464 (100%)	
Race‡						
Black	3 (3%)	6 (0.1%)		1 (2.6%)	1 (0.2%)	
Asian	1 (1%)	1 (0.0%)		1 (2.6%)	2 (0.4%)	
White	98 (96%)	4118 (99.9%)		36 (94.7%)	461 (99.4%)	

*Age at the time of sample analysis by Mayo Clinic Biobank or Mayo Clinic Genome Consortium;
†History of oral or intravenous steroid use;
‡Self-reported

Browser (University of California Santa Cruz, Santa Cruz, CA, USA) [33].

The location of protein binding sites was determined using the Human GRCh37/hg19 Assembly in the UCSC Genome Browser [33] with the Transcription Factor Chip Track [14,61,62] overlaid using the Track Data Hubs Feature [53].

The chromatin structure was assessed using the 15-state chromatin model within the Roadmap Epigenomics Project [35] on the Human hg19 assembly. The three-dimensional chromatin structure was assessed on the same gene track using experimental HiC data collected in HEK293 cells [66].

Datasets curated by The Genotype-Tissue Expression (GTEx) Project [19] were used to assess the functional impact of the newly identified SNPs on gene expression in varying tissues. The expression data and boxplots described here were obtained from the GTEx Portal (GTEx Analysis Release V7 [dbGaP Accession phs000424.v7.p2]).

When *PPARG* emerged as a potential candidate for involvement in the pathogenesis of ONFH, we used the Mayo Clinic electronic medical record and data abstraction interfaces to evaluate the clinical records of 9,638,296 individuals treated at Mayo Clinic (Fig. 1).

Risk association was tested between the use of antidiabetic thiazolidinediones, a class of *PPARG* agonists, and the development of ONFH. Specifically, the drugs rosiglitazone and pioglitazone were captured by validated medicine reconciliation platforms and evaluated against subsequent development of atraumatic ONFH as determined by ICD9 and ICD10 codes (Fig. 2D).

Population stratification was assessed using STRUCTURE (Stanford University, Palo Alto, CA) software. We used a logistic regression within Plink to test for an association with ONFH, assuming a log-additive genetic model adjusting for age, sex, subject source, and the top three principal components. We performed a gene-based level analyses using SNPs within *PPARG*. All genotyped and imputed SNPs were tested within *PPARG* (defined by its gene boundaries per Genome Browser +/- 75 kb) using sequence kernel association tests [25] with an unweighted linear kernel implemented in the sequence kernel association test package v1.0.9 [55] in R v3.1.1 [52]. Then, the top seven significant *PPARG* SNPs were used to form haplotypes. Associations between the haplotypes and ONFH were tested with logistic regression implemented in the haplo.stats package v1.6.11 [57] in R v3.1.1. The package locusZoom v1.3 [51] was used to visualize the single SNP GWAS results in the *PPARG* gene region. Logistic regression was also used in the validation cohort, but age and sex were the only covariates included in the models because the subjects were drawn from a single source (Mayo Clinic Biobank). Continuous covariates were compared using the Wilcoxon rank sum test and categorical covariates were compared using the Pearson chi-squared test. Data from the discovery cohorts and validation cohorts were analyzed separately as well as in aggregate through a meta-analysis of the two cohorts. For all three analyses, gene-level significance was set at $p < 3.33 \times 10^{-5}$ and individual SNP significance was set at $p < 5 \times 10^{-8}$, consistent with standard GWAS definitions [49].

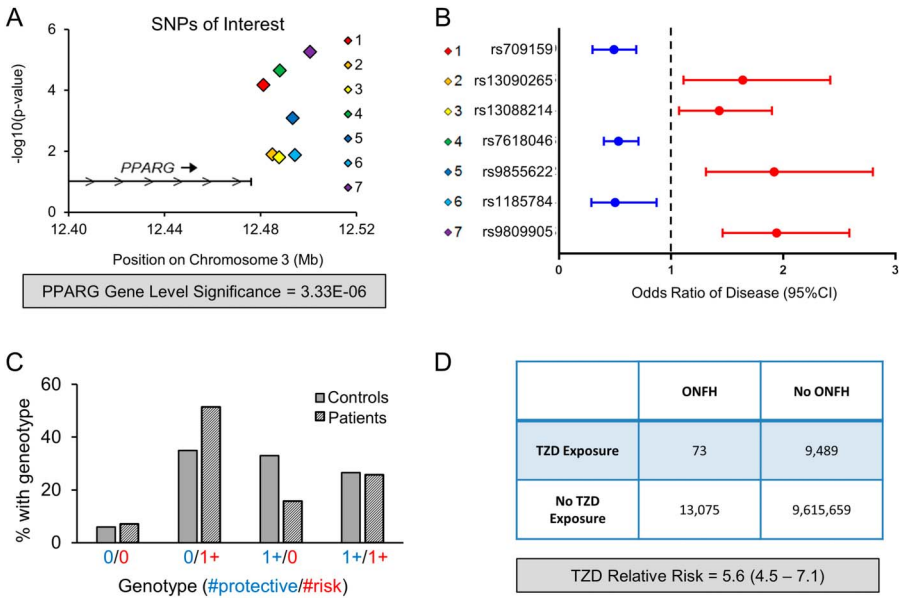

Fig. 2 The GWAS and clinical cohort assessment associate modulation of *PPARG* with increased ONFH risk is shown. (A) The schematic shows the location on chromosome 3 and $-\log_{10}$(p value) for the seven SNPs of interest with respect to the 3′ end of the *PPARG* locus with cumulative *PPARG* gene-level significance indicated below the graph. (B) Plot outlining the respective OR for ONfh development in patients harboring the indicated SNP. (C) The percentage of patients in the control or ONFH group found to have the indicated combinations of "Protective" (OR < 1) or "Risk" (OR > 1) alleles. (D) Table outlining the increased risk of ONFH with the use of TZD drugs that are *PPARG* agonists for diabetes management.

