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ORIGINAL RESEARCH

RAB40C Gene Polymorphisms Were Associated with Alcohol-Induced Osteonecrosis of the Femoral Head

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Introduction: Alcohol-induced osteonecrosis of the femoral head (ONFH), a progressive disease, is caused by excessive drinking and genetic factors. Currently, it remains to represent a significant challenge. The association between alcohol-induced ONFH and *RAB40C* gene polymorphisms may provide a direction for the mechanism of alcoholic ONFH.

Methods: A total of 201 alcohol-induced ONFH patients and 201 healthy controls were recruited in this case–control study. The polymorphisms of *RAB40C* gene were genotyped in blood samples by Agena MassARRAY RS1000. Pearson chi-square test was used to calculate difference in allele frequencies of gene polymorphisms between the cases and controls. Alcohol-induced ONFH risk was estimated using odds ratios (ORs) and 95% confidence intervals (CIs).

Results: In the overall analysis, the allele "G" of rs62030917 was significantly increased alcohol-induced ONFH risk (OR = 1.47, 95% CI = 1.07–2.02, $p = 0.017$) in the allele model. In the genetic analysis, rs62030917 also increased the risk of alcohol-induced ONFH in the dominant model (adjusted OR = 1.52, 95% CI=1.02–2.26, $p = 0.039$) and the log-additive model (adjusted OR = 1.42, 95% CI=1.05–1.93, $p = 0.025$). Age stratification analysis suggested that rs62030917 increased the risk of alcohol-induced ONFH among the individuals younger than 42 years old. Moreover, carriers of AA, GA and GG genotypes in rs2269556 had LDL-C levels that were significantly different ($p = 0.047$). Among them, carriers of GG genotype had the highest LDL-C levels.

Conclusion: This study revealed rs62030917 in *RAB40C* gene might increase the risk of alcohol-induced ONFH, providing a theoretical basis for the mechanism of *RAB40C* in alcohol-induced ONFH.

Keywords: alcohol-induced ONFH, *RAB40C*, single nucleotide polymorphisms

Introduction

Osteonecrosis of the femoral head (ONFH) is a disease in which the local death of osteocytes and bone marrow components is caused by venous congestion or impaired arterial blood supply or the femoral head rupture.[1,2] ONFH usually occurs between 30 and 50 years old.[1] It is reported that the risk factors of ONFH included corticosteroid use, trauma, alcohol consumption, coagulation abnormalities, hyperlipidemia, and smoking.[1] Numerous epidemiological and multicenter studies have shown that most ONFH patients have a history of alcohol abuse. It has been reported that alcohol can destroy bone homeostasis by directly

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inhibiting the proliferation and differentiation of bone marrow mesenchymal stem cells (BMSCs).[3] In particularly, alcohol significantly inhibited the proliferation and DNA synthesis of osteoprogenitor cells.[4] Therefore, alcohol is a factor that cannot be ignored to cause osteonecrosis of the femoral head.[5] At present, there are two main types of osteonecrosis: traumatic and non-traumatic.[2] Alcohol-induced ONFH belongs to non-traumatic osteonecrosis.[6] Epidemiological studies have reported that 20–45% of ONFH patients are associated with alcohol consumption.[1] Excessive drinking may lead to dyslipidemia, abnormal differentiation of BMSCs differentiation, and abnormal bone metabolism. In addition, alcohol has a significant dose effect on bone homeostasis.[7] However, each individual have different susceptibility, which may be related to genetic predispositions.[8] Some studied showed that *MMP-8* and *MMP-3* gene polymorphisms were related to the risk of alcohol-induced ONFH.[9]

RAB40C, also known as *RARL*/*RASL8C*, is located in 16p13.3. It is a member of the *RAS* oncogene family and encodes the protein RAB40C. The RAB family of small GTPases was regards as the cellular regulators of vesicular transport,[10] and RAB proteins were the key regulators of eukaryotic biofilm transport in all eukaryotes.[11] Studies have found that RAB40C was directly regulated by let-7a and played an important regulatory role in the biological role of gastric tumorigenesis.[12] Aberrant methylation in *RAB40C* may be related to the pathogenesis of prostate cancer.[13] Our previous work has proved that RAB40C is a novel lipid droplet-related RAB protein,[11] which is one of the RAB proteins regulating lipid droplets (LDs) homeostasis.[14] Lipid metabolism disorder is considered as the main factor of the pathogenesis of alcohol-induced ONFH.[15] Thus, the occurrence of alcohol-induced ONFH may be relevant to *RAB40C* gene.

