Variants in RETN gene are associated with Steroid-induced osteonecrosis of the femoral head risk among Han Chinese People

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Research article

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Abstract

Introduction Gene polymorhisms has an important influence on RETN gene expression level, and the increased level of resistin encoded in RETN will lead to metabolic disorder, especially lipid metabolism. Moreover, steroid-induced osteonecrosis of the femoral head (steroid-induced ONFH) is closely related to lipid metabolism level, so this study aims to explore the association of RETN Polymorphisms with susceptibility to steroid-induced ONFH in the Chinese Han Population.

Methods In this case-control study, eight single nucleotide polymorphisms (SNPs) of RETN were genotyped by Agena MassARRAY system in 199 steroid-induced ONFH patients and 200 healthy controls. The association between RETN polymorphisms and steroid-induced ONFH risk was evaluated using genetic models and haplotype analyses. Odds ratio (OR) and 95% confidence intervals (CIs) were calculated by logistic regression adjusted for age.

Results We found significant differences in the distribution of HDL-C, TG/HDL-C, and LDL-C/HDL-C between the patients and the control group ($p < 0.05$). In allele model and genotype model analysis, rs34861192, rs3219175, rs3745368 and rs1477341 could reduce the risk of steroid-induced ONFH. Further stratified analysis showed that rs3745367 was related to the clinical stage of patients, and rs1477341 was significantly correlated with an increased TG level and a decreased TC/ HDL-C level. The Linkage analysis showed that three SNPs (rs34861192, rs3219175) in RETN even significant linkage disequilibrium.

Conclusions Our results provide the firstly evidence that RETN gene polymorphisms were associated with a reduced risk of steroid-induced ONFH in Chinese Han Population.

Introduction

With the wide application of glucocorticoid in the treatment of rheumatic diseases, autoimmune diseases, hematopoietic system diseases and other diseases, steroid-induced osteonecrosis of the femoral head (steroid-induced ONFH) has become the most common non-traumatic osteonecrosis of the femoral head (ONFH) type in clinical practice [1, 2]. Epidemiological studies in East Asia have shown that 47.4% of all cases diagnosed as non-traumatic ONFH were directly related to steroids [3]. As a degenerative bone disease, it can cause the femoral head to collapse, which subsequently damage the hip joint and seriously reduces the patients quality of life and is difficult to reverse [3]. However, it is challenging to fully elucidate the pathogenesis of steroid-induced ONFH due to the various effects of steroids on multiple systems involved in osteoblast differentiation, osteoblast and osteoclast apoptosis, lipid metabolism, calcium metabolism and coagulation [4].

Some studies have shown that only a subset of patients develop ONFH within a few weeks of hormone therapy, suggesting that genetic factors may determine susceptibility to steroid-induced ONFH) [5]. Single-nucleotide polymorphisms (SNPs) are the most frequent variation that occurs in a single nucleotide at a specific position in the genome. Numerous SNPs have been identified through sequencing, and many of them in critical genes such as *MMP-8* [1], *MMP-9* [6], *MMP−14* [7],*ABCB1* [8], and *VEGFA* [9] were demonstrated to be associated with steroid-induced ONFH susceptibility.

RETN, also known as *ADSF* and *FIZZ3,* is located on chromosome 19p13. *RETN* gene encodes resistin, which is a hormone secreted by fat cells and belongs to the cystein-rich C-terminal domain proteins called resistin-like

molecules [10]. It was first observed in adipocytes, then in monocytes, macrophages, and the spleen, and more importantly, in human sebaceous glands and cultured sebaceous glands [11]. Some studies have demonstrated that elevated levels of resistin lead to metabolic disorders, which in turn are associated with diabetes, non-insulin dependent, acquired systemic lipodystrophy, as well as rheumatoid arthritis and the like [12–14]. Most importantly, it is the product of the secretion of fat cells and its related pathways include lipogenesis [15]. Elevated levels of resistin may also contribute to lipid metabolism disorders [14]. However, lipid metabolic disorders can lead to steroid-induced ONFH, so we believe that *RETN* can also induce steroid-induced osteonecrosis by affecting lipid metabolism.

Considering the importance of resistin expression in lipid metabolism and the influence of its promoter and intron regional polymorphism on *RETN* expression, we hypothesized that the polymorphisms site of *RETN* might influence steroid-induced osteonecrosis. Moreover, to our knowledge, there are no previous studies have investigated the association of risk of steroid-induced ONFH and *RETN* polymorphisms. Therefore, we conduct a case–control study to evaluate the possible relationship of *RETN* gene polymorphisms at allele, genotype, and haplotype interface with development of steroid-induced ONFH among Chinese Han Population.