Results

Genetic Variants Associated with ONFH

In the discovery cohort, we identified a cluster of seven SNPs related to *PPARG* that were associated with the differential risk of ONFH (Fig. 2A). These seven SNPs were in the top 1000 most genetically different SNPs on chromosome 3. Individual p values for these seven SNPs ranged from 1.03×10^{-5} to 1.56×10^{-2}; thus, no SNP achieved significance (cutoff $p < 5 \times 10^{-8}$). Gene-level analysis of *PPARG* achieved a p value of 3.33×10^{-6}, which was significant after whole-genome Bonferroni correction (cutoff = $p < 3.33 \times 10^{-5}$) (Fig. 2A). Thus, although no single SNP met the genome-wide significance cutoff of 5×10^{-8}, *PPARG* showed considerable variance associated with disease (Table 2).

All seven SNPs identified in the discovery cohort demonstrated similar p values and odds ratios (ORs) for disease when assessed in the validation cohort (Table 2). Four of the seven SNPs were associated with an increased risk of ONFH (OR, 1.43-1.94; $p = 1.58 \times 10^{-2}$-5.50×10^{-6}) and three were associated with a decreased risk of ONFH (OR, 0.49-0.53, $p = 1.32 \times 10^{-2}$-2.27×10^{-5}) (Table 2, Fig. 2B). Next, we analyzed patient genotypes at the individual level to investigate the prevalence of SNPs associated with increased versus decreased risk in the control and ONFH populations. We noted that a higher proportion of patients with ONFH (51%) possessed at least one SNP that was associated with increased risk and no SNPs that were associated with decreased risk compared with controls (35%, $p < 0.001$). Conversely, we found that a greater number of patients in the control group (33%) possessed at least one SNP associated with decreased risk and had no SNPs associated with increased risk than did patients with ONFH (16%, $p < 0.001$) (Fig. 2C).

Possible Mechanism by Which PPARG May Impact the Risk of ONFH

Given the strong association between *PPARG* genetic variance and ONFH development, a large institutional database was evaluated to determine differences in the risk of ONFH based on exposure to TZDs. These drugs are used widely for managing diabetes with a primary mechanism of action as *PPARG* agonists. In a database of nearly 10 million patients, TZD use increased the risk of ONFH development by a factor of 5.6 (95% CI, 4.5-7.1; $p < 0.001$) (Fig. 2D).

Although unexplored in relation to musculoskeletal tissue, four of the seven SNPs demonstrated an association with genes including *PPARG* in other tissues, suggesting their presence or absence had functional consequences (see Figure,

Table 2. GWAS analysis of significant SNPs in *PPARγ*

SNP	Position (bp)	A1	A2	Phase	MAF	MAF patients	MAF controls	Odds ratio (95% CI)	p value
rs709159	12481203	C	A	Discovery	0.2502	0.1716	0.2521	0.51 (0.21-0.34)	0.0014910
				Validation	0.2639	0.1316	0.2748	0.43 (0.22-0.85)	0.0145900
				Meta-analysis				0.49 (0.30-0.69)	0.0000676
rs13090265	12484985	G	A	Discovery	0.1165	0.1471	0.1158	1.94 (1.21-3.14)	0.0060970
				Validation	0.1265	0.1447	0.125	1.16 (0.59-2.29)	0.6724000
				Meta-analysis				1.64 (1.11 - 2.42)	0.0128400
rs13088214	12487827	C	T	Discovery	0.2705	0.3333	0.2689	1.54 (1.09-2.18)	0.0155800
				Validation	0.2988	0.34221	0.2953	1.21 (0.73-2.20)	0.4611000
				Meta-analysis				1.43 (1.07 - 1.90)	0.0158000
rs7618046	12488027	T	C	Discovery	0.4554	0.348	0.458	0.50 (0.35-0.71)	0.0001223
				Validation	0.4512	0.3289	0.4612	0.62 (0.38-1.00)	0.0541200
				Meta-analysis				0.53 (0.40-0.71)	0.0000227
rs9855622	12493347	T	C	Discovery	0.3895	0.4902	0.3865	2.22 (1.38-3.57)	0.0010170
				Validation	0.4183	0.4868	0.4106	1.48 (0.78-2.79)	0.2297000
				Meta-analysis				1.92 (1.31-2.80)	0.0008092
rs1185784	12494278	T	C	Discovery	0.1111	0.04902	0.1126	0.39 (0.19-0.78)	0.0083920
				Validation	0.0996	0.07895	0.1013	0.74 (0.31-1.73)	0.4812000
				Meta-analysis				0.50 (0.29-0.87)	0.0132100
rs9809905	12500651	G	T	Discovery	0.3895	0.4902	0.3865	2.22 (1.56-4.41)	0.0000103
				Validation	0.4183	0.4868	0.4106	1.51 (0.93-2.46)	0.0965200
				Meta-analysis				1.94 (1.46-2.59)	0.0000055

MAF = minor allele frequency (frequency that the second most common allele is observed in the population being studied), BP = base pair, A1 = minor allele, A2 = wild type allele

Supplemental Digital Content 1, http://links.lww.com/CORR/A158) [19]. To better understand how newly identified SNPs may be associated with the risk of ONFH, we observed their location within the genome in depth. First, we mapped the location of the variants in relation to evolutionarily conserved regions among vertebrates (Fig. 3A). We noted that SNP 1 (rs709159) and SNP 7 (rs9809905) fall in highly conserved regions. These regions share similar patterns of conservation among ambulatory vertebrates but diverge in birds and lower organisms (see Table, Supplemental Digital Content 2, http://links.lww.com/CORR/A164, and Figure, Supplemental Digital Content 3, http://links.lww.com/CORR/A165). Noting several evolutionary conserved regions proximal to the 3' end of *PPARG*, next, we searched for the presence of transcription factor binding domains in relation to our SNPs of interest (see Figure, Supplemental Digital Content 4, http://links.lww.com/CORR/A166). This demonstrated a substantial number of binding sites for the protein CTCF, an important regulator that establishes domain boundaries between accessible and non-accessible DNA in 3-D chromatin structures (Fig. 3B). We found that SNP 7 (rs9809905) is in the center of a highly enriched CTCF and CEBPB binding region (Fig. 3C). Because of the critical role of CTCF in controlling 3-D chromatin organization, we used the Epigenomics Roadmap [35] to assess the chromatin structure around the *PPARG* locus (Fig. 3D). In osteoblasts and bone marrow-derived mesenchymal stromal or stem cells (BMSCs), we observed regions of chromatin compaction and quiescence. However, in adipose tissue, adipose tissue-derived mesenchymal stromal or stem cells (AMSCs), and AMSCs directed into the adipogenic lineage, we observe regions of open chromatin and active transcription. Furthermore, HiC data show 3-D chromatin looping that links the 3' end of *PPARG,* which contain the newly identified SNPs with the 5' promoter region and transcriptional start site (Fig. 3D). Together, these observations suggest that the newly identified polymorphisms may have a functional association with the expression levels of *PPARG*. SNP 7 (rs9809905) is of particular interest because it has the highest OR of disease (OR = 1.95) and lowest p value ($p = 5.05 \times 10^{-6}$), and is located in an evolutionarily conserved protein binding region shown to be involved in 3-D chromatin interaction.

