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IGF-1 polymorphisms modulate the susceptibility to osteonecrosis of the femoral head among Chinese Han population

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Abstract

The study was performed to investigate the genetic associations of *IGF-1* polymorphisms rs35767, rs5742714, and rs972936 with susceptibility to osteonecrosis of the femoral head (ONFH) among Chinese Han population.

Totally, 101 ONFH patients and 128 healthy controls were enrolled. Hardy–Weinberg equilibrium (HWE) was detected with chi-square test in control group. Odds ratios (ORs) with 95% confidence intervals (95% CIs) were calculated to estimate the relationship between *IGF-1* polymorphisms and ONFH risk. Besides, hyplotype analysis was performed to examine linkage disequilibrium between the studied polymorphisms.

Genotype AA and allele A of polymorphism rs35767 were more frequent in control group, and offered protection for ONFH onset (AA: OR = 0.382, 95% CI = 0.158-0.923; A: OR = 0.650, 95% CI = 0.442–0.956). Furthermore, the negative relationship was also observed between ONFH risk and polymorphism rs5742714 under the comparisons CG vs CC, and G vs C (OR = 0.395, 95%CI = 0.199–0.787; OR = 0.346, 95%CI = 0.191–0.627). While the polymorphism rs972936 significantly enhanced the disease risk (CT vs CC: OR = 2.434, 95% CI = 1.184–5.003; TT vs CC: OR = 2.497, 95% CI = 1.040–5.990). Furthermore, haplotype analysis demonstrated that C-T (rs5742714–rs972936) could increase ONFH risk (OR = 2.177, 95% CI = 1.444–3.283), while G-T might be a protective factor for ONFH (OR = 0.472, 95% CI = 0.254–0.878).

IGF-1 polymorphisms rs35767, rs5742714, and rs972936 show significant association with ONFH risk.

Abbreviations: 95% CI = 95% confidence interval, CT = computed tomography, HWE = Hardy–Weinberg equilibrium, *IGF-1* = insulin-like growth factor-1, MRI = magnetic resonance imaging, ONFH = osteonecrosis of the femoral head, OR = odds ratio, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism, SNPs = single nucleotide polymorphisms.

Keywords: *IGF-1*, insulin-like growth factor, osteonecrosis of the femoral head, polymorphism, susceptibility

1. Introduction

Osteonecrosis of the femoral head (ONFH) is an intractable bone disease which can lead to the collapse of the femoral head and movement disorder of hip joint, severely reducing life quality of the patients.[1,2] This disease mainly affects middle-aged adults, if untreated, 75%–85% patients will present femoral head collapse.[3] ONFH cases could be divided into two types, traumatic and nontraumatic. The latter form is directly caused by deficiency of blood supply to the femoral head, but its exact etiology is still beyond totally illustrated.[4,5] Up to now, a variety of risk factors have been confirmed for initiation of nontrauma ONFH include the application of corticosteroid hormones, alcohol consumption, and other aspects (like decompression disease and sickle-cell anemia).[6–8] However, not all the individuals exposing to the risk factors will develop ONFH. It is generally considered that the initiation of ONFH is regulated by the interactions between certain risk factors and a series of genetic factors.[9] To explore disease-related genetic loci may be of great help for prevention and early diagnosis of ONFH in clinic.

Insulin-like growth factor-1 (*IGF-1*), a polypeptide containing 70 amino acid residues, exists almost all tissues in mammals, and can accelerate cell proliferation and differentiation, functioning as a mitosis promoter for many types of cell (including osteoblasts).[10]*IGF-1* pathway can regulate bone metabolism through diverse pathways. *IGF-1* can facilitate the synthesis and mineralization of bone matrix through mitosis-independent pathway, and affect bone metabolism through regulating the functions of bone cells.[11,12]*IGF-1* not only promotes the proliferation and conversion of bone marrow stromal cells to osteoblasts, but also elevates the synthesis of bone collagen.[13] In addition, *IGF-1* holds the capacity to suppress collagen degradation via inhibiting cell apoptosis, thus increasing the deposition of bone matrix.[14] Taken together, *IGF-1* plays critical roles in osteogenesis, bone restoration, and bone regeneration.[15] Animal experiments demonstrated that after treatment, the levels of *IGF-1* were significantly increased in ONFH rabbit model, revealing the close association of *IGF-1* expression with ONFH development.[16]*IGF-1*, the encoding