Materials And Methods

Study participants

The present hospital-based case control study recruited 199 patients diagnosed with steroid-induced ONFH and 200 unrelated control subjects at the orthopedic hospital of Inner Mongolia medical university (Inner Mongolia, China) from 2014 to 2019. These individuals were informed of the purpose of the study and signed informed consent. Steroid-induced ONFH was defined as long-term steroid intake of more than 16 mg per day or high-dose impulsive treatment with steroids lasting more than 1 week. X-ray examination and additional magnetic resonance imaging (MRI) and bone scan analyses were performed on each patient as necessary. We also used strict exclusion criteria to exclude the following patients: Patients with traumatic ONFH and other hip diseases; Patients who consumed more than 400ml of alcohol per week; Patients with a history of severe disease or severe chronic disease, such as renal insufficiency, diabetes, cardiovascular and cerebrovascular diseases, etc.

We collected epidemiological information from standardized questionnaires, and collected clinical information from medical records and imaging examinations to finally determine the control individuals. The included controls met the following criteria: they had no hip pain, and anteroposterior and frog-leg lateral pelvic radiographs did not show any lesions, no history of thromboembolic events, and no symptoms of hip disease, and not chronic diseases such as renal insufficiency, diabetes and cardiovascular and cerebrovascular diseases.

SNPs selection and genotyping

Individual demographics information and the clinical characteristics of patients were collected by well-trained interviewers. Subsequently, 5 mL of peripheral blood from each participant was collected by a specialized technician and stored into tubes containing ethylenediaminetetraacetic acid (EDTA). After centrifugation, the specimens were stored at −80 °C until further analysis. All volunteers signed an informed consent form explaining the research purpose of the blood withdrawal. Genomic DNA was isolated from peripheral whole

blood employing the Gold Mag-Mini Whole Blood Genomic DNA purification Kit (Gold Mag Co. Ltd., Xi'an, China) following the manufacturer's instructions and quantified by Nano Drop spectrophotometer 2000 C (Thermo Scientific, Waltham, Massachusetts, USA).

In this study, eight SNPs (rs7408174, rs34861192, rs34124816, rs3219175, rs3745367, rs3745368, rs3745369, rs1477341) were selected for genotyping the basis of their allele frequencies, location, and disease relevance through 1000 Genome Projects. These SNPs had minor allele frequencies > 5% in the 1000 Genome Projects (http://www.internationalgenome.org/). Polymerase chain reaction (PCR) extension primers were designed for these SNPS by MassARRAY Assay Design 3.0 software (Agena). SNP genotyping analysis was carried out at Agena MassARRAY RS1000 instrument (Shanghai, China) system according to the standard scheme recommended by the manufacturer, and data were managed and analyzed by Agena Typer 4.0 software [16]. In addition, about 10% of the total samples were randomly selected to repeat genotyping and the reproducibility was 100%.

Statistical analyses

All of the statistical analyses were performed with the SPSS statistical package, version 19.0 (SPSS Inc., Chicago, IL, USA). Hardy-Weinberg equilibrium (HWE) of each SNP in control group was tested by Fisher s exact test. Allele frequencies and genotype frequencies for each SNP of case and control subjects were evaluated using the Chi squared test. Odds ratios (ORs) and 95% confidence intervals (CIs) were determined using logistic regression analysis with adjustments for age and gender. The wild-type allele was used as a reference. Multiple genetic model analyses (codominant, dominant, recessive and log-additive) were applied using PLINK software (http://pngu.mgh.harvard.edu/purcell/plink/) to assess the association between SNPs and LDH. Finally, the Haploview software package (version 4.2) and the SHEsis software (http://analysis.bio-x.cn/myAnalysis.php) were used to analyze the pairwise linkage disequilibrium (LD), haplotype construction and genetic association of polymorphism loci. All *p* values in this study were two-sided, and *p*-value of less than 0.05 as the cutoff value for statistical significance.

Results

Results The basic characteristics of study subjects

The clinical information and the demographic characteristics of both the cases and controls are shown in Table 1. No significant difference was observed between the cases and controls in regards to age, gender, TC, TG, LDL-C, and TG/HDL-C. However, there were significant differences in the distribution of HDL-C, TG/HDL-C, and LDL-C/HDL-C between the two groups ($p < 0.05$).