In order to verify this hypothesis, this study aimed to search for the association between *RAB40C* single nucleotide polymorphisms (SNPs) and alcohol-induced ONFH susceptibility, so as to provide guidance for the potential treatment and prevention of the disease.

Materials and Methods

Ethical Statement

The ethical approval of this study is in line with the ethical principles of the Helsinki declaration on human medical research. Our study has been approved by the ethics committee of Hong Hui hospital in Xi ‘an, China, and all participants have signed informed consent before participating in the study.

Study Participants

All subjects were the Chinese Han population and included 201 patients and 201 controls. Participants underwent routine physical examinations including internal medicine, surgery, and specialized facial examinations. The diagnostic criteria for alcohol-induced ONFH are based on the clinical manifestations of hip, lumbar, and knee pain and mobility limitations. We further diagnosed the disease by MRI analysis and X-ray examination, such as deformities of the femoral head, hip stenosis, protuberances, or collapsed cartilage fractures.[16] The patients were diagnosed with ONFH after using plain radiographs in stage II, III, and IV of the Ficat Classification systems. Stage I is characterized by no radiological abnormalities. Only some joints are stiff and painful, usually with limited joint movement. The symptoms were relieved after rest, and no positive results were found on X-ray films. Occasionally, uniform or spotty osteoporotic areas could be seen. The second stage is characterized by bone reconstruction on X-ray film, with sparse bone and diffuse bone, but no change in the shape of femoral head or joint space. A plain or CT scan of the femoral head shows osteosclerosis, focal osteoporosis, or cystic changes. Stage III is characterized by continuous fracture of subchondral trabeculae with obvious cystic changes and sclerotic margin around it. The femoral head is flattened due to subchondral fracture, mainly in the load-bearing area. Stage IV is characterized by progressive enlargement of subchondral osteonecrosis, further compression and destruction of the femoral head and acetabulum, with narrowing of joint space and typical changes of osteoarthritis.[16] The enrolled patients had a history of drinking pure alcohol > 400 mL (320g/week, any alcoholic beverage) per week for six months or more. The inclusion criteria of the control group were as follows: (1) healthy, excluding asymptomatic ONFH (stage I) subjects; (2) age and BMI-matched Han Population in the control group; (3) no recent infection; (4) no other medical history; (5) no history of alcohol abuse.

SNP Selection and Genotyping

In 1000 genome project (http://www.internationalgenome.org/), we selected *RAB40C* candidate SNP sites with allele frequency (MAF) over 5%. Three SNPs (rs4984677,

rs62030917 and rs2269556) in *RAB40C* were finally identified in the case - control study. We isolated the genomic DNA from the whole blood sample using the Goldmag-mini Purification Kit (GoldMag Co. Ltd, Xi'an, China), and DNA concentration was measured using the NanoDrop 2000 (Thermo Scientific, Waltham, MA). Multiplexed SNP Mass EXTEND assay was designed by Agena MassARRAY Assay Design 4.0 software, and SNP genotyping was performed by Agena MassARRAY RS1000 (Agena, San Diego, CA, USA) according to the standard scheme.[17] We also used Agena Typer 4.0 software to analyze and manage our data.[18]

Data Analyses

We used Microsoft Excel (Microsoft, Redmond, WA) and SPSS Statistics (version 17.0, SPSS, Chicago, IL) to analyze the collected data. All the *p*-values in the study were two - tailed, and $p < 0.05$ was statistically significant. The chi-square test was used to evaluate the deviation of Hardy-Weinberg equilibrium (HWE). Pearson Chi - square test or Fisher's accurate test were also used to compare the allele frequency and genotype frequency of alcohol-induced ONFH patients with that of the control group. The association between polymorphisms in the *RAB40C* gene and the risk of alcohol-induced ONFH was calculated on the basis of logistic regression analysis. Four models (co-dominant, dominant, recessive, and log-additive) were established using PLINK version 1.07 software to assess the association between each sites and the alcohol-induced ONFH risk. The pairwise linkage disequilibrium (LD), haplotype construction, and genetic association of polymorphism loci were assessed using the Haploview software package (version 4.2).