Discussion

ONFH is a complex hip disorder with many described risk factors, yet up to 40% of patients are ultimately considered

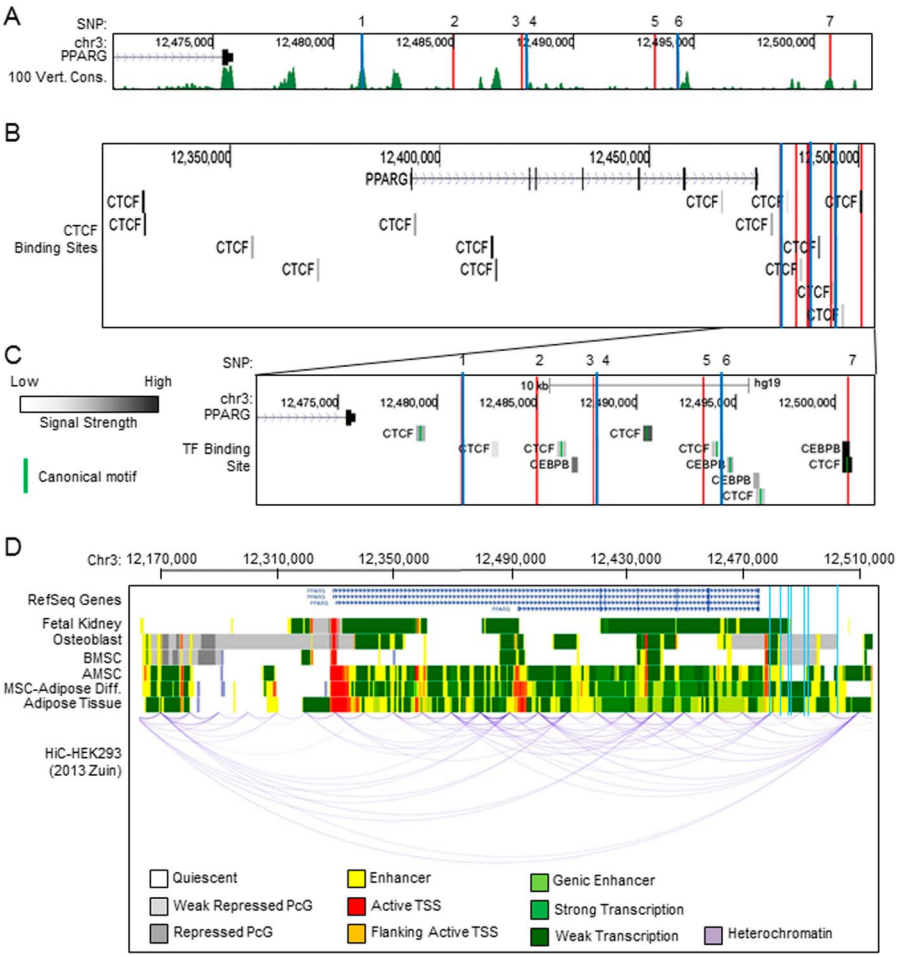

Fig. 3 Evolutionary conservation of SNP loci and chromatin structure with three-dimensional organization of *PPARG* is shown. (A) UCSC Genome Browser display of the 3′ *PPARG* region with SNP locations indicated (blue = protective, red = risk). The 100 vertebrate conservation track (green) and individual species conservation (black) is indicated below. (B) SNP locations are indicated (blue vertical lines) and track colors correspond to the chromatin state indicated at the bottom of the panel. UCSC Genome Browser display of CTCF binding sites with respect to the *PPARG* locus and SNP locations are indicated (blue = protective, red = risk). (C) An enhanced view of the 3′ end of *PPARG* depicting CTCF and CEBPβ binding sites is shown. (D) A chromatin state model for the indicated cell type (left) and HiC long-range interaction in HEK293 cells with respect to the *PPARG* locus is shown. Signal strength determined by ChIP assay and canonical binding motif regions are indicated as specified (left).

to have idiopathic disease [22]. The large number of patients with idiopathic disease is consistent with the concept we validated in this study, that genetic susceptibility may play a role in the pathogenesis of disease. GWAS was used as a screening tool to identify loci within the genome that may be linked with the risk of ONFH. GWAS is an ideal technology for preliminary work of this nature and provides a foundation for subsequent validation with complementary techniques. The identification of *PPARG* in this study as a critical modulator of the risk of ONFH is based on strong corroborating lines of evidence, including (1) genetic variance in loci governing the expression of *PPARG* and (2) pharmacologic modulation with common antidiabetic agents functioning through *PPARG* agonism. *PPARG* provides both a novel genetic marker and a potential pathway-targeting strategy for modulating disease progression.