The associations between RETN SNPs and steroid-induced ONFH

The allelic frequencies and basic information of the selected SNPs loci were shown in Table 2. Allele frequencies of all locus in controls were in accord with Hardy-Weinberg equilibrium (p 0.05). Using a chi-square test to assess the risk of genetic polymorphism in an allelic model and found rs34861192, rs3219175, rs3745368, rs1477341 were associated with a reduced risk of steroid-induced ONFH (rs34861192, OR = 1.64, 95% CI = 0.44 - 0.93, $p = 0.019$; rs3219175, OR = 0.61, 95% CI = 0.42 - 0.89, $p = 0.010$; rs3745368, OR = 0.62, 95% CI = 0.41 - 0.93, $p = 0.020$; rs1477341, OR = 0.74, 95% CI = 0.56 - 0.98, $p = 0.036$), respectively.

Associations between genotype frequencies and steroid-induced ONFH risk

As shown in Table 3, we further analyzed the association between genotype frequency and steroid-induced ONFH risk by four genetic models. We found that rs34861192 was associated with a lower steroid-induced ONFH risk in codominant, recessive, and log-additive models (AA vs GG: OR = 0.10, 95% CI = 0.01- 0.79, p = 0.0329; AA vs GG-AG: OR = 0.11, 95% CI = 0.01- 0.85, p = 0.035; A vs G: OR = 0.63, 95% CI = 0.43 - 0.93, p = 0.019). Rs3219175 was identified to a decrease the steroid-induced ONFH risk in codominant, dominant, recessive, and log-additive models (AA vs GG: OR = 0.10, 95% CI = 0.01 - 0.79, p = 0.029; AG-AA vs GG: OR = 0.63, 95% CI = 0.41 - 0.98, p = 0.038; AA vs GG-AG: OR = 0.11, 95% CI = 0.01 - 0.86, p = 0.036; A vs G: OR = 0.60, 95% CI = 0.41 - 0.88, p = 0.010). Rs3745368 was also related to decreasing steroid-induced ONFH risk in dominant and log-additive models (AG-AA vs GG: OR = 0.61, 95% CI = 0.38 - 0.95, p = 0.030; A vs G: OR = 0.61, 95% CI = 0.41 - 0.92, p = 0.019). In addition, the rs1477341polymorphism also showed an association with the steroid-induced ONFH risk in dominant and log - additive models (AT - AA vs TT: OR = 0.62, 95% CI = 0.40 - 0.96, p = 0.030; A vs T: OR = 0.73, 95% CI = 0.55 - 0.98, p = 0.036).

Relationship between RETN SNPs and clinical features of steroid-induced ONFH

We also explored the relationship between the *RETN* SNPs and steroid-induced ONFH clinical features, including gender, Hip lesions, clinical stages, and the expression level of lipid. We observed that rs3745367 shows association with the clinical stages in Table 4 (p = 0.049). As for the lipid metabolism level of patients, it was found that compared with AA genotype carriers, HDL-C level of TT genotype carriers in rs1477341 was significantly increased, while TC level was lower. Meanwhile, the TC/HDL-C ratio of TT genotype carriers was significantly lower than that of AA genotype carriers.

Associations between haplotype analyses and steroid-induced ONFH risk

The Linkage analysis showed that three SNPs (rs34861192, rs3219175) in *RETN* exhibited significant linkage disequilibrium (Figure 1).

GTEx database analysis

Through GTEx database analysis, rs34861192 and rs3219175 (p = 4.00E - 14, p = 1.60E^{-14}) sites were observed to be associated with decreased expression of *RETN* gene in Whole-Blood.

Discussion

It is well known that genetic studies have provided insights into many diseases, including steroid-induced ONFH. This study was designed to investigate the contribution of genetic variation in *RETN* gene to steroid-induced ONFH risk in a Chinese Han Population. Allele, genotype, and haplotype frequencies of eight SNPs in the *RETN* gene between steroid-induced ONFH patients and healthy controls were compared and stratification analyses were conducted. Our study found that the rs34861192, rs3219175, rs3745368, rs1477341 were associated with a reduced risk of steroid-induced ONFH. Stratified analysis indicated that rs3745367 was related to the clinical stages of steroid-induced ONFH. We also found that rs1477341 site mutation reduced TC level and increased HDL-C level in patients with steroid-induced ONFH, suggesting that this site may affect the risk of steroid-induced ONFH by affecting the expression level of lipoprotein in vivo. To our knowledge, this is the first research

to elucidate on the association of *RETN* genetic variants with the risk of steroid-induced ONFH in Chinese Han Population.

