Genome-wide association scans for idiopathic osteonecrosis of the femoral head in a Korean population

SEUNG-HOON BAEK[1], KANG-IL KIM[2], KYUNG-SIK YOON[3], TAE-HO KIM[4,5] and SHIN-YOON KIM[1,5]

[1]Department of Orthopedic Surgery, Graduate School of Medicine, Kyungpook National University, Daegu 41944; Departments of [2]Orthopedic Surgery and [3]Biochemistry and Molecular Biology, School of Medicine, Kyung Hee University, Seoul 02447; [4]Biomedical Research Institute, Kyungpook National University Hospital; [5]Skeletal Diseases Genome Research Center, Kyungpook National University, Daegu 41940, Republic of Korea

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Abstract. Osteonecrosis of the femoral head (ONFH) is a multifactorial disease and is associated with genetic predisposition, and exposure to certain risk factors. In particular, idiopathic ONFH in twins and the clustering of cases in families have indicated that genetic factors are involved. However, the majority of cases of ONFH are sporadic and various studies have demonstrated that differences in the study design and/or the ethnic groups analyzed leads to different results. The present study performed one of the first genome-wide association studies to identify genetic loci that may increase the risk of idiopathic ONFH. In total, 217 patients with idiopathic ONFH and 217 control samples, without ONFH, were genotyped using Axiom™ chips. Following quality control, 509,886 single-nucleotide polymorphisms (SNPs) were included in the association analysis to identify genetic variants that may influence susceptibility to idiopathic ONFH. The lowest P-value identified by the current study was for an association with rs220324 ($P=3.57x10^{-7}$), an SNP that is located near to the uromodulin-like 1 gene region on chromosome 21q22.3, although none of the SNPs reached the traditional genome-wide significance level of $5x10^{-8}$. However, the DnaJ heat shock protein family (Hsp40) member C6 (*DNAJC6)* locus, a region between 65.37 and 65.67 Mb located on chromosome 1p31.3, harbored a cluster of SNPs that were associated with idiopathic ONFH at a significance level of $P<1x10^{-5}$. Four variants, rs10493374, rs12032616, rs17127529 and rs6679032, with marginal associations were located in and around the *DNAJC6* locus and were in strong linkage disequilibrium with each other. In conclusion, the current study did not identify any SNPs that were associated with idiopathic ONFH at a genome-wide significance level, however, the results suggest that future studies should investigate the effects of SNPs in the *DNAJC6* gene on the idiopathic ONFH risk.

Correspondence to: Professor Shin-Yoon Kim, Department of Orthopedic Surgery, Graduate School of Medicine, Kyungpook National University, 101 Dongin-dong 2 Ga, Daegu 41944, Republic of Korea
E-mail: syukim@knu.ac.kr

Dr Tae-Ho Kim, Biomedical Research Institute, Kyungpook National University Hospital, 135 Dongdeok-ro, Daegu 41940, Republic of Korea
E-mail: kimth0929@ynu.ac.kr

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Introduction

Osteonecrosis is the death of bone tissue following microvascular injury, which results in bone resorption and, potentially, structural collapse. The disease typically affects the epiphyseal bone on the convex side of a joint, which may be due to a lack of collateral circulation and most commonly affects the femoral head, although it may also affect the humeral head, femoral condyles, proximal tibia, vertebra, and small bones of the hand and foot (1). Osteonecrosis of the femoral head (ONFH) is a complex multifactorial disease that is associated with genetic predisposition and exposure to certain environmental factors. Various etiological factors, including the use of corticosteroids, alcohol abuse, sickle cell anemia, radiation and Gaucher disease, are known to be implicated in the development of secondary osteonecrosis (2). It is possible for certain patients, those who have received high doses of corticosteroids or consumed excessive amounts of alcohol for a long period of time, to develop ONFH, however, there are also rare cases of ONFH that occur following short-duration corticosteroid treatment, thus indicating that differences exist in the susceptibility to risk factors and the genetic predisposition to ONFH between individuals (3,4). Idiopathic ONFH is diagnosed when individuals are not known to have been exposed to any of the known risk factors. The incidence or prevalence of idiopathic ONFH reflects ethnic differences (5). Idiopathic ONFH in identical twins and the clustering of cases in families indicate that genetic factors may be involved in development (6,7). Several studies have investigated genetic traits that may predispose an individual to ONFH development, including those involved in clotting disorders (8-10). However, many of the results of genetic studies investigating the association between genes involved in the clotting