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gene for this factor, is located at chromosome 12q23.2, and some of functional polymorphisms in this gene have been reported to be associated with production of *IGF-1*.[17,18] Rs35767, rs5742714, and rs972936 are three commonly studied single nucleotide polymorphisms (SNPs) in *IGF-1* gene, and all of them had been reported to be involved in several human diseases. For example, Zhang et al. suggested that *IGF1* rs35767 polymorphism might contribute to the risk of diabetic retinopathy through regulating serum concentration of *IGF1* in Chinese Han population.[19] Cao et al. reported that the genetic variants of rs5742714 polymorphism showed obvious association with *IGF1* serum level, moreover, rs5742714 may be a genetic predictor of susceptibility and prognosis for renal cell carcinoma among Chinese Han population.[20] Rs972936 also holds the capacity to regulate human diseases via influencing circulating levels of *IGF-1*.[21] Based on these studies, we hypothesized that these three polymorphisms of *IGF-1* gene might be involved in etiology of ONFH. However, no study has been performed to explore their relationships with ONFH among Chinese population.

In current study, a case-control study was conducted to investigate the genetic effects of *IGF-1* polymorphisms rs35767, rs5742714, and rs972936 on ONFH risk in Chinese Han population. The linkage disequilibrium between *IGF-1* polymorphisms was also detected to estimate their relationship with the disease etiology.

2. Materials and methods

2.1. Study subjects and ethics committee statement

In the present study, 101 ONFH patients were selected from the Department of Orthopedics in Qingdao Municipal Hospital between July 2014 and February 2017 as case group. Diagnosis of ONFH was made based on clinical demonstrations, magnetic resonance imaging (MRI), X-ray, computed tomography (CT), and radionuclide bone scan. ONFH induced by direct trauma would be excluded. In addition, individuals would be excluded from the study if they with the histories of rheumatoid arthritis, ankylosing spondylitis, hip joint-involving diseases (like hip dysplasia), cardiovascular and/or cerebrovascular diseases, metabolic disorders, or bone tumor. Meanwhile, 128 healthy individuals were recruited from the physical examination center during the same period as control group. The case and control groups were matched in age and gender.

All of the study participants were Chinese Han people living in the north region, without blood relationship. The study protocol was approved by the Ethics Committee of Qingdao Municipal Hospital. Each participant provided the written informed consent before sample collection.

2.2. DNA extraction and genotyping for IGF-1 polymorphisms

After fasting for 10–12 hours, 2 ml peripheral blood was collected from each participant using the sterile tubes containing EDTA anticoagulant. Genomic DNA was isolated from the blood samples using DNA extraction kit (TIANGEN Biotech, Beijing, China) according to the manufacturer's instruction. Genotyping for *IGF-1* polymorphisms (rs35767, rs5742714, and rs972936) was implemented through polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Primers for these polymorphisms were designed using Primer Premier 5.0 software, and synthesized by Shanghai Biological Engineering Technology Service Co., Ltd.(Shanghai Shi, China) (Table 1). PCR reaction was conducted in 10 μl mixtures containing 15 ng DNA, 5 pmol of each primer, 0.5 μl dNTP, 1.0 μl 10 × PCR Buffer and 8.0 μl double distilled water. The amplification conditions were as follows: predegeneration at 95°C for 10 minutes, followed by 30 cycles of degeneration at 95°C for 30 seconds, annealing at specific temperatures (Table 1) for 30 seconds and extension at 72°C for 1 minutes, and a final extension at 72°C for 10 minutes. Amplification products were digested using the corresponding specific restriction enzymes (Table 1). Subsequently, digested products were separated in 2% agarose gel stained with ethidium bromide, and visualized under ultraviolet light.

2.3. Statistical analysis

Genotype distribution of *IGF-1* polymorphisms in control group was examined to determine whether it conformed to Hardy–Weinberg equilibrium (HWE). Chi-square test was employed to compare the differences in genotype and allele frequencies between ONFH patients and healthy controls. Odds ratios (ORs) with 95% confidence intervals (95% CIs) were calculated to estimate the association of *IGF-1* polymorphisms with susceptibility to ONFH. All data syntheses were accomplished using SPSS 18.0 software (SPSS Inc., Chicago, IL), and $P < .05$ represented significant level. In addition, linkage disequilibrium between *IGF-1* polymorphisms was also detected to estimate their relationship with the disease incidence.

3. Results

3.1. Baseline features of study subjects

In this study, there were 62 males and 39 females in case group and 66 males and 62 females in control group, and the gender distribution did not show obvious differences between the two groups ($P = .137$). The average age was 43.15 ± 7.55 years in cases, while the data in control group was 44.59 ± 12.73, without significant difference ($P = .317$).