Results

This study contained 201 alcohol-induced ONFH patients with an average age of 42.68 ± 12.875 years old and 201 healthy controls with an average age of 42.87 ± 13.270 years old. The independent sample *t* test showed that no significant difference was between the case group and the control group. In the case group, there were 54 cases in stage I and II, 147 cases in stage III and IV. Moreover, 44 cases with unilateral lesions and 157 cases with bilateral lesions. The information of BMI, alcohol and tobacco use in both cases and controls were shown in the Table 1. Supplementary Table S1 listed the plasma lipoprotein and lipid levels between patients and controls, and the results of the table indicated that there was a significant difference in PLT levels between the two groups.

Table 1 Comparison of Clinical Data in Case and Control Groups

Characteristic	Case	Control	*p*-value
Number	201	201	
Age(years, Mean ± SD)	42.68 ± 12.88	42.87 ± 13.27	0.888[a]
BMI (kg/m^2, Mean ± SD)	21.15 ± 2.70	21.33 ± 2.87	0.383[a]
Drinking			0.688[b]
Yes	199(99%)	199(99%)	
Deletion	2(1%)	2(1%)	
Smoking			0.527[b]
No	20(10%)	25(12.4%)	
Yes	179(90%)	176(87.6%)	
Hip lesions			
Unilateral	44 (22%)		
Bilateral	157 (78%)		
Clinical stages			
I+II	54 (27%)		
III+IV	147 (73%)		

Notes: [a]Independent samples *t* test; [b]Pearson Chi-squared test. $p < 0.05$ indicates statistical significance.
Abbreviation: SD, standard deviation.

Supplementary Table S2 showed the primers were used for this study. We have successfully genotyped three SNPs of *RAB40C* gene, and the genotype frequency distribution of all SNPs in the control groups did not deviate from the HWE ($p > 0.05$). Table 2 showed the basic information of chromosome position, role, MAF (Minor allele frequency) of cases and controls and HWE *p*-value of the three SNPs located in *RAB40C* gene. In these three SNPs, the minor allele G of rs62030917 was significantly associated with an increased alcohol-induced ONFH risk (OR = 1.47, 95% CI = 1.07–2.02, $p = 0.017$).

Then, we used four genetic models (co-dominant, dominant, recessive, and log-additive models) to analyze the relationship between three SNPs and alcohol-induced ONFH risk (Table 3). The result indicated that carriers with G/A-G/G genotype in rs62030917 were more likely to have alcohol-induced ONFH risk compared with AA homozygous carriers (adjusted OR = 1.52, 95% CI=1.02–2.26, $p = 0.039$) in the dominant model. In the log-additive model, rs62030917 also increased the risk of alcohol-induced ONFH (adjusted OR = 1.42, 95% CI=1.05–1.93, $p = 0.025$).

In addition, we also analyzed the association between these loci and the risk of alcoholic osteonecrosis by age stratification and hip lesions stratification (Table 4). Our

Table 2 Basic Information of the Three SNPs in This Study

SNP ID	Gene	Chr	Position	Role	Alleles A/B	MAF		HWE *p*-value	OR (95% CI)	*p*[a]
						Case	Control			
Rs4984677	*RAB40C*	16p13.3	621,682	Intronic	A/G	0.392	0.356	1.000	1.17 (0.88–1.56)	0.290
Rs62030917	*RAB40C*	16p13.3	622,040	Intronic	G/A	0.295	0.221	0.218	1.47 (1.07–2.02)	0.017*
Rs2269556	*RAB40C*	16p13.3	625,215	UTR3	G/A	0.413	0.381	0.767	1.14 (0.86–1.52)	0.356

Notes: [a]Pearson Chi-squared test; *p< 0.05 indicates statistical significance.
Abbreviations: SNP, single nucleotide polymorphism; Chr, chromosome; Alleles A/B, Minor/Major alleles; HWE, Hardy Weinberg equilibrium; MAF, minor allele frequency; OR, odds ratio; 95% CI, 95% confidence interval.

