

Genetic associations of the visfatin G-948T polymorphism with obesity-related metabolic traits in an Iranian population

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Background: Obesity is a global public health problem. Visfatin, as an adipocytokine, is coded by a gene known as nicotinamide phosphoribosyltransferase. So far, results were conflicted regarding correlations of visfatin with obesity and metabolic variables. The present study aimed to explore the association between G-948T polymorphism of visfatin gene with obesity and lipid profile in a nationally representative sample of Iranian population. **Materials and Methods:** In this case-control study, we assessed 129 randomly selected patients with obesity and 182 healthy normal weight controls from participants of Isfahan Healthy Heart Program. Genomic DNA was isolated from peripheral blood cells, and high-resolution melt polymerase chain reaction was performed to explore the presence of G-948T polymorphism. **Results:** T carriers "GT + TT" were statistically more frequent in the obese patients than the controls ($P = 0.013$; odds ratio = 1.9, 95% confidence interval = 1.1–3.1). The serum levels of total cholesterol and low-density lipoprotein cholesterol (LDL-C) were significantly different between T carriers and GG homozygote genotype ($P = 0.03$ and 0.02, respectively). **Conclusion:** We concluded that visfatin G-948T polymorphism was correlated with obesity, total cholesterol, and LDL-C levels in our population.

Key words: G-948T, Iran, lipoprotein, obesity, polymorphism, visfatin

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INTRODUCTION

Visfatin, as an adipocytokine, is encoded by a gene, officially now known as nicotinamide phosphoribosyltransferase (NAMPT), but formerly known as pre-B-cell colony-enhancing factor (PBEF1) gene.^[1,2] Visfatin is a 52 kDa large protein and its gene is located on chromosome 7q22.2.2. It consists of 11 exons and 10 introns.^[3-5]

It is found that visfatin is expressed in adipocytes and other tissues such as skeletal muscle, liver, brain, bone marrow, and lymphocytes.^[5,6]

Visfatin is an adipokine, which is produced largely in visceral fat and has shown some insulin-mimetic properties.^[7-9] However, its insulin-mimetic action has been questioned,^[10] and nowadays, it seems that NAMPT participates in obesity and insulin resistance potentially as an inflammatory protein.^[8]

Visfatin is expressed by the macrophages infiltrating adipose tissue,^[11] tempted to hypothesize that visfatin might be involved in some obesity complications such as metabolic syndrome and/or Type 2 diabetes mellitus (T2DM).

Subsequent studies showed that elevated plasma levels of visfatin in obesity, T2DM, and metabolic syndrome

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are associated with inflammation-related atherogenic inflammatory diseases.^[12]

Several studies have shown increased level of visfatin gene expression and its plasma level in obesity.^[2,3,5,7,13] However, the relation between visfatin gene expression and obesity and T2DM has been inconsistent.^[3] Clinical studies have shown that plasma visfatin level elevated T2DM patient in comparison with healthy people.^[4,14,15] Visfatin has insulin-like effect as prevents the release of glucose from the liver, increases glucose absorption in adipocytes and monocytes, and enhances triglycerides synthesis,^[15] and it has been suggested that visfatin has a role in the pathophysiology of insulin resistance in obese people and patients with T2DM.^[16]

About 52 single nucleotide polymorphisms (SNPs) in the visfatin gene have been identified.^[11] It is possible that this genetic variation in PBEF1 contributes to these effects.^[5,11] We aimed to investigate the G-948T polymorphism in visfatin gene promoter and its correlation with obesity in a nationally representative sample of Iranian adolescent's population.

MATERIALS AND METHODS

Subjects

This project was conducted as a substudy of the Isfahan Healthy Heart Program (IHHP). It is a comprehensive action-oriented integrated community-based intervention program for noncommunicable diseases prevention and control. It was implemented in Central Iran. Details of data collection and sampling are published previously.^[17] For the current study, we included 129 obese (body mass index [BMI] >25 kg/m²) cases from the IHHP study population and 182 normal-weight (with BMI <25 kg/m²) participants as control. All patients gave their written consent to participate in the study. The study was approved by the Ethics Committee of the Isfahan University of Medical Sciences.

Physical examination and biochemical measurements

A team of trained health-care professionals and physicians recorded information and conducted the physical examination under standard protocols and using calibrated instruments. BMI was calculated as the weight (kg) divided by the height squared (m²).

The biochemical analysis was performed in the Isfahan Cardiovascular Research Institute laboratory, a collaborating center of the World Health Organization in Isfahan, which met the standards of the National Reference Laboratory too.