process and the risk of ONFH in Caucasians have not been replicated in other ethnic groups. For example, factor V Leiden and prothrombin G20210A mutations have not been identified in the Korean population (11,12). This suggests that geographic and ethnic differences exist in the prevalence of disease-associated genes and/or polymorphisms. In addition, Liu *et al* reported that all patients with familial ONFH in the study carried collagen type II α 1 chain (COL2A1) mutations, whereas no mutations were identified in the COL2A1 coding region in patients with sporadic ONFH (13). The majority of ONFH cases are sporadic, therefore, it is essential to screen candidate genes from a different perspective. Various genetic methods have been developed to identify disease-associated loci or causal variations associated with specific diseases. Genome-wide association studies (GWAS) have been facilitated by the availability of chip-based microarray techniques, which allow assays of more than a million single-nucleotide polymorphisms (SNPs) in order to identify disease-associated loci and potential causal variants associated with diseases. GWAS has been used to map a huge number of susceptibility genes for numerous complex diseases, including type 1 and type 2 diabetes, inflammatory bowel disease, prostate cancer and breast cancer (14-16). Since GWAS was first reported in 2005, 2,041 studies have been added to the Catalog of Published GWAS (http://www.genome.gov/26525384), however, to the best of our knowledge, GWAS of patients with idiopathic ONFH has not previously been performed. GWAS was performed in the present study to identify genetic variants that influence susceptibility to and outcomes of idiopathic ONFH in the Korean population.

Materials and methods

Study subjects. The study was initially performed at the GWAS discovery stage to identify SNPs potentially involved in the development of idiopathic ONFH in the Korean population. The ONFH patients were recruited consecutively from Kyungpook National University Hospital (Daegu, Korea) between 2002 and 2012, and written informed consent was obtained from all study participants prior to enrolment. This study was approved by the Institutional Review Board of the Kyungpook National University Hospital. All patients with ONFH were diagnosed by an orthopedist according to the diagnostic criteria of the Association Research Circulation Osseous classification system based on magnetic resonance imaging and plain radiographs (17). Based on etiological factors, patients were assigned to one of the following groups: Idiopathic, steroid-induced and alcohol-induced osteonecrosis. Only the patients in the idiopathic group were included in this study. Control subjects were defined by a lack of hip pain and the absence of any lesions with sclerotic margins or subchondral collapse consistent with ONFH in anteroposterior and frog-leg lateral pelvic radiographs. The GWAS included 217 idiopathic ONFH (152 males and 65 females aged 49.5±14.2 years) and 217 control (57 males and 160 females aged 54.7±12.8 years) samples.

Genome-wide SNP genotyping. Genomic DNA was extracted from whole human blood using the FlexiGene DNA kit (Qiagen, Inc., Valencia, CA, USA) according to the manufacturer's protocol. For each sample, genotyping was performed with the Axiom® 2.0 genome-wide ASI 1 Array kit (Affymetrix, Inc., Santa Clara, CA, USA) according to the standard protocols recommended by the manufacturer. Briefly, for each array, ~200 ng genomic DNA was amplified and fragmented randomly into fragments of 25-125 base pairs (bp). The initial amplification had a reaction volume of 40 μl, which contained 20 μl genomic DNA at a concentration of 10 ng/μl and 20 μl Denaturation Master Mix (reagent from Module 1(P/N901711) of the Axiom 2.0 reagent kit). The initial amplification was performed for 10 min at room temperature. Following the initial amplification, the incubated products were amplified with 130 μl Axiom® 2.0 Neutral Solution, 225 μl Axiom® 2.0 Amp Solution, and 5 μl Axiom® 2.0 Amp Enzyme. Amplification reactions were performed for 23±1 h at 37˚C. Amplification products were then used to amplify fragments of 200-1,100 bp. A fragmentation step reduced the amplified products into segments of ~25-50 bp, which were then end-labeled using reagents from Module 2-1(P/N901528) and Module 2-2(P/N901529) of the Axiom 2.0 Reagent kit. Following hybridization, the bound target was washed under stringent conditions to remove non-specific background and minimize any background noise caused by random ligation events (reagents from Module 4-1(P/N901278) and Module 4-2(P/N901276) of the Axiom 2.0 Reagent kit). Each polymorphic nucleotide was queried via a multi-color ligation event performed on the array surface. Following ligation, the arrays were stained and imaged using a GeneTitan® Multi-Channel Instrument (Affymetrix, Inc.). Images were analyzed using Genotyping Console™ software 4.14 (Affymetrix, Inc.).

Genotype quality control. Raw data in the form of CEL files were imported into Affymetrix Power Tools (version 1.16.1; Affymetrix, Inc.) and genotype calling was performed using Axiom GT1 algorithm (Affymetrix, Inc.). Samples with dish quality control (QC) value of >0.82 and call rate >0.95 were considered to have passed the quality control assessment. Downstream analysis was performed using PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/) version no. 1.07 (18). Markers were considered not to meet the quality control criteria and discarded if they were demonstrated to have any of the following: Call rate <95%, minor allele frequency of the control <1%, and deviations from the Hardy-Weinberg equilibrium with a P-value $<10^{-7}$. It was confirmed that the call rate per sample was >97%.