Table 3 Association Analysis Between SNPs and Alcohol-Induced ONFH Risk

SNP ID	Model	Genotype	Case	Control	Without Adjusted		Adjusted	
					OR (95% CI)	*p*	OR (95% CI)	*p*
rs4984677	Co-dominant	G/G	75 (37.69%)	83 (41.29%)	1		1	
		A/G	92 (46.23%)	93 (46.27%)	1.10 (0.72–1.67)	0.676	1.10 (0.72–1.67)	0.677
		A/A	32 (16.08%)	25 (12.44%)	1.42 (0.77–2.61)	0.263	1.42 (0.77–2.61)	0.263
	Dominant	G/G	75 (37.69%)	83 (41.29%)	1		1	
		A/G-A/A	124 (62.31%)	118 (58.71%)	1.16 (0.78–1.74)	0.461	1.16 (0.78–1.74)	0.461
	Recessive	G/G-A/G	167 (83.92%)	176 (87.56%)	1		1	
		A/A	32 (16.08%)	25 (12.44%)	1.35 (0.77–2.37)	0.299	1.35 (0.77–2.37)	0.299
	Log-additive	—	—	—	1.17 (0.88–1.55)	0.293	1.17 (0.88–1.55)	0.293
Rs62030917	Co-dominant	A/A	104 (52%)	125 (62.19%)	1		1	
		G/A	74 (37%)	63 (31.34%)	1.41 (0.92–2.16)	0.112	1.41 (0.92–2.16)	0.112
		G/G	22 (11%)	13 (6.47%)	2.03 (0.98–4.24)	0.058	2.04 (0.98–4.24)	0.058
	Dominant	A/A	104(52%)	125(62.19%)	1		1	
		G/A-G/G	96(48%)	76(37.81%)	1.52 (1.02–2.26)	0.039*	1.52 (1.02–2.26)	0.039*
	Recessive	A/A-G/A	178(89%)	188(93.53%)	1		1	
		G/G	22(11%)	13(6.47%)	1.79 (0.87–3.66)	0.112	1.79 (0.87–3.66)	0.112
	Log-additive	—	—	—	1.42 (1.05–1.93)	0.024*	1.42 (1.05–1.93)	0.025*
Rs2269556	Co-dominant	A/A	72(36%)	78(38.8%)	1		1	
		G/A	91(45.5%)	93(46.27%)	1.06 (0.69–1.63)	0.791	1.06 (0.69–1.63)	0.792
		G/G	37(18.4%)	30(14.93%)	1.34 (0.75–2.38)	0.326	1.34 (0.75–2.38)	0.327
	Dominant	A/A	72(36%)	78(38.81%)	1		1	
		G/A-G/G	128(64%)	123(61.19%)	1.13 (0.75–1.69)	0.562	1.13 (0.75–1.69)	0.563
	Recessive	A/A-G/A	163(81.5%)	171(85.07%)	1		1	
		G/G	37(18.5%)	30(14.93%)	1.29 (0.76–2.19)	0.338	1.29 (0.76–2.19)	0.339
	Log-additive	—	—	—	1.14 (0.86–1.50)	0.366	1.14 (0.86–1.50)	0.367

Notes: *p < 0.05 indicates statistical significance.
Abbreviations: SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval.