This study must be interpreted in light of important limitations. Most importantly, the sample size of ONFH patients (n = 140) was prohibitive for a GWAS capable of meeting individual SNP p value cutoffs $< 5 \times 10^{-8}$ thought to represent unequivocal whole-genome significance [49]. Nevertheless, several SNPs achieved p values that

are remarkable in the context of this initial cohort's sample size. On the contrary, *PPARG* achieved a high level of significance even after Bonferroni correction, despite the aforementioned modest sample size of patients, lending further credence to the strength of these findings. Furthermore, the results are supported by biologically intuitive and relevant implications for the identified gene and strong complementary clinical data confirming pharmacologic risk is mediated through *PPARG*. Second, the GWAS platforms from the discovery cohort were not uniform and thus required imputation and rigid exclusion criteria. We demonstrated excellent data quality through this process, but increased caution must be exercised when variant SNPs are identified through imputation at specific loci versus through uniform genotyping. Third, the genetic component of this study is restricted to GWAS evaluation. This method has strengths as an initial screening tool, but it lacks the capability to detect disease-related or SNP-induced changes in gene expression or chromatin modifications. Now that we have used GWAS to focus our search, we aim to use the advantages of RNA or ChIP sequencing technologies in future studies to complement the presented data. Furthermore, this study did not prove our proposed mechanism. Additional corroborative evidence will be required to substantiate these relationships. Investigations of the expression of *PPARG* in the femoral head tissue of patients with ONFH as well as cell line manipulation and experimentation with animal models are possible lines of exploration.

GWAS is a powerful screening tool for understanding the genetic contribution to disease. Although this technology may yield SNPs that are highly associated with a particular condition, findings are often difficult to interpret if the SNP is not related to a gene or pathway with known biological activity. Although our study lacks statistical power, a strength of our preliminary work lies in the molecular potential of the identified polymorphisms. *PPARG* has multiple intuitive biologic implications for the pathophysiology of ONFH (Fig. 4). First, *PPARG* functions in conjunction with the WNT pathway and governs whether mesenchymal stem cells differentiate toward adipose tissue or bone [37,59,64]. When active, *PPARG* controls the lineage allocation of stem cells by promoting adipogenic differentiation and suppressing osteogenic differentiation. This molecular ability is directly relevant to ONFH, which results from a combination of sentinel necrosis followed by an inability to regenerate trabecular bone [22,23,63]. Our findings also provide insight into likely mechanisms by which TZDs, as potent agonists of *PPARG*, increase the risk of ONFH. These agents are *PPARG*-activating and thus might shift the balance of the local regenerative cell population toward formation of adipose tissue instead of restorative bone [56]. Clinically, this principle was partly demonstrated before our study in large-scale evaluations of TZD use showing an elevated risk of atypical fracture [6,20,30,31,42,65]. Animal and in vitro studies have further confirmed that TZD use yields increased bone adiposity and decreased bone mineralization [2,43,54,58]. In retrospect, these collective findings might suggest that the earlier findings could be attributed to a previously unrecognized ONFH-like syndrome secondary to TZDs.

Steroids are a primary risk factor of ONFH development. *PPARG* has a high affinity for steroid receptors [26]. Therefore, it is possible that genetic- or pharmacologically-induced alterations in *PPARG* cause patient-specific sensitivity to steroids. Epigenetic modifications that depend on *PPARG* may explain why an even greater proportion of patients have ONFH and could impact the emerging landscape of individualized medicine for these patients. Furthermore, *PPARG* is a key regulator of lipid metabolism and vasculogenesis [1]. Previous reports have shown that statins are protective for patients with ONFH, and avascular lesions are pathognomonic for the disease [44,46,50]. These critical relationships warrant further investigation to further clarify the extent of *PPARG*'s role in the pathophysiology of ONFH.

Although a great deal of work is required to clarify the role of *PPARG* in ONFH, we summarize our current proposed mechanism as follows (Fig. 4). In a healthy state, *PPARG* maintains low levels of expression, yielding normal bone architecture. In ONFH, we suggest that pathologic upregulation of *PPARG,* either through SNPs or pharmacologic modulation with TZDs, may alter pathways leading to osteonecrosis in bone. In normal bone physiology, *PPARG* is expressed at low levels, which encourages a decreased adipocyte/osteoblast ratio for healthy bone formation. However, upregulation of *PPARG* leads to a high adipocyte/osteoblast ratio in the MSC lineage and simultaneously acts on the hematopoietic lineage to increase osteoclastogenesis; together, these mechanisms yield net bone loss and increased adiposity and edema. Substantial adiposity in bone may also lead to increased pressure in the femoral head with a subsequent "compartment-syndrome-like condition" and disruption of the vascular supply. Furthermore, *PPARG* acts on a variety of critical metabolic pathways that, if disrupted, have intuitive biologic implications for the pathophysiology of osteonecrosis. *PPARG* interacts with steroid receptors, holding particular relevance because steroids are the primary exogenous risk factor of the development of osteonecrosis. *PPARG* also interacts with lipid metabolism, which has long been proposed as a critical pathway for osteonecrosis susceptibility as evidenced by studies demonstrating attenuated osteonecrosis progression with the administration of statin drugs. *PPARG* also plays a critical role in local vascular formation; indeed, "avascular necrosis" is the historical term for this disease because a disrupted vascular supply is uniformly responsible for

