IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
December 2017 Vol.:11, Issue:1

Resistin Gene Polymorphism (420c/G) As a Predictor for Ischemic Heart Diseases

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Asraa Ali Kadhum*[1], Majid Kadhum Hussain[2], Riyadh. Dh. Al-zu[3]

[1]*College of medicine, University of Karbala, Karbala, Iraq.*

[2] *Department of Biochemistry, College of Medicine, University of Kufa, Najaf,*

[3] *College of Medicine, University of Kerbala, Kerbala, Iraq.*

Submission: 25 November 2017
Accepted: 3 December 2017
Published: 30 December 2017

Keywords: ischemic heart diseases (IHD), resistin, SNP single nucleotide polymorphism 420C>G.

ABSTRACT

Background: Ischemic heart diseases are the most common type of cardiovascular diseases, includes stable and unstable angina and myocardial infarction a chronic condition that narrows arteries by building fat-filled bulges in the arterial walls. Resistin is a cytokine secreted by macrophages in human and has an anti-insulinemic activity. It has molecular weight is 12.5 kD and its length is 108 amino acids The role of resistin in the development of ischemic heart disease is not well understood globally. **Aim**: To assess the association of the −420C>G SNP of resistin gene with the ischemic heart disease in the general population**. Methods**: A case control study was performed at which 150 patients with ischemic heart disease and 150 healthy individuals were observed. Genotyping for SNP 420C>G in the resistin gene was performed by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. **Results:** The genotype and allele frequencies of resistin gene polymorphism in IHD and control persons were examined under the co-dominant, dominant and recessive models with the use of multinominal logistic regression analysis. Neither genotype distribution nor the minor allele frequency showed insignificant changes among the IHD patients and the control group. **Discussion:** The results showed that resistin gene polymorphism 420C>G (homozygous GG and heterozygous CG genotype) was insignificantly associated with IHD subject. **Conclusion:** The −420C>G SNP of resistin gene is not associated with ischemic heart disease.

INTRODUCTION

Ischemic heart disease is the number one killer in the developed world, with over 7.4 million deaths (1). It causes about one-third or more of all deaths in people over age 35 (2). Ischemic heart disease (IHD) is the most common type of cardiovascular diseases (3), is a chronic condition that narrows arteries by building fat-filled bulges in the arterial walls that develop slowly over time. These bulges are called atherosclerotic plaques (4). It is estimated that nearly one-half of all middle-aged men and one-third of middle-aged women may develop the disease (*Wong, 2014)*. There are three main types of ischemic heart disease Stable angina, Unstable angina, Myocardial infarction (5). Resistin is a polypeptide hormone secreted by adipocytes, belongs to a family of cysteine-rich secretory proteins (6). Its molecular weight is 12.5 kD and its length is 108 amino acids in humans (7). It is considered a pro-inflammatory molecule and plays a role in the inflammatory response (8). Resistin plays important regulatory function in insulin resistance and diabetes in a variety of biological: metabolic syndrome, atherosclerosis and cardiovascular disease (9), (10), (11). The normal plasma resistin concentration range in healthy humans is between 2–40 ng/ml (12), (13), (14) (15), (16). Resistin is encoding by RETN gene locates at chromosome position 19p13.3 (17). RELM gene family are an N-terminal signal peptide, a variable middle region, and a C-terminal cysteine-rich sequence (18). SNPs are distributed along the genome at a distance of 0.3–1 kb, making SNPs the most frequent variations in the genome (19). Several SNPs in RETN i.e., –420C>G (rs1862513), +299 (G>A) (3745367),SNP-358 (rs3219175) (20), rs7308752 (G/G) ,rs516115 (C/C) , rs7139228, rs7956537, rs3138167, (21), 3'UTR +62G>A (rs 3745368) (22) SNP-420C > G (rs1862513) in the promoter is one of the most studied SNPs of RETN gene (23),(24),(25),(26). It has been demonstrated an association of the -420C>G SNP with coronary artery disease, arteriosclerosis and cerebrovascular disease (27). Researchers have focused on the -420C > G polymorphism (rs1862513). Because it locates within the 5′ flanking region of the RETN gene. This region is involved in the recruitment of the nuclear transcription factor Sp1/3 (25), (28).