Table 4 Correlation Analysis of Rs62030917, Rs2269556 and Alcohol-Induced ONFH After Stratificated by Age and Hip Lesions in Different Genetic Models

SNP	Model	Genotype	≤42		>42		Unilateral		Bilateral	
			OR(95% CI)	*p*	OR(95% CI)	*p*	OR(95% CI)	*p*	OR(95% CI)	*p*
Rs62030917	Co-dominant	A/A	1		1		1		1	
		G/A	1.24 (0.67–2.28)	0.494	1.61 (0.88–2.91)	0.120	2.20 (1.08–4.45)	0.036*	1.25 (0.79–1.97)	0.343
		G/G	3.00 (1.08–8.30)	0.035*	1.21 (0.40–3.65)	0.742	3.20 (1.08–9.48)	0.029*	1.79 (0.82–3.92)	0.144
	Dominant	A/A	1		1		1		1	
		G/A-G/G	1.51 (0.86–2.66)	0.152	1.53 (0.87–2.68)	0.139	2.37 (1.22–4.61)	0.011*	1.34 (0.88–2.05)	0.178
	Recessive	A/A-G/A	1		1		1		1	
		G/G	2.77 (1.03–7.47)	0.044*	1.01 (0.34–3.00)	0.984	2.28 (0.82–6.38)	0.116	1.65 (0.77–3.55)	0.197
	Log-additive	—	1.53 (1.00–2.33)	0.048	1.31 (0.84–2.04)	0.239	1.90 (1.18–3.08)	0.009*	1.30 (0.94–1.80)	0.114
	Alleles	G/A	1.62 (1.04–2.54)	0.033*	1.33 (0.84–2.09)	0.224	2.01 (1.23–3.29)	0.005*	1.34 (0.95–1.88)	0.095
Rs2269556	Co-dominant	A/A	1		1		1		1	
		G/A	0.94 (0.50–1.73)	0.831	1.20 (0.65–2.20)	0.565	1.59 (0.72–3.51)	0.247	0.96 (0.61–1.52)	0.870
		G/G	1.19 (0.53–2.66)	0.671	1.51 (0.66–3.48)	0.333	2.83 (1.13–7.10)	0.027*	1.07 (0.57–2.00)	0.841
	Dominant	A/A	1		1		1		1	
		G/A-G/G	1.00 (0.56–1.78)	0.995	1.27 (0.71–2.25)	0.419	1.90 (0.90–3.97)	0.090	0.99 (0.64–1.52)	0.955
	Recessive	A/A-G/A	1		1		1.00		1	
		G/G	1.23 (0.59–2.57)	0.574	1.37 (0.64–2.92)	0.422	2.13 (0.99–4.60)	0.053	1.09 (0.61–1.94)	0.774
	Log-additive	—	1.06 (0.72–1.57)	0.762	1.22 (0.82–1.82)	0.325	1.68 (1.06 −2.67)	0.028*	1.02 (0.75–1.37)	0.913
	Alleles	G/A	1.07 (0.71–1.59)	0.758	1.22 (0.82–1.82)	0.320	1.70 (1.07–2.71)	0.023*	1.02 (0.75–1.38)	0.913

Notes: *$p < 0.05$ indicates statistical significance.

Abbreviations: SNP, single nucleotide polymorphism; OR, odds ratio, 95% CI, 95% confidence interval.

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results suggested that rs62030917 increased the risk of alcohol-induced ONFH among people ≤ 42 years old shown in the allele model (OR = 1.62, 95% CI = 1.04–2.54, p = 0.033), –the co-dominant model (OR = 2.99, 95% CI = 1.08–8.30, p = 0.035), the recessive model (OR = 2.77, 95% CI = 1.03–7.47, p = 0.044) and –the log-additive model (OR = 1.53, 95% CI = 1.00–2.33, p = 0.048). In patients with unilateral lesions vs controls, rs62030917 conveyed a increasing risk of alcohol-induced ONFH in the allele model (OR = 2.01, 95% CI = 1.23–3.29, p = 0.005), the co-dominant model (OR = 2.20, 95% CI = 1.08–4.45, p = 0.036; OR = 3.20, 95% CI = 1.08–9.48, p = 0.029), the dominant model (OR = 2.37, 95% CI = 1.22–4.61, p = 0.011) and the log-additive model (OR = 1.90, 95% CI = 1.18–3.08, p = 0.009). Likewise, rs2269556 also increased the risk of alcohol-induced ONFH (patients with unilateral lesions vs controls) in the allele model (OR = 1.70, 95% CI = 1.07–2.71, p = 0.023), the co-dominant model (OR = 2.83, 95% CI = 1.13–7.10, p = 0.027) and the log-additive model (OR = 1.68, 95% CI = 1.06–2.67, p = 0.028).