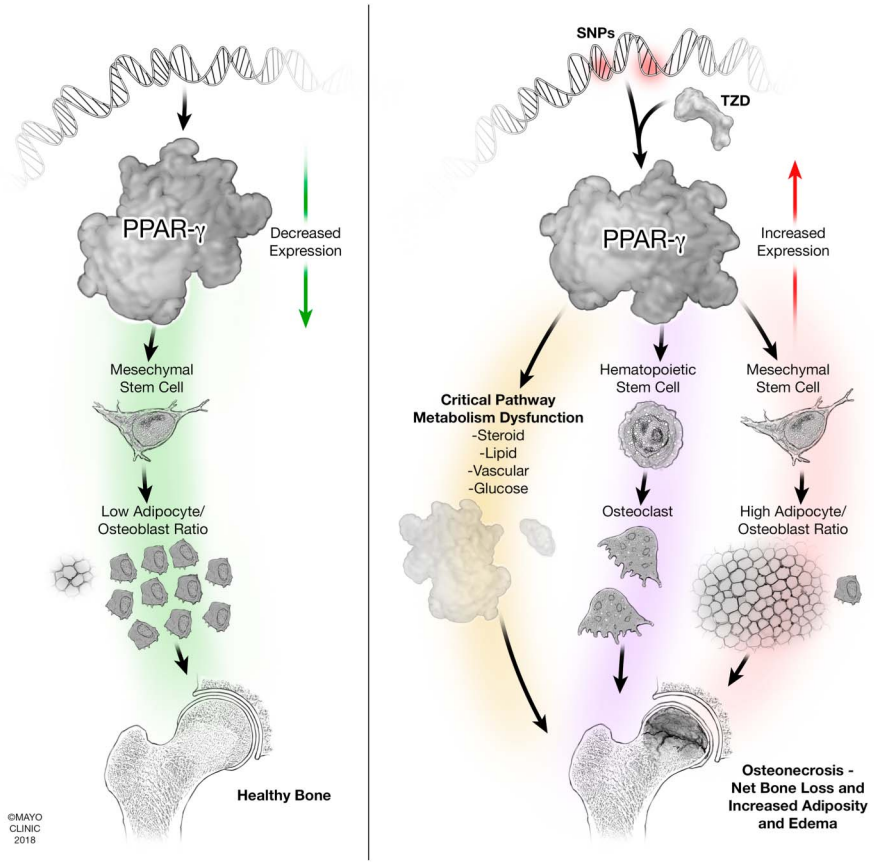

Fig. 4 Proposed mechanisms for *PPARG* involvement in the pathophysiology of ONFH are shown. (A) *PPARG* function in a healthy state with low levels of expression yielding normal bone architecture. (B) Pathologic upregulation of *PPARG,* either through SNPs or pharmacologic modulation with TZD drugs, altering pathways leading to osteonecrosis in bone.

traumatic osteonecrosis and has been shown histologically in a subset of patients with atraumatic disease.

Multilevel molecular analysis of the chromosomal regions in which the SNPs reside shows that our newly identified SNPs are in a region that has many molecular properties consistent with a crucial regulatory region for controlling the expression of *PPARG*. Specifically, we mapped SNP 7 (rs9809905) to an evolutionarily conserved DNA region containing binding sites for the key regulators CTCF and CEBPB. Previous studies have elucidated the dynamic binding patterns of CTCF during adipogenesis and identified it as a key regulator of *PPARG* transcriptional activation [11,12]. Additionally, analysis of HiC 3-D chromatin interaction data demonstrates that this region is looped back to the 5' promoter region of *PPARG*. CTCF has been well-established as a mediator of chromatin looping between regulatory and promoter regions [9], and augmented CTCF binding has been shown to reactivate previously silenced genes [10]. These data suggest that the increased OR associated with SNP 7 (rs9809905) may stem from its functional impact on the expression of *PPARG*. It is also important to note the tissue and cell-specific variability of the chromatin state near *PPARG* when considering the functional impact of these SNPs. In bone or bone-derived cells (osteoblasts and BMSCs), chromatin is compacted at both the 3' and 5' region, resulting in quiescent or weak transcription of *PPARG*. Conversely, in adipose or adipose-derived cells, chromatin is open and active at the 3' and 5' regions of *PPARG*, resulting in active gene transcription. This dichotomy in chromatin states is reflected in the *PPARG* expression levels within respective tissues. *PPARG* is well-described as a master regulator of adipogenesis [38] that drives adipocyte differentiation and development of fat tissue. Therefore, the expression of *PPARG* must be tightly regulated depending on the cell phenotype. This is likely why we observed chromatin compaction in nonadipogenic bone cells and open chromatin in adipose-derived cells. Our results suggest that SNPs that alter the appropriate regulation and expression of *PPARG* could lead to improper differentiation of mesenchymal stem cells, giving rise to ONFH. This could explain the combination of human and animal data showing atypical fractures following TZD administration, marked by increased bone

marrow adiposity and edema leading to fracture. We reason that the location of SNP 7 (rs9809905) in a highly conserved CTCF binding site may augment the binding of CTCF in this region. In osteoblasts, this may lead to relaxation of an otherwise compacted region of chromatin at the 3' end of *PPARG* and promote looping to the 5' promoter. In osteoblasts and preosteoblasts, which express low basal *PPARG* mRNA levels, SNP-induced expression of *PPARG* may be detrimental to proper cell differentiation or function, resulting in compromised bone integrity. Therefore, it is intuitive that SNP 7 (rs9809905) has the highest OR for ONFH and has the highest incidence among the diseased population. Our followup pharmacosurveillance using the Mayo Clinic's electronic medical record yielded complementary results in that patients receiving TZD (a *PPARG* agonist) had an increased risk of ONFH development by a factor of 5.6 (95% CI, 4.5-7.1; $p < 0.001$). This finding confirms that elevated *PPARG* expression is associated with ONFH. In light of this finding, we reason that these newly identified SNPs, particularly rs980990, may have some association with the risk of ONFH. Because *PPARG* is already capable of being manipulated pharmacologically with TZDs, theoretically, an inhibitor of *PPARG* either locally or systemically, could benefit patients with early-stage ONFH.

This study identified *PPARG* as a potential modulator of the risk of ONFH through genetic variance and pharmacologic upregulation with TZDs. The primary mechanism by which *PPARG* is known to function is through a shift in mesenchymal stem cell differentiation towards the formation of adipose tissue rather than bone formation. Furthermore, known interactions of *PPARG* with the well-established risk factors of ONFH—steroid use and impaired vasculogenesis—provide further interest for a possible role in the pathogenesis of ONFH. Nevertheless, considerable additional investigation is needed to confirm or refute these proposed relationships. RNA sequencing of femoral head tissues with and without ONFH as well as cellular manipulation and animal model recapitulation of disease are potential avenues for exploration. Given previous success with the pharmacologic modulation of *PPARG*, therapeutic intervention and prevention of ONFH may be possible for patients with this condition, who are typically younger and often have limited options.