The *RTEN* gene encodes a resistin, which is also considered to be a biomarker or mediator of metabolic and inflammatory diseases [17, 18]. Some studies have shown that elevated levels of serum resistin lead to metabolic disorders, including obesity, insulin resistance, type 2 diabetes, hypertension, dyslipidemia and atherosclerotic cardiovascular disease [19–21]. Moreover, it has been shown that the plasma resistin concentration was determined to a large extent by polymorphisms of *RETN.* For example, one study showed that plasma resistin was significantly correlated with rs34861192, rs34124816, rs3219175, rs3745367, and rs3745369 [22]. The study of H. found that rs34861192 and rs3745368 polymorphisms of *RETN* as robust and independent determinants of plasma resistin concentration [23]. An Epigenome-wide association study suggests that SNPs rs34861192 and rs3219175 in the *RETN* promoter region may influence circulating resistin levels by affecting DNAm at cg02346997 and the abundance of *RETN* mRNA in monocytes [3]. In addition, the minor (A) allele of rs34861192 was also found to be associated with a lower plasma resistin level (R2 = 0.010) [23]. It also has been confirmed that GG genotype carriers of *RETN* rs3219175 and rs3481192 SNPs exhibited higher levels of log resistin than A allele carriers [24]. After multiple testing corrections, the locus rs1477341 also had a strong correlation with resistin level, and all minor alleles were correlated with higher level [25]. In our study, we found that the frequency of the minor (A) allele of rs34861192, rs3219175 and rs1477341 were 0.136, 0.132 and 0.405, respectively. All three sites were associated with a lower risk of steroid-induced ONFH. Therefore, we speculated that these three sites may reduce the occurrence of metabolic disorder by inducing a lower level of resistin, ultimately reducing the risk of steroid-induced ONFH. Our study also found that rs3745368 SNPs may reduce the risk of steroid-induced ONFH. Because the single nucleotide polymorphism (SNPs) in the 3-untranslated region (3 UTR) of the gene could affect gene expression and disease susceptibility [26], we speculate that rs3745368 SNP in the 3 UTR of the *RETN* gene might affect the expression of the RETN gene, thereby affecting the risk of steroid-induced ONFH development.

Moreover, the plasma resistin levels have been shown to be associated with serum concentrations of HDL-cholesterol and triacylglycerol, IRI and BMI [23]. In our study, we found that rs1477341 was significantly correlated with elevated HDL-C levels, and the HDL-C level of TT carriers was significantly higher than that of AA carriers. Besides, our study also found that rs1477341 can reduce the risk of steroid-induced ONFH. These results further suggested the correlation between this site and resistin levels, HDL/C levels, metabolic disorders, and osteonecrosis. As for linkage disequilibrium analysis, it was also found that rs34861192 and rs3219175 were all located in the same LD segment, and all two SNPs were strongly correlated with circulating resistin levels [27]. Similarly, our study also found that rs34861192 and rs3219175 were located in the same LD block.

Inevitably, our study has some limitations could not be ignored. Firstly, the inherent selecting bias and information bias were the unavoidable problems, because this is a hospital-based, single - center study. Secondly, Secondly, the number of cases in our study is not large enough to rule out false-negative results, so a larger sample size is needed for further confirmation. Third, our current study is a foundational case-control study that requires further functional studies to understand the underlying genetic mechanism of steroid-induced ONFH. Despite the limitations mentioned above, our current results provide scientific evidence for *RETN* gene and steroid-induced ONFH in future studies.

Conclusions

To sum up, the present study confirmed for the first time that the *RETN* poly- morphisms rs34861192, rs3219175, rs3745368 and rs1477341 were associated with a reduced risk of steroid-induced ONFH, which may provide new data to facilitate earlier diagnosis and promote early prevention, and shed light on the pathogenesis of osteonecrosis for the further study.

Declarations

Ethics approval and consent to participate

The use of human blood sample and the protocol in this study were strictly comply with the criterion of the Declaration of Helsinki and were approved by the Ethics Committee of the orthopedic hospital of Inner Mongolia medical university (Inner Mongolia, China). Each participant signed the informed consent form after fully understanding the purpose of our study.

Consent for publication

Written informed consent was obtained from the patient for publication of this report.

Availability of data and materials

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

FA and JZW: conceived and designed the experiments; TLZ: write articles the article; HYG and JQW: performed the experiments; CL and YT: analyzed the data; CM, JZ and KZW: contributed reagents/materials/analysis tools. All authors contributed significantly to the final draft of the paper and agreed to submit the manuscript for publication.

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References

1.Du J, Jin T, Cao Y, Chen J, Guo Y, Sun M, Li J, Zhang X, Wang G, Wang J: *Association between genetic polymorphisms of MMP8 and the risk of steroid-induced osteonecrosis of the femoral head in the population of northern China. Medicine* 2016, *95(*37):e4794.