Table 1

Primers designed for genotyping *IGF-1* polymorphisms.

Polymorphism	Primers		Annealing temperature (°C)	Enzyme
	Forward	Reverse		
rs35767	5′-CCAGGATAACACAAAGAGCCAGAGTAG-3′	5′-TAAAATAAGGTTGCAAAAGCCCAGAGC-3′	61	EcoRII
rs5742714	5′-TTAATTTCCCCTGCTACTTTGAAACCAG-3′	5′-GTCATTAGGATTGATATTCCTCTGCCAT-3′	61	HinfI
rs972936	5′-ATGGAAGTGTTTTCTGCACTGTGT-3′	5′-CTCTCTTGTGTCTGGCTGTGGCTC-3′	58	AluI

Table 2

Association between *IGF-1* polymorphisms and the susceptibility to osteonecrosis of the femoral head.

	Case (n = 101)		Control (n = 128)					
Genotype/allele	No.	%	No.	%	P_{HWE}	*P*	OR	95% CI
rs35767					.777			
GG	45	44.55	42	32.81		Ref		
GA	47	46.54	64	50.00		.189	0.685	0.390–1.205
AA	9	8.91	22	17.19		.029	0.382	0.158–0.923
G	137	67.82	148	57.81		Ref		
A	65	32.18	108	42.19		.028	0.650	0.442–0.956
rs5742714					.106			
CC	86	85.15	85	66.41		Ref		
CG	14	13.86	35	27.34		.007	0.395	0.199–0.787
GG	1[a]	1.00	8	6.25		.051	0.124	0.015–1.009
C	186	92.08	205	80.08		Ref		
G	16	7.92	51	19.92		<.001	0.346	0.191–0.627
rs972936					.127			
CC	13	12.87	34	26.56		Ref		
CT	67	66.34	72	56.25		.014	2.434	1.184–5.003
TT	21	20.79	22	17.19		.038	2.497	1.040–5.990
C	93	46.04	140	54.69		Ref		
T	109	53.96	116	45.31		.066	1.415	0.977–2.048

Notes: a: The number of genotype/allele was less than 5, and the adjusted *P* values were selected.
CI = confidence interval, OR = odds ratio, $P_{HWE} = P$ value for Hardy–Weinberg equilibrium.

3.2. HWE examinations

The genotype distributions of *IGF-1* polymorphisms in control group were revealed to be in line with HWE law ($P > .05$ for all) (Table 2), suggesting that the controls were representative for general population.

3.3. Associations of IGF-1 polymorphisms with the susceptibility to ONFH

The genotype and allele distributions of the three polymorphisms in *IGF-1* gene were shown in Table 2.

For rs35767 polymorphism, AA genotype, and A allele exerted higher frequencies in control group than that in case group (17.19% vs 8.91%; 42.19% vs 32.18%), which suggested their association with decreased disease risk (OR = 0.382, 95% CI = 0.158-0.923, $P = .029$; OR = 0.650, 95% CI = 0.442-0.956, $P = .028$).

Meanwhile, CG genotype of polymorphism rs5742714 showed low frequency in case group, compared with control group (13.96% vs 27.34%), suggesting its association with decreased risk of ONFH (OR = 0.395, 95%CI = 0.199–0.787, $P = .007$). The frequency of G allele was also significantly lower in case group than that in control group. Allele G was a protective factor for ONFH (OR = 0.346, 95%CI = 0.191–0.627, $P < .001$).

For polymorphism rs972936, CT, and TT exhibited obviously higher frequencies in case group (66.34% vs 56.25%; 20.79% vs 17.19%), and these differences were statistically significant, demonstrating their promoting effect on ONFH susceptibility (CT: OR = 2.434, 95% CI = 1.184–5.003, $P = .014$; TT: OR = 2.497, 95% CI = 1.040–5.990, $P = .038$). The distributions of polymorphism rs972936 alleles did not show significant differences between case and control group ($P = .066$).

3.4. Haplotype analysis results

Strong linkage disequilibrium was detected between the polymorphisms rs5742714 and rs972936, and three haplotypes were constructed for them, namely C-C, C-T, and G-T (Table 3). Using C-C as reference, we analyzed the relationships of the other two haplotypes with ONFH risk, and found that C-T could significantly increase the disease risk (OR = 2.177, 95% CI = 1.444–3.283, $P < .001$), while G-T might be a protective factor for ONFH initiation (OR = 0.472, 95% CI = 0.254–0.878, $P = .016$).