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References

1. Ahmadian M, Suh JM, Hah N, Liddle C, Atkins AR, Downes M, Evans RM. PPARgamma signaling and metabolism: the good, the bad and the future. *Nat Med*. 2013;19:557-566.
2. Ali AA, Weinstein RS, Stewart SA, Parfitt AM, Manolagas SC, Jilka RL. Rosiglitazone causes bone loss in mice by suppressing osteoblast differentiation and bone formation. *Endocrinology*. 2005;146:1226-1235.
3. Bielinski SJ, Chai HS, Pathak J, Talwalkar JA, Limburg PJ, Gullerud RE, Sicotte H, Klee EW, Ross JL, Kocher JP, Kullo IJ, Heit JA, Petersen GM, de Andrade M, Chute CG. Mayo Genome Consortia: a genotype-phenotype resource for genome-wide association studies with an application to the analysis of circulating bilirubin levels. *Mayo Clin Proc*. 2011;86:606-614.
4. Bozic KJ, Zurakowski D, Thornhill TS. Survivorship analysis of hips treated with core decompression for nontraumatic osteonecrosis of the femoral head. *J Bone Joint Surg Am*. 1999;81:200-209.
5. Chao YC, Wang SJ, Chu HC, Chang WK, Hsieh TY. Investigation of alcohol metabolizing enzyme genes in Chinese alcoholics with avascular necrosis of hip joint, pancreatitis and cirrhosis of the liver. *Alcohol Alcohol*. 2003;38:431-436.
6. Chen HH, Horng MH, Yeh SY, Lin IC, Yeh CJ, Muo CH, Sung FC, Kao CH. Glycemic control with thiazolidinedione is associated with fracture of T2DM patients. *PLoS One*. 2015;10:e0135530.
7. Cruess RL. Experience with steroid-induced avascular necrosis of the shoulder and etiologic considerations regarding osteonecrosis of the hip. *Clin Orthop Relat Res*. 1978:86-93.
8. Cruess RL. Steroid-induced osteonecrosis: a review. *Can J Surg*. 1981;24:567-571.
9. de Wit E, Vos ES, Holwerda SJ, Valdes-Quezada C, Verstegen MJ, Teunissen H, Splinter E, Wijchers PJ, Krijger PH, de Laat W. CTCF Binding polarity determines chromatin looping. *Mol Cell*. 2015;60:676-684.
10. Deng W, Rupon JW, Krivega I, Breda L, Motta I, Jahn KS, Reik A, Gregory PD, Rivella S, Dean A, Blobel GA. Reactivation of developmentally silenced globin genes by forced chromatin looping. *Cell*. 2014;158:849-860.
11. Dubois-Chevalier J, Oger F, Dehondt H, Firmin FF, Gheeraert C, Staels B, Lefebvre P, Eeckhoute J. A dynamic CTCF chromatin binding landscape promotes DNA hydroxymethylation and transcriptional induction of adipocyte differentiation. *Nucleic Acids Res*. 2014;42:10943-10959.
12. Dubois-Chevalier J, Staels B, Lefebvre P, Eeckhoute J. The ubiquitous transcription factor CTCF promotes lineage-specific epigenomic remodeling and establishment of transcriptional networks driving cell differentiation. *Nucleus*. 2015;6:15-18.
13. Ficat RP. Idiopathic bone necrosis of the femoral head. Early diagnosis and treatment. *J Bone Joint Surg Br*. 1985;67:3-9.
14. Gerstein MB, Kundaje A, Hariharan M, Landt SG, Yan KK, Cheng C, Mu XJ, Khurana E, Rozowsky J, Alexander R, Min R, Alves P, Abyzov A, Addleman N, Bhardwaj N, Boyle AP, Cayting P, Charos A, Chen DZ, Cheng Y, Clarke D, Eastman C, Euskirchen G, Frietze S, Fu Y, Gertz J, Grubert F, Harmanci A, Jain P, Kasowski M, Lacroute P, Leng JJ, Lian J, Monahan H, O'Geen H, Ouyang Z, Partridge EC, Patacsil D, Pauli F, Raha D, Ramirez L, Reddy TE, Reed B, Shi M, Slifer T, Wang J, Wu L, Yang X, Yip KY, Zilberman-Schapira G, Batzoglou S, Sidow A, Farnham PJ, Myers RM, Weissman SM, Snyder M. Architecture of the human regulatory network derived from ENCODE data. *Nature*. 2012;489:91-100.
15. Glueck CJ, Freiberg R, Glueck HI, Tracy T, Stroop D, Wang Y. Idiopathic osteonecrosis, hypofibrinolysis, high plasminogen activator inhibitor, high lipoprotein(a), and therapy with Stanozolol. *Am J Hematol*. 1995;48:213-220.