Genotyping of pre-B-cell colony-enhancing factor 1 single nucleotide polymorphisms

After leukocyte separation, DNA was extracted using conventional techniques. The genomic DNA was separated from blood using genomic DNA isolation kit (GeNetBio, Korea) according to the manufacturer's protocol. Primers were designed by Beacon Designer 7.5 (PREMIER Biosoft International, USA) and synthesized by TIB MOLBIOL (Germany). Oligonucleotide sequences were as follows: Forward 5'-GGCAGACATTGATTATCCC-3' and reverse is 5'-GAGGAGTAAGGCTACTTAAAGCG-3'. Genotyping was done by high-resolution melt (HRM). Our tests were done by Rotor-Gene 6000 instrument (Corbett Life Science, Australia). Polymerase chain reactions (PCRs) were carried out in duplicate in 20 µL of final volume using the Type-it HRM kit (Qiagen, Germany), HRM PCR buffer, HotStarTaq Plus DNA Polymerase, nucleotides and EvaGreen dye, and 30 ng DNA. The PCR program consisted of an initial denaturation activation step at 95°C for 5 min, followed by a 40-cycle program (denaturation at 95°C for 15 s, annealing conditions 55°C for 5 s, 72°C for 15 s, and HRM step from 70°C to 95°C rising at 0.1°C/s). The HRM curves were checked and selected samples. Normalized and temperature-shifted melting curves from HRM, suggestive of SNP, were distinguished, and the samples were subjected to direct sequencing.

Statistical analysis

All statistical analyses were performed using SPSS software (SPSS Inc. version 16, Chicago, IL, USA). We evaluated the differences of demographic variables between case and controls by independent sample *t*-test. The relative associations between G-948T genotypes and BMI risk were assessed using multiple logistic regression method to calculate odds ratios and 95% of confidence intervals controlling for age and gender. A χ^2 test was performed for the study polymorphisms to determine if the sample demonstrated Hardy-Weinberg equilibrium. Furthermore, an independent sample *t*-test was used for comparing variable means between genotypes. A two-tailed *P* < 0.05 was considered statistically significant.

RESULTS

In this study, the relationship between G-948T genetic polymorphism and level of lipid profile and other factors related to obesity was performed. The G-948T SNP was genotyped in 129 cases and 182 controls. 51.2% of cases and 35.2% of controls are female. 48.8% of cases and 64.8% of controls are male. Demographic characteristics of cases are reported in Table 1.

Other biochemical and physical characteristics including weight, waist circumference, fasting blood

sugar, total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), systolic blood pressure, and DBS of cases and control groups are shown in Table 1.

As demonstrated in Table 2, the frequency of GT and TT genotypes was higher in cases compared to controls.

Moreover, in cases, the ratio of GT + TT genotype to GG genotype was considerably higher than controls.

G-948T genotype distributions were in agreement with the Hardy-Weinberg equilibrium ($\chi^2 = 0.44$, $df = 1$, $P = 0.5$).

Demographic and biochemical characteristics of cases carry G-948T polymorphism as shown in Table 3.

The present data have shown that the level of BMI was higher in people with GT and TT genotype than GG genotype; hence, it demonstrates that carrying the T allele (GT + TT) is associated with higher level of BMI and also higher level of LDL and cholesterol [Table 3].

DISCUSSION

Obesity is a growing public health problem worldwide. Abdominal obesity, especially, is strongly associated with dyslipidemia and increased risk for T2DM, metabolic syndrome, and coronary artery disease. The role of adipose tissue in the complications such as obesity-linked insulin resistance, metabolic syndrome, and diabetes has been allied to fat-derived adipokines. Visfatin was identified in 2005 as an adipocytokine exerts insulin-like actions on glucose metabolism and is increased in morbid obesity.

Obesity is a complex multifactorial disorder that occurs due to interactions between genetic and nongenetic factors.

The results of previous studies showed conflicting results in case of the relation between visfatin and metabolic syndrome.^[13,18,19]

This study has investigated whether G-948T gene polymorphism has an association with obesity and comorbidities such as dyslipoproteinemia in Iranian population. Our findings suggested that variations in this polymorphism were correlated with obesity, total cholesterol, and LDL-C levels in carriers of T allele.