2.Wang A, Ren M, Wang J: *The pathogenesis of steroid-induced osteonecrosis of the femoral head: A systematic review of the literature. Gene* 2018.

3.Nakatochi M, Ichihara S, Yamamoto K, Ohnaka K, Kato Y, Yokota S, Hirashiki A, Naruse K, Asano H, Izawa H *et al: Epigenome-wide association study suggests that SNPs in the promoter region of RETN influence plasma resistin level via effects on DNA methylation at neighbouring sites. Diabetologia* 2015, *58(*12):2781–2790.

4.Aimaiti A, Wufuer M, Wang YH, Saiyiti M, Cui L, Yusufu A: *Can bisphenol A diglycidyl ether (BADGE) administration prevent steroid-induced femoral head osteonecrosis in the early stage? Medical hypotheses* 2011, *77(*2):282–285.

5.Zhang Y, Yin J, Ding H, Zhang C, Gao YS: *Vitamin K2 Ameliorates Damage of Blood Vessels by Glucocorticoid: a Potential Mechanism for Its Protective Effects in Glucocorticoid-induced Osteonecrosis of the Femoral Head in a Rat Model. International journal of biological sciences* 2016, *12(7*):776–785.

6.Du J, Liu W, Jin T, Zhao Z, Bai R, Xue H, Chen J, Sun M, Zhang X, Wang G *et al: A single-nucleotide polymorphism in MMP9 is associated with decreased risk of steroid-induced osteonecrosis of the femoral head. Oncotarget* 2016, *7(*42):68434–68441.

7.Qi Y, Wang J, Sun M, Ma C, Jin T, Liu Y, Cao Y, Wang J: *MMP–14 single-nucleotide polymorphisms are related to steroid-induced osteonecrosis of the femoral head in the population of northern China. Molecular genetics & genomic medicine* 2018.

8.Zhang Y, Kong X, Wang R, Li S, Niu Y, Zhu L, Chen W, Lin N: *Genetic association of the P-glycoprotein gene ABCB1 polymorphisms with the risk for steroid-induced osteonecrosis of the femoral head in Chinese population. Molecular biology reports* 2014, *41(*5):3135–3146.

9.Ma W, Xin K, Chen K, Tang H, Chen H, Zhi L, Liu H: *Relationship of common variants in VEGFA gene with osteonecrosis of the femoral head: A Han Chinese population based association study. Scientific reports* 2018, *8(*1):16221.

10.Kim HJ, Lee YS, Won EH, Chang IH, Kim TH, Park ES, Kim MK, Kim W, Myung SC: *Expression of resistin in the prostate and its stimulatory effect on prostate cancer cell proliferation. BJU international* 2011, *108(*2 Pt 2):E77–83.

11.Younis S, Blumenberg M, Javed Q: *Resistin gene polymorphisms are associated with acne and serum lipid levels, providing a potential nexus between lipid metabolism and inflammation. Archives of dermatological research* 2016, *308(*4):229–237.

12.Hornik B, Dulawa J, Szewieczek J, Durmala J: *Physical activity increases the resistin concentration in hemodialyzed patients without metabolic syndrome. Diabetes, metabolic syndrome and obesity: targets and therapy* 2019, *12:*43–57.

13.Johnson C, Drummer Ct, Virtue A, Gao T, Wu S, Hernandez M, Singh L, Wang H, Yang XF: *Increased Expression of Resistin in MicroRNA–155-Deficient White Adipose Tissues May Be a Possible Driver of Metabolically Healthy Obesity Transition to Classical Obesity. Frontiers in physiology* 2018, *9:*1297.

14.Ohmori R, Momiyama Y, Kato R, Taniguchi H, Ogura M, Ayaori M, Nakamura H, Ohsuzu F: *Associations between serum resistin levels and insulin resistance, inflammation, and coronary artery disease. Journal of the American College of Cardiology* 2005, *46(*2):379–380.

15.Ikeda Y, Tsuchiya H, Hama S, Kajimoto K, Kogure K: *Resistin affects lipid metabolism during adipocyte maturation of 3T3-L1 cells. The FEBS journal* 2013, *280(*22):5884–5895.

16.Gabriel S, Ziaugra L, Tabbaa D: *SNP genotyping using the Sequenom MassARRAY iPLEX platform. Current protocols in human genetics* 2009, *Chapter 2:*Unit 2.12.

17.Lehrke M, Reilly MP, Millington SC, Iqbal N, Rader DJ, Lazar MA: *An inflammatory cascade leading to hyperresistinemia in humans. PLoS medicine* 2004, *1(*2):e45.