16. Glueck CJ, Freiberg RA, Boppana S, Wang P. Thrombophilia, hypofibrinolysis, the eNOS T-786C polymorphism, and multifocal osteonecrosis. *J Bone Joint Surg Am*. 2008;90:2220-2229.
17. Glueck CJ, Freiberg RA, Sieve L, Wang P. Enoxaparin prevents progression of stages I and II osteonecrosis of the hip. *Clin Orthop Relat Res*. 2005:164-170.
18. Glueck CJ, Glueck HI, Welch M, Freiberg R, Tracy T, Hamer T, Stroop D. Familial idiopathic osteonecrosis mediated by familial hypofibrinolysis with high levels of plasminogen activator inhibitor. *Thromb Haemost*. 1994;71:195-198.
19. GTExConsortium. The Genotype-Tissue Expression (GTEx) project. *Nat Genet*. 2013;45:580-585.
20. Habib ZA, Havstad SL, Wells K, Divine G, Pladevall M, Williams LK. Thiazolidinedione use and the longitudinal risk of fractures in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab*. 2010;95:592-600.
21. Hernigou P, Bachir D, Galacteros F. The natural history of symptomatic osteonecrosis in adults with sickle-cell disease. *J Bone Joint Surg Am*. 2003;85-A:500-504.
22. Houdek MT, Wyles CC, Martin JR, Sierra RJ. Stem cell treatment for avascular necrosis of the femoral head: current perspectives. *Stem Cells Cloning*. 2014;7:65-70.
23. Houdek MT, Wyles CC, Packard BD, Terzic A, Behfar A, Sierra RJ. Decreased osteogenic activity of mesenchymal stem cells in patients with corticosteroid-induced osteonecrosis of the femoral head. *J Arthroplasty*. 2016;31:893-898.
24. Hungerford DS, Zizic TM. Alcoholism associated ischemic necrosis of the femoral head. Early diagnosis and treatment. *Clin Orthop Relat Res*. 1978:144-153.
25. Ionita-Laza I, Lee S, Makarov V, Buxbaum JD, Lin X. Sequence kernel association tests for the combined effect of rare and common variants. *Am J Hum Genet*. 2013;92:841-853.
26. Issemann I, Green S. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature*. 1990;347:645-650.
27. Ito H, Matsuno T, Omizu N, Aoki Y, Minami A. Mid-term prognosis of non-traumatic osteonecrosis of the femoral head. *J Bone Joint Surg Br*. 2003;85:796-801.
28. Jones LC, Hungerford DS. Osteonecrosis: etiology, diagnosis, and treatment. *Curr Opin Rheumatol*. 2004;16:443-449.
29. Jones LC, Mont MA, Le TB, Petri M, Hungerford DS, Wang P, Glueck CJ. Procoagulants and osteonecrosis. *J Rheumatol*. 2003;30:783-791.
30. Kahn SE, Haffner SM, Heise MA, Herman WH, Holman RR, Jones NP, Kravitz BG, Lachin JM, O'Neill MC, Zinman B, Viberti G, Group AS. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N Engl J Med*. 2006;355:2427-2443.
31. Kahn SE, Zinman B, Lachin JM, Haffner SM, Herman WH, Holman RR, Kravitz BG, Yu D, Heise MA, Aftring RP, Viberti G, Diabetes Outcome Progression Trial Study G. Rosiglitazone-associated fractures in type 2 diabetes: an Analysis from A Diabetes Outcome Progression Trial (ADOPT). *Diabetes Care*. 2008;31:845-851.
32. Kaneshiro Y, Oda Y, Iwakiri K, Masada T, Iwaki H, Hirota Y, Kondo K, Takaoka K. Low hepatic cytochrome P450 3A activity is a risk for corticosteroid-induced osteonecrosis. *Clin Pharmacol Ther*. 2006;80:396-402.
33. Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, Haussler D. The human genome browser at UCSC. *Genome Res*. 2002;12:996-1006.
34. Koo KH, Lee JS, Lee YJ, Kim KJ, Yoo JJ, Kim HJ. Endothelial nitric oxide synthase gene polymorphisms in patients with nontraumatic femoral head osteonecrosis. *J Orthop Res*. 2006;24:1722-1728.
35. Kundaje A, Meuleman W, Ernst J, Bilenky M, Yen A, Heravi-Moussavi A, Kheradpour P, Zhang Z, Wang J, Ziller MJ, Amin V, Whitaker JW, Schultz MD, Ward LD, Sarkar A, Quon G, Sandstrom RS, Eaton ML, Wu YC, Pfenning AR, Wang X, Claussnitzer M, Liu Y, Coarfa C, Harris RA, Shoresh N, Epstein CB, Gjoneska E, Leung D, Xie W, Hawkins RD, Lister R, Hong C, Gascard P, Mungall AJ, Moore R, Chuah E, Tam A, Canfield TK, Hansen RS, Kaul R, Sabo PJ, Bansal MS, Carles A, Dixon JR, Farh KH, Feizi S, Karlic R, Kim AR, Kulkarni A, Li D, Lowdon R, Elliott G, Mercer TR, Neph SJ, Onuchic V, Polak P, Rajagopal N, Ray P, Sallari RC, Siebenthall KT, Sinnott-Armstrong NA, Stevens M, Thurman RE, Wu J, Zhang B, Zhou X, Beaudet AE, Boyer LA, De Jager PL, Farnham PJ, Fisher SJ, Haussler D, Jones SJ, Li W, Marra MA, McManus MT, Sunyaev S, Thomson JA, Tlsty TD, Tsai LH, Wang W, Waterland RA, Zhang MQ, Chadwick LH, Bernstein BE, Costello JF, Ecker JR, Hirst M, Meissner A, Milosavljevic A, Ren B, Stamatoyannopoulos JA, Wang T, Kellis M. Integrative analysis of 111 reference human epigenomes. *Nature*. 2015;518:317-330.
36. Lavernia CJ, Sierra RJ, Grieco FR. Osteonecrosis of the femoral head. *J Am Acad Orthop Surg*. 1999;7:250-261.
37. Lee MJ, Chen HT, Ho ML, Chen CH, Chuang SC, Huang SC, Fu YC, Wang GJ, Kang L, Chang JK. PPARgamma silencing enhances osteogenic differentiation of human adipose-derived mesenchymal stem cells. *J Cell Mol Med*. 2013;17:1188-1193.
38. Lefterova MI, Haakonsson AK, Lazar MA, Mandrup S. PPARgamma and the global map of adipogenesis and beyond. *Trends Endocrinol Metab*. 2014;25:293-302.
39. Liu YF, Chen WM, Lin YF, Yang RC, Lin MW, Li LH, Chang YH, Jou YS, Lin PY, Su JS, Huang SF, Hsiao KJ, Fann CS, Hwang HW, Chen YT, Tsai SF. Type II collagen gene variants and inherited osteonecrosis of the femoral head. *N Engl J Med*. 2005;352:2294-2301.
40. Manolio TA. Genomewide association studies and assessment of the risk of disease. *N Engl J Med*. 2010;363:166-176.
41. Manolio TA. Bringing genome-wide association findings into clinical use. *Nat Rev Genet*. 2013;14:549-558.
42. McDonough AK, Rosenthal RS, Cao X, Saag KG. The effect of thiazolidinediones on BMD and osteoporosis. *Nat Clin Pract Endocrinol Metab*. 2008;4:507-513.
43. Mieczkowska A, Basle MF, Chappard D, Mabilleau G. Thiazolidinediones induce osteocyte apoptosis by a G protein-coupled receptor 40-dependent mechanism. *J Biol Chem*. 2012;287:23517-23526.
44. Mont MA, Hungerford DS. Non-traumatic avascular necrosis of the femoral head. *J Bone Joint Surg Am*. 1995;77:459-474.
45. Mont MA, Jones LC, Einhorn TA, Hungerford DS, Reddi AH. Osteonecrosis of the femoral head. Potential treatment with growth and differentiation factors. *Clin Orthop Relat Res*. 1998:S314-335.
46. Mont MA, Zywiel MG, Marker DR, McGrath MS, Delanois RE. The natural history of untreated asymptomatic osteonecrosis of the femoral head: a systematic literature review. *J Bone Joint Surg Am*. 2010;92:2165-2170.
47. Olson JE, Ryu E, Johnson KJ, Koenig BA, Maschke KJ, Morrisette JA, Liebow M, Takahashi PY, Fredericksen ZS, Sharma RG, Anderson KS, Hathcock MA, Carnahan JA, Pathak J, Lindor NM, Beebe TJ, Thibodeau SN, Cerhan JR. The Mayo Clinic Biobank: a building block for individualized medicine. *Mayo Clin Proc*. 2013;88:952-962.
48. Paria N, Copley LA, Herring JA, Kim HK, Richards BS, Sucato DJ, Rios JJ, Wise CA. The impact of large-scale genomic methods in orthopaedic disorders: insights from genome-wide association studies. *J Bone Joint Surg Am*. 2014;96:e38.