Statistical analysis. Statistical analyses were performed using PLINK (version no. 1.07) (18) and SAS (version no. 9.1.3; SAS Institute, Cary, NC, USA). To test for allelic and genotype associations between SNPs and ONFH, 2x2 and 2x3 contingency tables were constructed, which were compared using Cochran-Armitage trend, Chi-squared, and Jonckheere-Terpstra tests. The strength of association was estimated by the odds ratio and the 95% confidence interval, which were calculated using Cornfield methods. Three genetic models of inheritance (recessive, dominant, and codominant) for SNPs were assessed. Genotypes were given codes of 0, 1 and 2; 0, 1 and 1; and 0, 0 and 1 in the codominant, dominant, and recessive models, respectively. The quantile-quantile (Q-Q) plot demonstrates close agreement

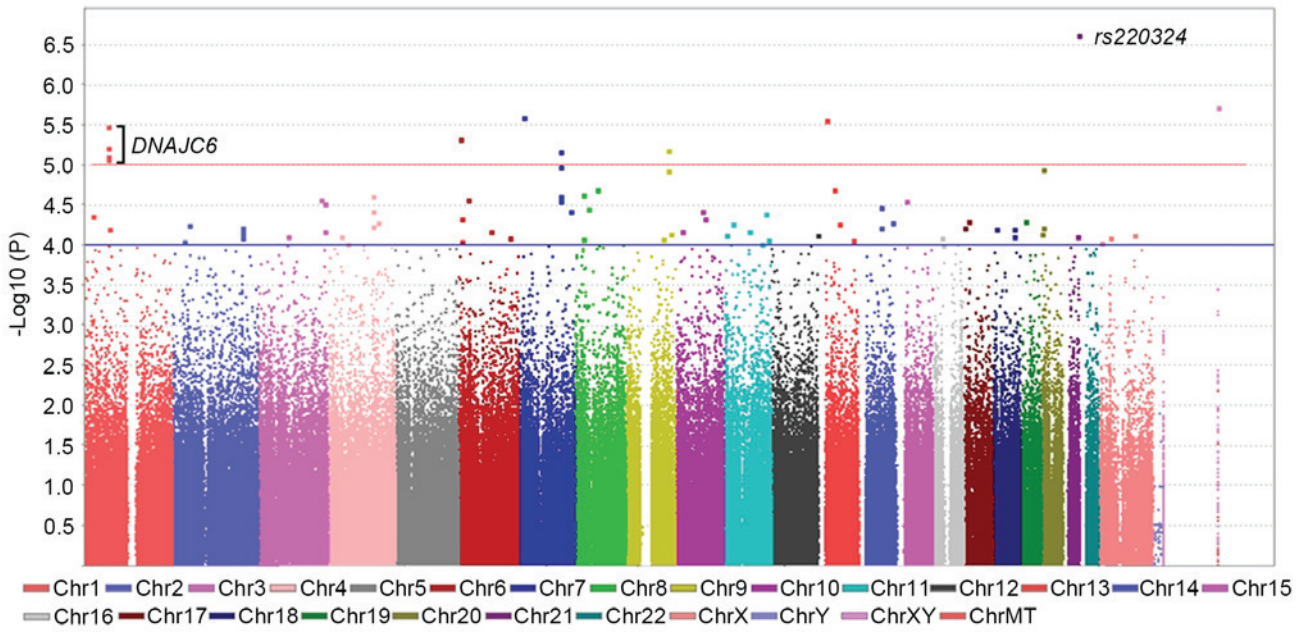

Figure 1. Genome-wide SNP association signals for idiopathic ONFH. Manhattan plot, logarithm of P-values vs. Chr coordinates, of SNP associations in idiopathic ONFH. The red and blue lines denote P-values of $1x10^{-5}$ and $1x10^{-4}$, respectively. SNP, single-nucleotide polymorphism; ONFH, osteonecrosis of the femoral head; Chr, chromosome.