18.Reilly MP, Lehrke M, Wolfe ML, Rohatgi A, Lazar MA, Rader DJ: *Resistin is an inflammatory marker of atherosclerosis in humans. Circulation* 2005, *111(7*):932–939.

19.McTernan CL, McTernan PG, Harte AL, Levick PL, Barnett AH, Kumar S: *Resistin, central obesity, and type 2 diabetes. Lancet (London, England)* 2002, *359(*9300):46–47.

20.Degawa-Yamauchi M, Bovenkerk JE, Juliar BE, Watson W, Kerr K, Jones R, Zhu Q, Considine RV: *Serum resistin (FIZZ3) protein is increased in obese humans. The Journal of clinical endocrinology and metabolism* 2003, *88(*11):5452–5455.

21.Verma S, Li SH, Wang CH, Fedak PW, Li RK, Weisel RD, Mickle DA: *Resistin promotes endothelial cell activation: further evidence of adipokine-endothelial interaction. Circulation* 2003, *108(*6):736–740.

22.Onuma H, Tabara Y, Kawamura R, Tanaka T, Ohashi J, Nishida W, Takata Y, Ochi M, Yamada K, Kawamoto R *et al: A at single nucleotide polymorphism–358 is required for G at –420 to confer the highest plasma resistin in the general Japanese population. PloS one* 2010, *5(*3):e9718.

23.Asano H, Izawa H, Nagata K, Nakatochi M, Kobayashi M, Hirashiki A, Shintani S, Nishizawa T, Tanimura D, Naruse K *et al: Plasma resistin concentration determined by common variants in the resistin gene and associated with metabolic traits in an aged Japanese population. Diabetologia* 2010, *53(*2):234–246.

24.Apalasamy YD, Rampal S, Salim A, Moy FM, Su TT, Majid HA, Bulgiba A, Mohamed Z: *Polymorphisms of the resistin gene and their association with obesity and resistin levels in Malaysian Malays. Biochemical genetics* 2015, *53(*4–6):120–131.

25.Hivert MF, Manning AK, McAteer JB, Dupuis J, Fox CS, Cupples LA, Meigs JB, Florez JC: *Association of variants in RETN with plasma resistin levels and diabetes-related traits in the Framingham Offspring Study. Diabetes* 2009, *58(*3):750–756.

26.Fu Y, Yu Y, Wu Y, You Y, Zhang Y, Kou C: *Association between Two Resistin Gene Polymorphisms and Metabolic Syndrome in Jilin, Northeast China: A Case-Control Study. Disease markers* 2017, *2017:*1638769.

27.Osawa H, Tabara Y, Ohashi J, Kawamura R, Onuma H, Makino H: *Is rs34861192 or rs1862513 a more promising variant for determining plasma resistin in an aged Japanese population? Diabetologia* 2010, *53(*4):795–797.

Tables

Table 1: Characteristics of the participants.

Variables	Cases (n=199)	Controls (n=200)	*p* value
Sex N			0.966[b]
Male	115	116	
Female	84	84	
Age, years (Mean ± SD)	41.15 ± 12.934	41.20 ± 8.662	0.961[a]
Clinical stages			
Stage II	47		
Stage III	94		
Stage IV	58		
Hip lesions			
unilateral	59		
bilateral	140		
TC (mmol/L)	4.50±0.923	4.54±0.814	0.703
TG (mmol/L)	1.84±1.455	1.76±1.076	0.569
HDL-C (mmol/L)	1.07±0.269	1.15±0.214	0.000[a]
LDL-C (mmol/L)	2.64±0.765	2.56±0.728	0.285
TC/HDL-C	4.41±1.178	4.04±0.856	0.000[a]
TG/HDL-C	1.94±1.930	1.64±1.149	0.059
LDL-C/HDL-C	2.58±0.821	2.30±0.767	0.001[a]

TC: Total cholesterol, TG: Triglycerides, LDL-C: Low-density lipoprotein-cholesterol, LDL-C: High-density lipoprotein-cholesterol.

p^{a} value was calculated by Independent samples t test.

p^{b} value was calculated by Chi-squared test.

$^{*}p < 0.05$ indicates statistical significance.

Table 2: Basic information of candidate SNPs of *RETN* gene in this study.