49. Pearson TA, Manolio TA. How to interpret a genome-wide association study. *Jama*. 2008;299:1335-1344.
50. Pritchett JW. Statin therapy decreases the risk of osteonecrosis in patients receiving steroids. *Clin Orthop Relat Res*. 2001:173-178.
51. Pruim RJ, Welch RP, Sanna S, Teslovich TM, Chines PS, Gliedt TP, Boehnke M, Abecasis GR, Willer CJ. LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics*. 2010;26:2336-2337.
52. R Core Team (2014). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing V, Austria. URL http://www.R-project.org/. Date Accessed: January 15, 2018.
53. Raney BJ, Dreszer TR, Barber GP, Clawson H, Fujita PA, Wang T, Nguyen N, Paten B, Zweig AS, Karolchik D, Kent WJ. Track data hubs enable visualization of user-defined genome-wide annotations on the UCSC Genome Browser. *Bioinformatics*. 2014;30:1003-1005.
54. Samadfam R, Awori M, Benardeau A, Bauss F, Sebokova E, Wright M, Smith SY. Combination treatment with pioglitazone and fenofibrate attenuates pioglitazone-mediated acceleration of bone loss in ovariectomized rats. *J Endocrinol*. 2012;212:179-186.
55. wcfLMaMWSS-SSKATRpvhC Seunggeun Lee.
56. Shockley KR, Lazarenko OP, Czernik PJ, Rosen CJ, Churchill GA, Lecka-Czernik B. PPARgamma2 nuclear receptor controls multiple regulatory pathways of osteoblast differentiation from marrow mesenchymal stem cells. *J Cell Biochem*. 2009;106:232-246.
57. Sinnwell JP, Schaid DJ. haplo.stats: Statistical analysis of haplotypes with traits and covariates when linkage phase is ambiguous. Available at: https://cran.r-project.org/web/packages/haplo.stats/index.html. 2014. Date Accessed: January 15, 2018.
58. Smith SY, Samadfam R, Chouinard L, Awori M, Benardeau A, Bauss F, Guldberg RE, Sebokova E, Wright MB. Effects of pioglitazone and fenofibrate co-administration on bone biomechanics and histomorphometry in ovariectomized rats. *J Bone Miner Metab*. 2015;33:625-641.
59. Takada I, Kouzmenko AP, Kato S. Wnt and PPARgamma signaling in osteoblastogenesis and adipogenesis. *Nat Rev Rheumatol*. 2009;5:442-447.
60. Tokuhara Y, Wakitani S, Oda Y, Kaneshiro Y, Masada T, Kim M, Kadoya Y, Azuma T, Takaoka K. Low levels of steroid-metabolizing hepatic enzyme (cytochrome P450 3A) activity may elevate responsiveness to steroids and may increase risk of steroid-induced osteonecrosis even with low glucocorticoid dose. *J Orthop Sci*. 2009;14:794-800.
61. Wang J, Zhuang J, Iyer S, Lin X, Whitfield TW, Greven MC, Pierce BG, Dong X, Kundaje A, Cheng Y, Rando OJ, Birney E, Myers RM, Noble WS, Snyder M, Weng Z. Sequence features and chromatin structure around the genomic regions bound by 119 human transcription factors. *Genome Res*. 2012;22:1798-1812.
62. Wang J, Zhuang J, Iyer S, Lin XY, Greven MC, Kim BH, Moore J, Pierce BG, Dong X, Virgil D, Birney E, Hung JH, Weng Z. Factorbook.org: a Wiki-based database for transcription factor-binding data generated by the ENCODE consortium. *Nucleic Acids Res*. 2013;41:D171-176.
63. Wyles CC, Houdek MT, Crespo-Diaz RJ, Norambuena GA, Stalboerger PG, Terzic A, Behfar A, Sierra RJ. Adipose-derived mesenchymal stem cells are phenotypically superior for regeneration in the setting of osteonecrosis of the femoral head. *Clin Orthop Relat Res*. 2015;473:3080-3090.
64. Yuan Z, Li Q, Luo S, Liu Z, Luo D, Zhang B, Zhang D, Rao P, Xiao J. PPARgamma and Wnt signaling in adipogenic and osteogenic differentiation of mesenchymal stem cells. *Curr Stem Cell Res Ther*. 2016;11:216-225.
65. Zhu ZN, Jiang YF, Ding T. Risk of fracture with thiazolidinediones: an updated meta-analysis of randomized clinical trials. *Bone*. 2014;68:115-123.
66. Zuin J, Dixon JR, van der Reijden MI, Ye Z, Kolovos P, Brouwer RW, van de Corput MP, van de Werken HJ, Knoch TA, van IWF, Grosveld FG, Ren B, Wendt KS. Cohesin and CTCF differentially affect chromatin architecture and gene expression in human cells. *Proc Natl Acad Sci U S A*. 2014;111:996-1001.