Linkage disequilibrium (LD) blocks composed of rs62030917 and rs2269556 were found in unilateral lesions (Figure 1) and older than 42 years old groups (Figure 2), respectively. However, there are no statistically significant correlation between the risk of alcohol-induced ONFH and haplotype. Comparative analysis of plasma

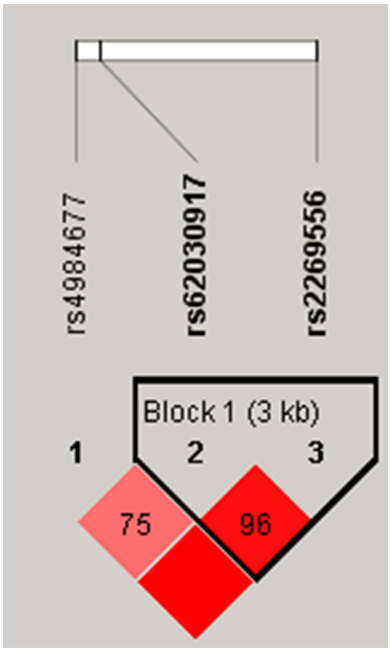

Figure 1 In patients with unilateral lesions, LD plots containing three SNPs from *RAB40C*.

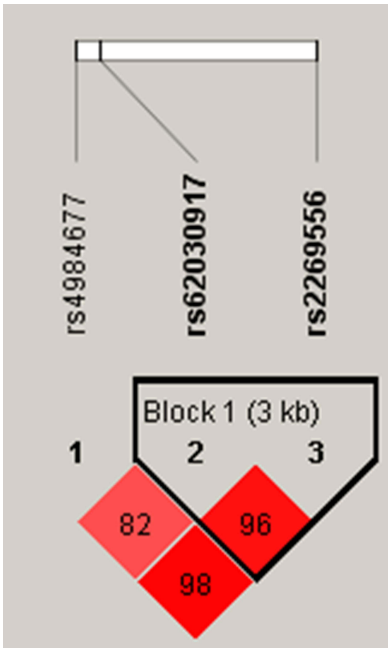

Figure 2 In patients older than 42 years old, LD plots containing three SNPs from *RAB40C*.

lipoprotein and lipid levels (TC, TG, HDL-C, LDL-C and PLT) in carrier with different genotypes of rs4984677, rs62030917 and rs2269556 was shown in Table 5. The results showed that carriers of AA, GA and GG genotypes in rs2269556 had LDL-C levels of 2.55 ± 0.71 mmol/L, 2.78 ± 0.83 mmol/L and 2.95 ± 1.08 mmol/L, respectively, which were significantly different (p = 0.047). Among them, carriers of GG genotype had the highest LDL-C levels.

Discussion

The pathogenesis of non-traumatic osteonecrosis is vascular injury, osteocyte death, or defective bone repair.[19,20] Long term excessive drinking can lead to dyslipidemia and then induce ONFH.[21,22] In recent years, people began to pay attention to the relationship between hereditary susceptibility and alcohol-induced ONFH.[23–25] Our study on the relationship *RAB40C* gene polymorphisms and alcohol-induced ONFH is the novel study.