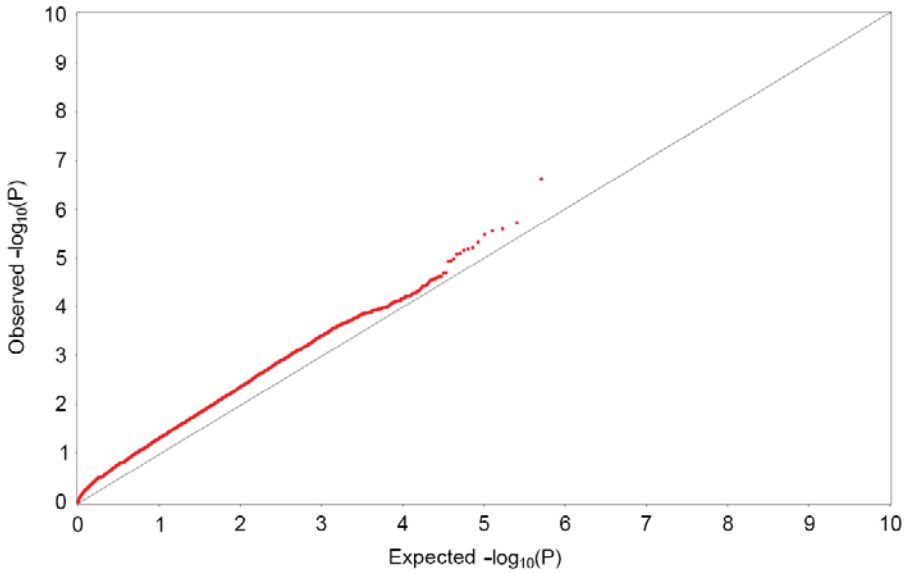

Figure 2. Quantile-quantile plot of genome-wide association study results for idiopathic osteonecrosis of the femoral head. The horizontal axis indicates the expected -log10 (P-values). The vertical axis indicates the observed -log10 (P-values). The gray line represents y=x.

between two distributions if the population stratification and cryptic relatedness are controlled adequately. Undetected population stratification or cryptic relatedness results in deviation from the null across the entire distribution; whereas, large-effect susceptibility loci generate deviations at the highly significant end of the range (19). Regional association plots were created using LocusZoom (version no. 1.3; https://statgen.sph.umich.edu/locuszoom/genform.php?type=yourdata; University of Michigan, Ann Arbor, MI, USA). Haploview (version no. 4.2; http://www.broad.mit.edu/mpg/haploview; Broad Institute, Cambridge, MA, USA) was used to determine linkage disequilibrium (LD) in the genomic region using an accelerated expectation-maximization algorithm (20).

Results

Genotype data for 217 idiopathic ONFH cases (152 male, 65 female) and 217 control (57 male, 160 female) subjects was analyzed. Based on stringent quality control criteria, 509,886 SNPs were selected for association analysis for idiopathic ONFH. A Manhattan plot of the association analyses for all SNPs was produced using the chromosomal positions (x-axis) and negative logarithm of P-values (y-axis) for each

Table I. All 78 SNPs with P<1x10^{-4} according to GWAS analysis of ONFH cases and controls.

rsSNP number	Chr.	Position[a]	Nearest Gene[b]	Allele[c]		MAF		OR	95% CI	P-value
				1	2	ONFH	Control			
rs220324	21	43586699	*UMODL1, ABCG1*	T	C	0.33	0.484	0.35	0.23-0.52	2.35E-07
rs306896	X	154979339	*SPRY3*	T	C	0.595	0.444	-	-	1.89E-06
rs1432486	7	13481626	Intergenic/Unknown	C	T	0.491	0.333	1.93	1.47-2.54	2.52E-06
rs9285223	13	23124968	Intergenic/Unknown	C	T	0.204	0.288	-	-	2.77E-06
rs17127529	1	65799440	***DNAJC6***	T	C	0.311	0.463	0.52	0.40-0.69	3.27E-06
rs17548629	6	3114457	*RIPK1*	C	T	0.183	0.318	0.48	0.35-0.66	4.69E-06
rs10493374	1	65783231	***DNAJC6***	G	T	0.15	0.276	0.46	0.33-0.65	6.05E-06
rs12376144	9	119488641	*ASTN2*	C	A	0.394	0.254	-	-	6.53E-06
rs7794364	7	117508506	*CTTNBP2*	C	T	0.201	0.095	-	-	6.82E-06
rs6679032	1	65764742	***DNAJC6***	A	G	0.152	0.276	0.47	0.34-0.66	7.94E-06
rs12032616	1	65703484	***DNAJC6****, AK4*	A	G	0.16	0.286	0.47	0.34-0.66	8.32E-06
rs1012084	7	117529421	Intergenic/Unknown	G	A	0.2	0.097	-	-	1.05E-05
rs6037866	20	4521824	Intergenic/Unknown	T	C	0.229	0.365	-	-	1.12E-05
rs756118	9	119487330	*ASTN2*	T	C	0.392	0.256	-	-	1.16E-05
rs3794431	13	44544511	*DGKZP1*	A	G	0.171	0.293	0.5	0.36-0.69	2.00E-05
rs16927830	8	62658110	*ASPH, NKAIN3, MIR4470*	T	C	0.585	0.47	2.76	1.71-4.44	2.04E-05
rs2466224	8	22785361	*PEBP4*	A	C	0.277	0.411	-	-	2.37E-05
rs28407041	4	127437492	Intergenic/Unknown	C	T	0.449	0.32	2.33	1.57-3.45	2.40E-05
rs4730811	7	117582806	Intergenic/Unknown	A	G	0.21	0.108	-	-	2.45E-05
rs7775145	6	25419880	*LRRC16A*	G	A	0.47	0.353	2.39	1.58-3.60	2.66E-05
rs10513755	3	177115806	Intergenic/Unknown	C	G	0.558	0.482	1.36	1.04-1.77	2.71E-05
rs11631923	15	25764385	*LOC105370737*	T	C	0.32	0.449	-	-	2.75E-05
rs6466637	7	117576675	Intergenic/Unknown	A	G	0.21	0.109	-	-	2.79E-05
rs9882205	3	186570398	*ADIPOQ*	A	G	0.347	0.472	0.42	0.28-0.64	3.05E-05
rs16887454	8	38413475	*LOC105379383*	G	A	0.217	0.309	0.45	0.30-0.66	3.52E-05
rs10999727	10	72960515	Intergenic/Unknown	G	A	0.468	0.354	2.33	1.55-3.49	3.71E-05
rs11098882	4	127447572	Intergenic/Unknown	A	C	0.38	0.25	1.84	1.38-2.46	3.74E-05
rs17170575	7	147218996	*CNTNAP2, MIR548I4*	C	T	0.243	0.204	20.7	2.75-156	3.76E-05
rs1886461	14	61944882	*PRKCH*	A	G	0.498	0.388	2.43	1.59-3.72	3.93E-05
rs11216126	11	116617240	Intergenic/Unknown	A	C	0.24	0.131	2.08	1.46-2.97	4.06E-05
rs7514144	1	20735869	*LOC339505*	T	C	0.124	0.23	0.48	0.33-0.68	4.37E-05
rs3804483	6	6637677	*LY86*	C	T	0.267	0.391	0.45	0.30-0.66	4.62E-05
rs1515416	10	79364940	*KCNMA1*	A	C	0.385	0.279	4.64	2.09-10.3	4.64E-05
rs2969183	17	11344773	*SHISA6*	G	A	0.067	0.014	5.43	2.21-13.4	5.00E-05