MATERIALS AND METHODS

Study subjects

A case-control study of 300 subjects (150 IHD and 150 control) was conducted to study the association of 420C\G SNP Resistin with ischemic heart disease. The patient population

included 150 subjects (90 men and 60 women) with Ischemic Heart disease who attended the cardiology center in Najaf governorate from January to April 2017.The Inclusion criteria were:(1) Those patients who were diagnosed by physicians as having(IHD) ;(2) Age of subjects was >40 years old. The exclusion criteria were: (1) Patient with liver disease; (2) Patient with renal dysfunction (3) Patients with diabetes mellitus or abnormal glucose tolerance test. The control group included 150 apparently healthy subjects (74 men and 76 women) randomly selected from the general population. The exclusion criteria were :(1) No past medical history of IHD ;(2) No family history of IHD ;(3) Matched to patients with regard to age, sex, and geographical Distribution ;(4) BMI < 30 kg/m2 and more than 18.5 kg/m2 ;(5) Fasting total cholesterol < 200 mg/dl ;(6) Fasting total triglycerides < 150 mg/dl. All cases complete a detailed questionnaire that included information about age, sex, family history, drug history, medical history and other relevant information, for all subject weight, height, BMI was measured. Informed consent has been taken from all subjects. Karbala Medical College Ethical Committee has approved the study protocol.

Genotypic data

Peripheral blood samples of (IHD) and control group were collected in EDTA-anticoagulant tube and DNA was extracted from whole-blood samples using the Reliaprep genomic DNA extraction Kit (Promega, U.S.A). Then DNA concentration and purity were measured by UV absorption at 260 and 280 nm (Bio Drop, U. K.). Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) for resistin gene using thermocycler (Biometra, Germany). The primer sequences were obtained from (29): forward 5-TGT-CAT-TCT-CAC-CCA-GAG-ACA-3 .and reverse 5-TGG-GCT-CAG-CTA-ACC-AAA-TC-3 .Amplification was performed in a total volume of 23 µlwhich contained 12.5 µl of Go Taq Green Master Mix, (Promega Corporation, Madison, WI), 1µl of each primer (One Alpha, U.S.A.),3.5 µl of nuclease free water and 5 µl of DNA template.Cycling conditions were 95ºC for 4 min followed by 30 cycles of 94ºC for 45 Sec, 60ºC for 45 Sec, 72ºC for 45Sec and a final extension of 72ºC for 7 min.Amplification product of a resistin gene was533bp.The product digested with 10 u of restriction enzyme (Bbs1) (Biolabs) and 2% agarose gels. To determine genotyping error rate, we performed random duplication in 20% of the samples.

STATISTICAL ANALYSIS

Genotype frequencies were tested for Hardy-Weinberg equilibrium by X^2 test using online software web-Assorted(30). Genetic power was calculated using the online software OSSE (31). Genotype and allele frequencies in IHD and control group were tested by multinomial logistic regression analysis with and without adjustment for age, sex and (BMI) using SPSS.

RESULTS

Result of digestion with restriction enzyme (Bbs1) for resistin gene (420C>G) including 533 bp band for the wild-type (CC) genotype, for the heterozygous genotype (CG) three bands 533,323 and 210bp and for homozygous genotype (GG) two bands 323,210 bp as shown in fig.1.

The genotype and allele frequencies of resistin gene polymorphism in IHD and control persons were examined under the co-dominant, dominant and recessive models with the use of multinominal logistic regression analysis. Neither genotype distribution nor the minor allele frequency showed significant changes among the comparison of the of IHD patients with the control group. Genotype and allele frequencies of resistin gene are shown in Table 1.