Ooi *et al.* showed that visfatin and its genetic variants were associated with adiposity, obesity-related morbidities, and adverse cardiometabolic parameters in severely obese children.^[20]

Table 1: Demographic and biochemical characteristics of cases

Variables	Case (BMI >25), n=129	Control (BMI <25), n=182	P
Age (years)	38±11	30±9	0.04
Sex (%)			
Female	66 (51.2)	64 (35.2)	0.005
Male	63 (48.8)	118 (64.8)	
Weight (kg)	88±12	65±7	<0.000
Waist circumference (cm)	104±11	81±6	<0.000
FBS (mg/dl)	89±10	85±9	0.017
Total cholesterol (mg/dl)	202±37	173±26	<0.000
Triglyceride (mg/dl)	183±11	88±29	<0.000
HDL-C (mg/dl)	43±9	53±8	<0.000
LDL-C (mg/dl)	122±30	102±24	<0.000
SBP (mmHg)	118±15	107±12	<0.000
DBP (mmHg)	77±9	71±8	<0.000

Values are expressed as mean±SD. P values are two-tailed. BMI=Body mass index; FBS=Fasting blood sugar; HDL-C=High-density lipoprotein cholesterol; LDL-C=Low-density lipoprotein cholesterol; SBP=Systolic blood pressure; DBP=Diastolic blood pressure; SD=Standard deviation

Table 2: Allele and genotype frequencies

Polymorphism	Control (BMI <25), n=182 (%)	Case (BMI >25), n=129 (%)	OR (95% CI)	P
G-948T				
G	151 (83.0)	95 (73.6)		
T	31 (17.0)	34 (26.4)	1.7 (0.9-3.1)	0.067
GG	129 (70.9)	71 (55.0)		
GT	47 (25.8)	48 (37.2)	1.7 (1-3.01)	0.05
TT	6 (3.3)	10 (7.8)	3.3 (1.0-10.1)	0.034
GG	129 (70.9)	71 (55.0)		
GT + TT	53 (29.1)	58 (45.0)	1.9 (1.1-3.1)	0.013

Results adjusted by age and sex. BMI=Body mass index; OR=Odds ratio; CI=Confidence interval

Table 3: Demographic and biochemical characteristics of participants

Variables	G-948T		P
	GG	GT + TT	
Triglyceride (mg/dl)	124±96	134±71	0.3
Cholesterol (mg/dl)	181±23	190±36	0.03
HDL (mg/dl)	49±9	48±10	0.4
LDL (mg/dl)	108±27	115±30	0.02
FBS (mg/dl)	86±10	88±9	0.4
Waist circumference (cm)	90±14	92±13	0.09
SBP (mmHg)	110±15	113±14	0.18
DBP (mmHg)	74±9	74±9	0.5
BMI (kg/m ²)	26±5	27±5	0.01

BMI=Body mass index; FBS=Fasting blood sugar; HDL=High-density lipoprotein; LDL=Low-density lipoprotein; SBP=Systolic blood pressure; DBP=Diastolic blood pressure

Böttcher *et al.* have shown that the ratio of visceral/sc visfatin messenger RNA (mRNA) expression was associated with G-498T polymorphisms.^[21]

Plasma levels of visfatin are elevated in obese patients, patients with metabolic syndrome, T2DM, nonalcoholic fatty liver disease, and coronary artery disease. Visfatin/NAMPT levels also correlated with obesity in children, and this marker was associated with BMI and waist circumference.^[22-24]

Motawi *et al.* reported higher frequencies of G-948T among T2DM patients compared with controls.^[25]

However, Körner *et al.* reported no association of -948G polymorphism or their haplotypes with BMI, waist-to-hip ratio, or parameters of glucose, insulin, or lipid metabolism and showed that the G-948T variant was just associated with significantly higher diastolic BP in obese children.^[26]

Chang *et al.* showed that the VAT visfatin mRNA expression was positively correlated with fasting triglyceride and total cholesterol levels.^[27]

Johansson *et al.* found that obese carriers of the G-948T variant allele had significantly higher levels of HDL-C.^[3]

Interestingly, it has been shown that pericardial fat thickness, as well as visceral obesity, was associated with increased visfatin levels in morbid obese patients.^[28] The plasma levels of visfatin are closely related by body fat mass.^[29]

However, two other studies showed no association between the G-948T SNP with BMI, waist circumference, serum glucose levels, or lipid metabolism.^[5,26]

CONCLUSION

The recent report has shown an ascending trend in the prevalence of obesity in Iran.^[30] To the best of our knowledge, this is the first study investigating the association of the G-948T SNP with obesity and dyslipidemia in Iranian patients. Our study demonstrates a clear association with obesity, total cholesterol, and LDL-C levels, in apparent contrast to some of the previous studies. The reasons for these discrepancies are unclear but may have been caused by the following factors: (i) ethnic heterogeneity may affect visfatin level or visfatin sensitivity; (ii) criteria for recruitment were different in the various studies, thus differences in confounding factors such as age and lifestyle may affect visfatin levels and other traits of interest and obscure the results.