Table I. Continued.

rsSNP number	Chr.	Position[a]	Nearest Gene[b]	Allele[c]		MAF		OR	95% CI	P-value
				1	2	ONFH	Control			
rs714919	19	15151667	Intergenic/Unknown	G	A	0.272	0.384	0.45	0.31-0.67	5.02E-05
rs34299628	4	142506694	Intergenic/Unknown	G	A	0.214	0.326	0.45	0.31-0.67	5.09E-05
rs11629059	14	96093508	Intergenic/Unknown	G	A	0.565	0.482	2.56	1.61-4.08	5.16E-05
rs9569891	13	59150370	Intergenic/Unknown	C	T	0.623	0.486	1.74	1.33-2.29	5.32E-05
rs731365	11	24334057	*LOC105376595*	G	T	0.317	0.451	0.57	0.43-0.75	5.47E-05
rs7561623	2	45694417	*SRBD1*	G	A	0.249	0.141	2.03	1.43-2.87	5.61E-05
rs28558216	4	127437795	Intergenic/Unknown	C	T	0.376	0.249	1.82	1.36-2.43	5.86E-05
rs1902249	2	198521356	*RFTN2*	T	C	0.604	0.467	1.74	1.33-2.27	5.94E-05
rs3783786	14	61937224	*PRKCH*	G	A	0.599	0.468	-	-	5.94E-05
rs297745	20	4473609	Intergenic/Unknown	C	T	0.224	0.346	-	-	6.00E-05
rs9789059	17	129225	*RPH3AL*	C	T	0.349	0.418	0.32	0.18-0.57	6.10E-05
rs547810	18	58202247	Intergenic/Unknown	G	C	0.376	0.264	2.19	1.49-3.23	6.14E-05
rs1965881	18	9659377	Intergenic/Unknown	C	T	0.528	0.393	-	-	6.17E-05
rs17130890	1	69683475	*LOC105378786*	T	C	0.178	0.294	0.52	0.38-0.72	6.19E-05
rs9444695	6	90253629	*ANKRD6*	G	A	0.588	0.456	1.7	1.30-2.23	6.63E-05
rs6719832	2	198533369	*RFTN2*	A	G	0.602	0.468	2.38	1.55-3.66	6.69E-05
rs11253891	10	16332723	*LOC102724039*	G	A	0.157	0.267	0.51	0.36-0.71	6.72E-05
rs3774262	3	186571814	*ADIPOQ*	G	A	0.357	0.235	1.85	1.26-2.70	6.73E-05
rs1660849	11	71100302	*FLJ42102, SHANK2*	C	T	0.521	0.405	2.59	1.61-4.18	6.78E-05
rs6136898	20	2047818	Intergenic/Unknown	C	T	0.126	0.051	-	-	7.24E-05
rs7869304	9	124946021	*MORN5*	A	G	0.336	0.461	0.45	0.30-0.67	7.25E-05
rs7974644	12	130593579	Intergenic/Unknown	G	A	0.618	0.484	-	-	7.43E-05
rs916220	X	102254602	Intergenic/Unknown	G	A	0.346	0.198	3.31	1.81-6.07	7.44E-05
rs11820502	11	5688024	*TRIM5*	C	G	0.336	0.465	0.34	0.19-0.59	7.51E-05
rs6708239	2	198525084	*RFTN2*	A	G	0.604	0.47	1.72	1.31-2.25	7.57E-05
rs6778265	3	81985745	*LOC105377179*	C	T	0.12	0.221	0.48	0.33-0.70	7.65E-05
rs10009706	4	38079475	*TBC1D1*	G	A	0.332	0.446	0.31	0.17-0.57	7.67E-05
rs17657655	18	58229575	Intergenic/Unknown	A	C	0.346	0.228	-	-	7.77E-05
rs2835984	21	39188787	*KCNJ6, TMPRSS3*	A	T	0.302	0.41	0.3	0.16-0.56	7.82E-05
rs9967823	2	198531529	*RFTN2*	A	G	0.604	0.47	1.72	1.31-2.25	7.88E-05
rs7192193	16	26171345	Intergenic/Unknown	G	A	0.519	0.424	2.63	1.61-4.29	7.94E-05
rs1091272	X	32622348	*DMD*	G	T	0.476	0.356	5.24	2.16-12.7	7.98E-05
rs9496856	6	144411489	Intergenic/Unknown	A	C	0.101	0.164	-	-	8.09E-05
rs7032668	9	105027288	Intergenic/Unknown	C	T	0.168	0.265	0.45	0.31-0.67	8.23E-05