The coding region of *RAB40C* contains 281 amino acids, which is longer than most small GTPases because it contains a unique SOCS box domain between the conserved GTPase domain and the pre-acylated C-terminal. Special SOCS box domain interacted with the elongated protein B/C and Cul5 modules, and binded with the ring finger proteins to form active ubiquitin ligases, which

Table 5 Comparative Analysis of Plasma Lipoprotein and Lipid Levels in Carrier with Different Genotypes of Rs4984677, Rs62030917 and Rs2269556

SNP		TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	PLT (10^9/L)
Rs4984677	AA(n=32)	4.70± 1.05	1.69± 0.85	1.03± 0.25	2.91± 1.06	228.53±71.16
	AG(n=92)	4.74± 0.91	1.88± 1.20	1.06± 0.24	2.77± 0.84	229.51±53.58
	GG(n=75)	4.50± 0.86	1.99± 1.53	1.02± 0.22	2.58± 0.71	230.13±60.63
	p	0.244	0.551	0.366	0.120	0.964
Rs62030917	AA(n=104)	4.59± 0.89	2.02± 1.42	1.03± 0.23	2.65± 0.84	230.94±62.13
	GA(n=74)	4.73± 0.92	1.73± 1.17	1.08± 0.25	2.81± 0.78	227.58±53.90
	GG(n=22)	4.63± 1.07	1.85± 0.88	0.99± 0.24	2.82± 1.13	232.41±75.66
	p	0.611	0.331	0.225	0.387	0.916
Rs2269556	AA(n=72)	4.52± 0.88	2.04± 1.55	1.02± 0.22	2.55± 0.71	228.54±66.49
	GA(n=91)	4.72± 0.89	1.81± 1.20	1.07± 0.24	2.78± 0.83	231.65±52.81
	GG(n=37)	4.76± 1.06	1.79± 0.85	1.03± 0.26	2.95± 1.08	227.43±67.48
	p	0.296	0.472	0.396	0.047*	0.918

Notes: *$p < 0.05$ indicates statistical significance.
Abbreviations: TC, Total cholesterol; TG, Triglycerides; LDL-C, Low-density lipoprotein-cholesterol; HDL-C, High-density lipoprotein-cholesterol; ApoA1, Apolipoprotein A1; ApoB, Apolipoprotein B; PLT, platelet count.

mediated a series of cellular processes.[26–28] Our previous work has proved that RAB40C is a novel LDs-related RAB protein.[11] This is because there is a unique SOCS box domain of RAB40C, which is necessary for LDs cluster.[14] As the proadipocytes differentiate into adipocytes, the expression of *RAB40C* increased. During the formation and maturation of LDs in adipocytes, RAB40C gradually accumulated to the surface of LDs. RAB40C knockout moderately reduced the size of LDs, suggesting that RAB40C is involved in the biological genetic process of LDs.[29]

Alcohol induces cell differentiation into adipocytes.[30] With the increase of alcohol exposure time and concentration, the number of adipocytes was increased.[31] In the alcohol treatment group, intracellular lipid deposition also occurred, which eventually leaded to the death of osteocytes.[31,33] These effective findings suggested that alcohol can directly induce adipogenesis, reduce bone marrow mesenchymal osteogenesis, and produce intracellular lipid deposition leading to osteocyte death, which may be related to the occurrence of alcohol-induced ONFH.[31] In view of the research, rs62030917 of *RAB40C* significantly increased the risk of alcohol-induced ONFH. In subjects with unilateral lesions, rs62030917 and rs2269556 increased the alcohol-induced ONFH risk. Rs62030917 increased the risk of alcohol-induced ONFH in people ≤ 42 years old.

To sum up, the expression of *RAB40C* gene increases the risk of alcohol-induced ONFH, which indicates that the gene polymorphisms of *RAB40C* may increase its risk of disease. There are limitations in the scope and quantity of sample selection in this study. In this study, patients and people from Northwest China were selected, which may be biased. In addition, the sample size of this study is small, and further verification of our results needs larger samples to support. Finally, some polymorphic loci with alcohol-induced ONFH have been screened out at the DNA level, and the relationship between polymorphisms and gene expression level has not yet been evaluated. Therefore, the relationship between polymorphisms and gene expression level needs to be evaluated at the RNA level and protein level. The study only provides a direction for alcohol-induced ONFH research.

Conclusion

Our study indicated that *RAB40C* gene polymorphism rs62030917 significantly increased the risk of alcohol-induced ONFH and it may be a risk locus. Our study

provides a direction for the research on the mechanism of alcoholic osteonecrosis, but it still needs intensive study.

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