A larger sample size would increase the power of our study and would allow the investigation of the possible involvement of visfatin variants and obesity and dyslipidemia. Further studies are needed to clarify the molecular mechanism of visfatin on obesity development and lipid metabolism.

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Conflicts of interest

There are no conflicts of interest.

AUTHORS' CONTRIBUTIONS

SHJ and RD had substantial contributions to conception and design of the study, analysis of the data, and drafting of the paper. LR, HN, AR, and NS had contributions to data collection and analysis. They also contributed to drafting of the paper.

REFERENCES

1. Bobbert P, Kühl U, Poller W, Rauch U, Schultheiss HP, Skurk C. Nicotinamide phosphoribosyltransferase/pre-B-cell colony enhancing factor/visfatin plasma levels and clinical outcome in patients with dilated cardiomyopathy. *J Card Fail* 2015;21:330-8.
2. Kocelak P, Olszanecka-Glinianowicz M, Owczarek A, Bozentowicz-Wikarek M, Brzozowska A, Mossakowska M, *et al.* Plasma visfatin/nicotinamide phosphoribosyltransferase levels in hypertensive elderly – Results from the PolSenior substudy. *J Am Soc Hypertens* 2015;9:1-8.
3. Johansson LM, Johansson LE, Ridderstråle M. The visfatin (PBEF1) G-948T gene polymorphism is associated with increased high-density lipoprotein cholesterol in obese subjects. *Metabolism* 2008;57:1558-62.
4. Tokunaga A, Miura A, Okauchi Y, Segawa K, Fukuhara A, Okita K, *et al.* The -1535 promoter variant of the visfatin gene is associated with serum triglyceride and HDL-cholesterol levels in Japanese subjects. *Endocr J* 2008;55:205-12.
5. Zhang YY, Gottardo L, Thompson R, Powers C, Nolan D, Duffy J, *et al.* A visfatin promoter polymorphism is associated with low-grade inflammation and type 2 diabetes. *Obesity (Silver Spring)* 2006;14:2119-26.
6. Sethi JK, Vidal-Puig A. Visfatin: The missing link between intra-abdominal obesity and diabetes? *Trends Mol Med* 2005;11:344-7.
7. Dogru T, Sonmez A, Tasci I, Bozoglu E, Yilmaz MI, Genc H, *et al.* Plasma visfatin levels in patients with newly diagnosed and untreated type 2 diabetes mellitus and impaired glucose tolerance. *Diabetes Res Clin Pract* 2007;76:24-9.
8. Kadoglou NP, Gkontopoulos A, Kapelouzou A, Fotiadis G, Theofilogiannakos EK, Kottas G, *et al.* Serum levels of vaspin and visfatin in patients with coronary artery disease-Kozani study. *Clin Chim Acta* 2011;412:48-52.
9. Shaker O, El-Shehaby A, Zakaria A, Mostafa N, Talaat S, Katsiki N, *et al.* Plasma visfatin and retinol binding protein-4 levels in patients with type 2 diabetes mellitus and their relationship to adiposity and fatty liver. *Clin Biochem* 2011;44:1457-63.
10. Stephens JM, Vidal-Puig AJ. An update on visfatin/pre-B cell colony-enhancing factor, an ubiquitously expressed, illusive cytokine that is regulated in obesity. *Curr Opin Lipidol*