Table I. Continued.

rsSNP number	Chr.	Position[a]	Nearest Gene[b]	Allele[c]		MAF		OR	95% CI	P-value
				1	2	ONFH	Control			
rs17088580	8	22578660	*PEBP4, LOC100507139*	G	A	0.154	0.263	-	-	8.24E-05
rs12574001	11	124112644	*OR8G5*	C	T	0.134	0.06	2.74	1.64-4.58	8.71E-05
rs7326256	13	99788540	Intergenic/Unknown	G	A	0.062	0.141	0.37	0.22-0.61	8.71E-05
rs1971866	2	30262542	*LOC101929418*	G	C	0.507	0.384	2.28	1.51-3.46	8.81E-05
rs3804486	6	6637995	*LY86*	G	A	0.267	0.387	0.47	0.32-0.68	8.98E-05
rs5979043	X	9164003	*FAM9B*	C	G	0.339	0.214	7.89	2.41-25.8	9.37E-05
rs564569	11	107706991	*SLC35F2*	G	A	0.502	0.371	1.71	1.31-2.24	9.59E-05
rs1459821	4	53340238	Intergenic/Unknown	T	G	0.074	0.154	0.38	0.23-0.63	9.62E-05
rs566238	11	107706832	*SLC35F2*	C	T	0.502	0.371	1.71	1.31-2.24	9.75E-05
rs12933766	16	29922011	*KCTD13, ASPHD1*	C	T	0.375	0.281	3.61	1.83-7.13	9.89E-05

[a]Chromosome positions are based on NCBI build 37. [b]Gene is defined as the gene containing the SNP or the closest genes up to 100 kb up/downstream of the SNP. [c]Allele 1 refers to a major allele, while Allele 2 refers to a minor allele. - no data due to codominant genetic model. SNP, single nucleotide polymorphism; GWAS, genome-wide association study; ONFH, patients with osteonecrosis of femoral head; Chr, chromosome; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval.

SNP (Fig. 1). In the Q-Q plot (Fig. 2), minimal deviation from the expected P-value distribution was observed, and only at the upper tail of the distribution, suggesting that population stratification was adequately controlled. All of the association signals with $P<1x10^{-4}$ (78 SNPs) are shown in Table I. Although none of the SNPs reached the accepted genome-wide significance level of $5x10^{-8}$, 11 SNPs on chromosomes 1, 6, 7, 9, 13 and 21 had the strongest associations with increased susceptibility to idiopathic ONFH at a significance level of $P<10^{-5}$ (Fig. 1 and Table I). The most significant association was detected at rs220324 ($P=3.57x10^{-7}$), which is located in an intergenic region between the uromodulin-like 1 (*UMODL1*) gene and the ATP-binding cassette, sub-family G, member 1 (*ABCG1*) gene region on chromosome 21q22.3. Given that certain disease-associated genes may be enriched among the top ranked genes in GWAS, the present study then filtered the SNPs to identify chromosomal regions that harbored clusters of associated SNPs at a less stringent criterion ($P<1x10^{-5}$). It was observed that DnaJ heat shock protein family (Hsp40) member C6 (*DNAJC6)*, a region between 65.37 and 65.67 Mb in the chromosome 1p31.3 region, harbored a cluster of SNPs, which were associated with idiopathic ONFH at a significance level of $P<1x10^{-5}$. Four significant SNPs, rs10493374, rs12032616, rs17127529 and rs6679032 in the *DNAJC6* gene region were revealed to be in strong LD with each other (Table II), and all had associations with idiopathic ONFH at a significance level of $P<1x10^{-5}$. The regional association plot and LD plot for these loci are presented in Fig. 3.