SNP ID	Gene	Band	Alleles A/B	MAF		HWE-p^a	OR (95%CI)	p^b-value
				Case	Control			
rs7408174	*RETN*	19	C/T	0.265	0.233	0.556	1.19(0.86-1.64)	0.287
rs34861192	*RETN*	19	A/G	0.136	0.198	0.654	0.64(0.44-0.93)	0.019*
rs34124816	*RETN*	19	A/C	0.075	0.100	1.000	0.73(0.45-1.20)	0.219
rs3219175	*RETN*	19	A/G	0.132	0.200	0.659	0.61(0.42-0.89)	0.010*
rs3745367	*RETN*	19	A/G	0.324	0.390	0.882	0.75(0.56-1.00)	0.052
rs3745368	*RETN*	19	A/G	0.113	0.171	1.000	0.62(0.41-0.93)	0.020*
rs3745369	*RETN*	19	C/G	0.303	0.337	0.636	0.85(0.63-1.15)	0.305
rs1477341	*RETN*	19	A/T	0.405	0.480	0.479	0.74(0.56-0.98)	0.036*

SNP: single nucleotide polymorphism, HWE: Hardy-Weinberg equilibrium, OR: odds ratio, 95% CI: 95% confidence interval, MAF: minor allele frequency.

* $p < 0.05$ indicates statistical significance.

p^a and p^b were calculated by Chi-squared test.

Table 3: Analysis of the association between SNPs of RETN gene and steroid-induced ONFH risk.

SNP ID	Model	Genotype	Control	Case	OR (95% CI)	*p*-value
rs34861192	Codominant	GG	130	146	1	0.029*
		AG	61	52	0.76(0.49-1.18)	
		AA	9	1	0.10(0.01-0.79)	
	Dominant	GG	130	146	1	0.071
		AG-AA	70	53	0.67(0.44-1.03)	
	Recessive	GG-AG	191	198	1	0.035*
		AA	9	1	0.11(0.01-0.85)	
	Log-additive	---	---	---	0.63(0.43-0.93)	0.019*
rs3219175	Codominant	GG	129	146	1	0.029*
		AG	62	50	0.71(0.46-1.11)	
		AA	9	1	0.10(0.01-0.79)	
	Dominant	GG	129	146	1	0.038*
		AG-AA	71	51	0.63(0.41-0.98)	
	Recessive	GG-AG	191	196	1	0.036*
		AA	9	1	0.11(0.01-0.86)	
	Log-additive	---	---	---	0.60(0.41-0.88)	0.010*
rs3745368	Codominant	GG	137	156	1	0.131
		AG	56	41	0.64(0.40-1.02)	
		AA	6	2	0.29(0.06-1.45)	
	Dominant	GG	137	156	1	0.030*
		AG-AA	62	43	0.61(0.38-0.95)	
	Recessive	GG-AG	193	197	1	0.17
		AA	6	2	0.32(0.06-1.63)	
	Log-additive	---	---	---	0.61(0.41-0.92)	0.019*
rs1477341	Codominant	TT	51	68	1	0.051
		AT	105	90	0.64(0.41-1.02)	
		AA	43	32	0.56(0.31-1.00)	
	Dominant	TT	51	68	1	0.030*
		AT-AA	148	122	0.62(0.40-0.96)	
	Recessive	TT-AT	156	158	1	0.239
		AA	43	32	0.74(0.44-1.23)	
	Log-additive	---	---	---	0.73(0.55-0.98)	0.036*

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

p value adjusted for age was calculated by logistic regression.

* $p < 0.05$ indicates statistical significance.

Table 4: The association of genotypes in *RETN* genes with the clinical phenotypes.

SNP	genotype	Gender		p	Hip lesions		p	Clinical stages			p
		Male	Female		Unilateral	Bilateral		Stage II	Stage III	Stage IV	
rs7408174	CC	8	6	0.775	5	9	0.285	3	5	6	0.694
	CT	42	35		18	59		19	39	19	
	TT	64	43		36	71		24	50	33	
rs34861192	AA	0	1	0.465	0	1	0.547	0	1	0	0.193
	AG	29	23		18	34		15	28	9	
	GG	86	60		41	105		32	65	49	
rs34124816	AA	94	77	0.105	51	120	0.650	41	78	52	0.557
	CA	20	6		8	18		6	14	6	
	CC	1	1		0	2		0	2	0	
rs3219175	AA	0	1	0.470	0	1	0.592	0	1	0	0.216
	AG	28	22		17	33		15	26	9	
	GG	86	60		41	105		31	66	49	
rs3745367	AA	12	9	0.775	3	18	0.263	3	14	4	0.049*
	AG	48	39		27	60		24	44	19	
	GG	55	36		29	62		20	36	35	
rs3745368	AA	1	1	0.308	0	2	0.650	0	2	0	0.613
	AG	28	13		12	29		9	21	11	
	GG	86	70		47	109		38	71	47	
rs3745369	CC	8	6	0.876	4	10	0.059	2	11	1	0.161
	CG	50	40		20	70		20	41	29	
	GG	54	37		35	56		24	41	26	
rs1477341	AA	24	8	0.063	9	23	0.809	6	21	5	0.090
	AT	48	42		25	65		21	43	26	
	TT	35	33		22	46		17	25	26	

p value was calculated by logistic regression.