- 2006;17:128-31.
11. Curat CA, Wegner V, Sengenès C, Miranville A, Tonus C, Busse R, *et al.* Macrophages in human visceral adipose tissue: Increased accumulation in obesity and a source of resistin and visfatin. *Diabetologia* 2006;49:744-7.
 12. Romacho T, Sánchez-Ferrer CF, Peiró C. Visfatin/Nampt: An adipokine with cardiovascular impact. *Mediators Inflamm* 2013;2013:946427.
 13. Fukuhara A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K, *et al.* Visfatin: A protein secreted by visceral fat that mimics the effects of insulin. *Science* 2005;307:426-30.
 14. Saboori S, Hosseinzadeh-Attar MJ, Yousefi Rad E, Hosseini M, Mirzaei K, Ahmadvand Z. The comparison of serum vaspin and visfatin concentrations in obese and normal weight women. *Diabetes Metab Syndr* 2015;9:320-3.
 15. Esteghamati A, Alamdari A, Zandieh A, Elahi S, Khalilzadeh O, Nakhjavani M, *et al.* Serum visfatin is associated with type 2 diabetes mellitus independent of insulin resistance and obesity. *Diabetes Res Clin Pract* 2011;91:154-8.
 16. Rezvan N, Hosseinzadeh-Attar MJ, Masoudkabar F, Moini A, Janani L, Mazaherioun M. Serum visfatin concentrations in gestational diabetes mellitus and normal pregnancy. *Arch Gynecol Obstet* 2012;285:1257-62.
 17. Rabiei K, Kelishadi R, Sarrafzadegan N, Abedi HA, Alavi M, Heidari K, *et al.* Process evaluation of a community-based program for prevention and control of non-communicable disease in a developing country: The Isfahan Healthy Heart Program, Iran. *BMC Public Health* 2009;9:57.
 18. Berndt J, Klötting N, Kralisch S, Kovacs P, Fasshauer M, Schön MR, *et al.* Plasma visfatin concentrations and fat depot-specific mRNA expression in humans. *Diabetes* 2005;54:2911-6.
 19. Chen MP, Chung FM, Chang DM, Tsai JC, Huang HF, Shin SJ, *et al.* Elevated plasma level of visfatin/pre-B cell colony-enhancing factor in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2006;91:295-9.
 20. Ooi SQ, Chan RM, Poh LK, Loke KY, Heng CK, Chan YH, *et al.* Visfatin and its genetic variants are associated with obesity-related morbidities and cardiometabolic risk in severely obese children. *Pediatr Obes* 2014;9:81-91.
 21. Böttcher Y, Teupser D, Enigk B, Berndt J, Klötting N, Schön MR, *et al.* Genetic variation in the visfatin gene (PBEF1) and its relation to glucose metabolism and fat-depot-specific messenger ribonucleic acid expression in humans. *J Clin Endocrinol Metab* 2006;91:2725-31.
 22. Moschen AR, Gerner RR, Tilg H. Pre-B cell colony enhancing factor/NAMPT/visfatin in inflammation and obesity-related disorders. *Curr Pharm Des* 2010;16:1913-20.
 23. Stastny J, Bienertova-Vasku J, Tomandl J, Tomandlova M, Zlamal F, Forejt M, *et al.* Association of genetic variability in selected regions in visfatin (NAMPT) gene with anthropometric parameters and dietary composition in obese and non-obese Central-European population. *Diabetes Metab Syndr* 2013;7(3):166-71. [doi: 10.1016/j.dsx.2013.06.001].
 24. Belo VA, Luizon MR, Lacchini R, Miranda JA, Lanna CM, Souza-Costa DC, *et al.* The effects of NAMPT haplotypes and metabolic risk factors on circulating visfatin/NAMPT levels in childhood obesity. *Int J Obes (Lond)* 2015;39:130-5.
 25. Motawi TM, Shaker OG, El-Sawalhi MM, Abdel-Nasser ZM. Visfatin -948G/T and resistin -420C/G polymorphisms in Egyptian type 2 diabetic patients with and without cardiovascular diseases. *Genome* 2014;57:259-66.
 26. Körner A, Böttcher Y, Enigk B, Kiess W, Stumvoll M, Kovacs P. Effects of genetic variation in the visfatin gene (PBEF1) on obesity, glucose metabolism, and blood pressure in children. *Metabolism* 2007;56:772-7.
 27. Chang YC, Chang TJ, Lee WJ, Chuang LM. The relationship of visfatin/pre-B-cell colony-enhancing factor/nicotinamide phosphoribosyltransferase in adipose tissue with inflammation, insulin resistance, and plasma lipids. *Metabolism* 2010;59:93-9.
 28. Malavazos AE, Ermetici F, Cereda E, Coman C, Locati M, Morricone L, *et al.* Epicardial fat thickness: Relationship with plasma visfatin and plasminogen activator inhibitor-1 levels in visceral obesity. *Nutr Metab Cardiovasc Dis* 2008;18:523-30.
 29. Olszanecka-Glinianowicz M, Madej P, Zdun D, Bozentowicz-Wikarek M, Sikora J, Chudek J, *et al.* Are plasma levels of visfatin and retinol-binding protein 4 (RBP4) associated with body mass, metabolic and hormonal disturbances in women with polycystic ovary syndrome? *Eur J Obstet Gynecol Reprod Biol* 2012;162:55-61.
 30. Rahmani A, Sayehmiri K, Asadollahi K, Sarokhani D, Islami F, Sarokhani M. Investigation of the prevalence of obesity in Iran: A systematic review and meta-analysis study. *Acta Med Iran* 2015;53:596-607.