Discussion

ONFH usually affects young adults and, without treatment, progresses to the collapse of femoral head leading to osteoarthritis and destruction of the hip joint (21). ONFH is one of the most common diseases of the hip joint in Korea, where its incidence is relatively high compared with other countries (5,22). Approximately 50-60% of total primary hip replacements are thought to be the result of ONFH in Korea (5). In Korea, non-traumatic ONFH is associated with idiopathic factors in 36%, alcoholism in 35%, steroids in 14% and other factors in 15% of cases (23). In a previous study, Kang *et al* (22) reported the prevalence of ONFH in Korea using medical claims data obtained from the Korean National Health Insurance Service (Wonju, Korea). The estimated average number of annual cases was 14,103, thus, indicating an average prevalence of 28.91 per 100,000 over a 5-year period (2002-2006). It was demonstrated that 32.4% had a history of alcohol abuse and 14.6% of cases were associated with steroid usage. In recent years, the clinical significance of ONFH for hip diseases has received attention, however, the details of its pathogenesis and epidemiology are not well understood; although it is generally assumed that venous thrombosis resulting in blood flow obstruction to the femoral head, mediated by thrombophilia and/or hypofibrinolysis, is important in the development of ONFH (24,25). Several studies have investigated the genetic traits that may predispose subjects to ONFH development. However, contrary to expectations, the results of various studies have demonstrated that differences are observed between reports with different study designs and/or ethnic groups involved in the analyses.

Table II. Linkage disequilibrium relationships (|D'| and r^2) between the four SNPs that had strong associations with the *DNAJC6* gene.

	SNP |D'|			
SNP r^2	*rs12032616*	*rs6679032*	*rs10493374*	*rs17127529*
rs12032616		0.860	0.860	0.749
rs6679032	0.687		1	1
rs10493374	0.685	1		1
rs17127529	0.256	0.428	0.426	

SNP, single nucleotide polymorphism; DNAJC6, DnaJ heat shock protein family (Hsp40) member C6.