* $p < 0.05$ indicates statistical significance.

Table 5: The association of genotypes in *RETN* genes with the clinical phenotypes.

SNP	Genotype	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	TC/HDL-C	TG/HDL-C	LDL-C/HDL-C
rs34861192	AA(n=1)	4.84	2.91	1.08	2.61	4.48	2.69	2.42
	AG(n=52)	4.71±0.93	1.97±1.32	1.05 ± 0.27	2.75±0.77	4.72±2.48	2.13±1.79	2.75 ±0.86
	GG(n=146)	4.43±0.91	1.78±1.50	1.07±0.27	2.60±0.76	4.30±1.15	1.87±1.98	2.52 ±0.81
	P	0.156	0.551	0.073	0.587	0.090	0.653	0.670
rs3219175	AA (n=1)	4.84	2.91	1.08	2.61	4.48	2.69	2.42
	AG (n=50)	4.75±0.93	2.01±1.33	1.05±0.28	2.77±0.78	4.76±1.23	2.17±1.82	2.77±0.87
	GG (n=146)	4.42±0.91	1.78±1.50	1.07±0.27	2.59±0.76	4.29±1.15	1.87±1.98	2.51±0.80
	P	0.097	0.492	0.835	0.356	0.058	0.587	0.152
rs3745368	AA (n=2)	4.11±0.64	1.13±0.01	1.14±0.23	2.20±0.80	4.38±1.67	1.18±0.26	2.40±1.37
	AG (n=41)	4.37±0.95	1.76±1.61	1.04±0.22	2.56±0.81	4.35±1.23	1.92±2.24	2.55±0.88
	GG (n=156)	4.54±0.91	1.87±1.42	1.07±0.28	2.66±0.76	4.43±1.17	1.96±1.86	2.59±0.80
	P	0.453	0.722	0.718	0.531	0.927	0.853	0.912
rs1477341	AA(n=32)	2.00±1.40	4.41±0.82	1.01±0.22	2.50±0.71	4.52±1.15	2.17±1.69	2.56±0.84
	AT(n=90)	1.96±1.47	4.57±0.94	1.04±0.25	2.71±0.78	4.58±1.16	2.10±1.98	2.70±0.79
	TT(n=68)	1.62±1.53	4.45±0.97	1.13±0.30	2.58±0.78	4.12±1.19	1.65±2.06	2.39±0.84
	P	0.302	0.588	0.049*	0.315	0.049*	0.292	0.066

p value was calculated by logistic regression.

* $p < 0.05$ indicates statistical significance.

Figures

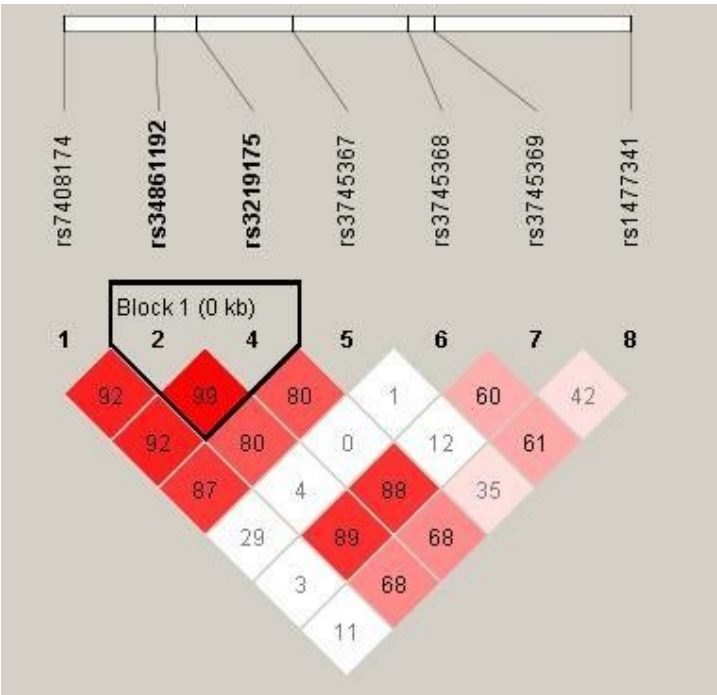

Figure 1

Haplotype block map for the eight SNPs in the RETN gene. Block 1 includes rs34861192 and rs3219175 with D'= 1 (100%) for the corresponding variants.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementtableS1.docx