4. Discussion

ONFH is a pathological progress induced by the abnormalities of blood supply to the femoral head.[2] *IGF-1* can affect bone tissues from several pathways, mainly involving osteogenesis and bone metabolism.[22] Dysregulation of *IGF-1* may lead to osteoblast

Table 3

Haplotype analysis for *IGF-1* polymorphisms rs5742714 and rs972936.

Locus1-locus2	Case (2n = 202)	Control (2n = 256)	*P*	OR (95% CI)
C-C	93 (46.04)	140 (54.69)	Ref	
C-T	94 (46.53)	65 (25.39)	<.001	2.177 (1.444–3.283)
G-T	16 (7.92)	51 (19.92)	.016	0.472 (0.254–0.878)

Notes: Locus1, rs5742714; locus2, rs972936.
CI = confidence interval, OR = odds ratio, Ref = reference.

aging and metabolic bone diseases.[23] In this study, we investigated the genetic association of *IGF-1* gene polymorphisms with ONFH risk in Chinese Han population. Analysis results demonstrated that *IGF-1* polymorphisms rs35767 and rs5742714 might offer protection for the risk of ONFH, while polymorphism rs972936 could increase the susceptibility to the disease.

IGF-1 is generally considered as a gene implicating in etiology of ONFH. In *IGF-1*-knockout mice, osteoblasts is defective, and its cell numbers is also decreased.[24] In a rabbit model of ONFH, the expression profile of *IGF-1* shows close association with reparative process of the disease.[25] According to existing documents, there are some functional polymorphisms in *IGF-1* gene which could influence the transcriptional activity and protein production, like rs35767, rs5742714, and rs972936.[19–21] Moreover, a series of studies have demonstrated that these three polymorphisms were involved in various human diseases. For instance, Marini et al. reported that the individuals carrying rs35767 T allele showed low risk of anemia compared with C allele.[26] A related study carried out among Japanese population demonstrated that rs5742714 polymorphism in combination with overweight could affect development of pancreatic cancer.[27] Among Chinese pregnant women, those carrying GA/AA genotypes were more likely to have spontaneous preterm infants.[28] However, the effects of these three polymorphisms on individual susceptibility to ONFH had been rarely reported in Chinese Han population.

In current study, we selected polymorphisms rs35767, rs5742714, and rs972936 as the targets to explore the genetic association of *IGF-1* gene with ONFH risk among Chinese people. As for genotype and allele frequencies, significant differences were observed in all the three studied polymorphisms between ONFH and control groups. Specifically, genotype AA and allele A of polymorphism rs35767 were more frequent in healthy controls than that in ONFH patients, showing protective effects for the disease; meanwhile, rs5742714 also exhibited a negative relationship with the disease occurrence under the contrasts of CG vs CC and G vs C; while rs972936 polymorphism was significantly related to increased risk of ONFH. These results were in line with the relevant studies carried out in other types of human disease.[26–28] In addition, strong linkage disequilibrium was observed between polymorphisms rs5742714 and rs972936. Haplotype analysis demonstrated that the haplotype C-T dramatically elevated the susceptibility to ONFH when compared to C-C, while G-T decreased the individual susceptibility to ONFH among Chinese Han population.

Despite of the encouraging results, there were still some limitations in the present study which need to be addressed here. Firstly, the sample size in the current study was relatively small that might influence the accuracy of the final results. Secondly, the interactions between our studied polymorphisms and other relevant aspects on the disease susceptibility were not discussed in this study. Additionally, the molecular mechanisms underlying the functional roles of *IGF-1* polymorphisms on risk of ONFH remained poorly known. Further well-designed studies with a large sample size will be required to address the above issues.

ONFH is a multifactorial disease representing a complex interplay of genetic anomalies and environmental factors. Knowledge of genetic variations thought to be involved in the disease may be useful in determining individuals considered at higher risk to develop ONFH with the ultimate goal of preventing the multiple hit effect. Polymorphism rs972936 remain one of the strongest association with hereditary ONFH, therefore, screening an individual at risk for ONFH for the presence of genetic risk factors may be beneficial in evaluating treatment options. As our society is moving towards a more preventive and personalized medicine, genetic studies will likely become of greater value and ONFH is a superb example of the potential applicability of translational research from basic science to patient care.

In conclusion, *IGF-1* polymorphisms rs35767 and rs5742417 may offer protective effects for ONFH susceptibility while polymorphism rs972936 may enhance the disease risk among Chinese Han population.

Author contributions

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