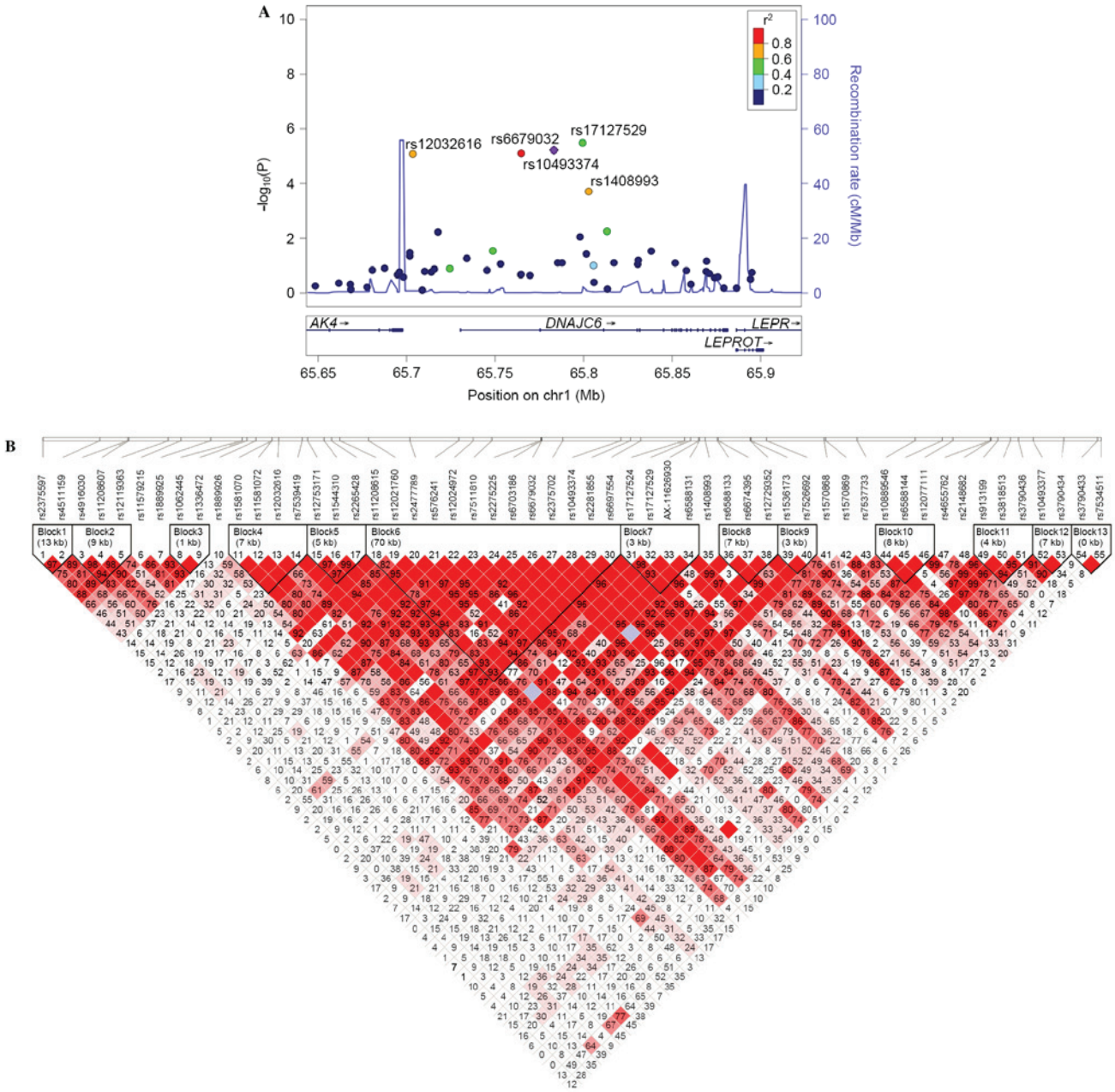

Figure 3. Regional plot of SNPs in the *DNAJC6* locus and the LD relationship among these SNPs. (A) Data are shown for the *DNAJC6* locus around the four associated SNPs. The rs17127529 SNP in the regional plot was the SNP with the most significant association with idiopathic ONFH in the *DNAJC6* gene. The blue curve highlights the recombination rates based on 1000 Genomes Asian data. (B) The strength of the LD relationship (D') between the most strongly associated SNP and the other SNPs is represented by the red color intensity based on genome-wide association study data from the present study. SNP, single-nucleotide polymorphism; LD, linkage disequilibrium; Chr, chromosome.

The present study conducted a GWAS in the Korean population to identify new susceptibility loci associated with idiopathic ONFH. To the best of our knowledge, it is the first high-density GWA scan of ONFH in a Korean population, and the lowest P-value, and most significant association, this study identified was obtained for the association of idiopathic ONFH with rs220324 ($P=3.57x10^{-7}$), which is located in an intergenic region between the *UMODL1* gene and *ABCG1* gene region on chromosome 21q22.3. Notably, the cholesterol transporter ABCG1 is involved in cholesterol homeostasis, where it promotes cholesterol efflux from cells and regulates intracellular cholesterol homeostasis (26). However, none of the SNPs identified by the present study satisfied the recognized GWA criteria ($P<1x10^{-8}$). Given that certain disease-associated genes may be enriched among the top ranked genes in GWAS, the present study searched for chromosomal regions containing two or more significant SNPs within 100 kb of the significant marker SNPs using a less stringent P-value, and the LD structure of the region was analyzed using GWAS data. Subsequently, a chromosomal block containing genes that clustered with SNPs thought to be associated with ONFH ($P<1x10^{-5}$) was identified. The four variants, rs10493374, rs12032616, rs17127529 and rs6679032, with modest associations were located in and around the *DNAJC6* locus. A previous GWAS of the anticoagulant pathway also detected significant associations between certain SNPs in the *DNAJC6* gene and free protein S plasma levels (27). The protein C anticoagulant pathway regulates blood coagulation by preventing the formation of thrombi. This pathway has two main plasma components, protein C and protein S. Deficiencies in antithrombin, protein C and protein S, or an impaired anticoagulant pathway, increase the incidence of thromboembolic disorders (27). Notably, a number of researchers have suggested that intravascular coagulation and microcirculatory thrombotic occlusion may provide a common pathway to non-traumatic osteonecrosis. It is assumed that venous thrombosis with blood flow obstruction to the femoral head, mediated by thrombophilia and/or hypofibrinolysis, leads to increased intraosseous pressure, reduced arterial flow and hypoxia, which appear to be important in the development of ONFH (24,25). The exact function of *DNAJC6* remains unknown, however, the protein encoded by *DNAJC6* resembles a tyrosine-protein phosphatase auxilin, which is an enzyme that promotes the uncoating of clathrin-coated vesicles, thus, it potentially has a role in endocytosis. Endocytosis itself, which is followed by partial proteolysis, is involved in coagulation via molecular modification of factor V and factor VIII (27,28). Thus, a similar mechanism involving *DNAJC6* and free protein S plasma levels may be associated with ONFH, although validation of this hypothesis requires further investigation. When a less stringent P-value ($P<1x10^{-4}$) was applied to select candidate regions for further investigation, in phase 2 of the present study, several chromosomal regions that clustered with SNPs thought to be associated with idiopathic ONFH were discovered (data not shown). The present study performed one of the first GWAS of idiopathic ONFH in Koreans and aimed to identify genetic variants that influence susceptibility to idiopathic ONFH. The results indicated that several SNPs in the *DNAJC6* gene are potentially associated with idiopathic ONFH. Further investigations, including replicate studies, are required to confirm whether *DNAJC6* is an ONFH susceptibility gene, but the preliminary findings of the present study provide novel insight into the genetic factors associated with the risk of ONFH.

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