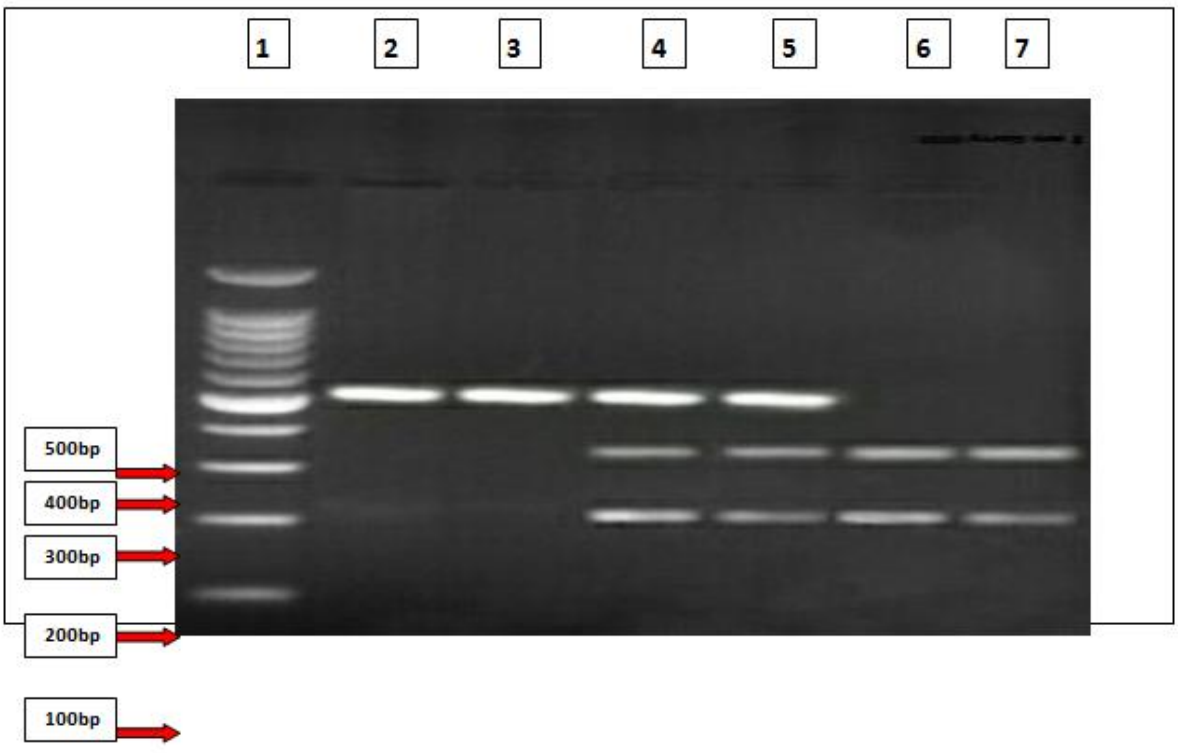

Fig.1. Results of resistin gene polymorphism product on agarose gel electrophoresis.

Line 1: DNA Marker

Line 2 and 3: CC genotype (533) bp CG

Line 4 and 5:CG genotype (533,323,210) bp

Line 6 and 7: GG genotype (323,210)bp

Table.1. Genotype and allele frequency of 420C>G polymorphism of resistin gene and association of this variant with IHD in the study subjects.

Resistin(420C\G) (C/G)	Control n=150	IHD n=150	Unadjusted OR (95% CI)	P value	Adjusted OR (95%CI)	P value
CC(Reference)	96	88				
CG	22	27	1.39 (0.71-2.52)	0.36	1.22 (0.63-1.92)	0.33
GG	32	35	1.19 (0.68-2.08)	0.53	1.32 (0.73-1.92)	0.48
MAF%	86(18%)	97(20.6%)	1.10 (0.62-1.93)	0.85		

DISCUSSION

Genotype frequencies of resistin gene 420C>G were consistent with Hardy–Weinberg equilibrium (HWE) in control individuals and IHD subjects (P 0.05). The calculated genetic power to detect a significant difference at level of 0.05 was (10%).The analyzed SNP (420C>G) locates in the promoter region of the gene, so it can direct the changes of resistin action through its concentration or via other routes. It has been found that 2/3 of serum resistin concentration is related to ethnic variation (32). Resistin levels could not be determined due to logistic difficulties. Results revealed insignificant variation of the genotype distribution of the −420C>G SNP of resistin gene in patients with ischemic heart disease and the control group. Moreover, the minor allele frequency G was found to be insignificantly altered in the two investigated groups. These results suggested the absence of the association of this SNP with the occurrence of the disease in our population. However, it is very critical to consider the factors that may affect the results. One of the principal factors is the genetic power of the study, it was found to be 10%. This is a low value to obtain significant difference and the subsequent decision making. It is worthy to consider the cause for obtaining such finding. The serious factor that leads to this observation is the sample size. It was recruited to be 150 patients and 150 healthy individuals. This population was selected

randomly since there were no previous data dealing with the polymorphism of resistance genes in our population as well as the periphery of Iraq. However, the selection depended on other populations. The insignificant changes of the genotype frequencies of the G allele were evident among the two investigated groups. In addition the, the minor allele frequencies did not significantly change during a comparable assessment. Thus, the little difference of the AMF among the two groups will lead to the low obtained genetic power. To solve this problem it is reasonable to increase the sample size, but the increment is too high that could not be tolerated under the current logistic conditions. Anyhow, the present findings could be considered as a pilot data for further studies conducted on resistin gene polymorphisms in numerous pathological conditions. Several studies have demonstrated that resistin is insignificantly associated with adiposity or metabolic traits, at least in the absence of obesity or type 2 diabetes (32),(34). Consistent with our results, a study of the −420 variant in Europeans found no correlation with carotid atherosclerosis in a cohort study and no association with myocardial infarction in a case-control study (35).

CONCLUSION

The −420C>G SNP of resistin gene is not associated with ischemic heart disease in the population of Kerbala and Najaf. The findings are pilot for further studies try to assess −420C>G SNP of resistin gene in Iraq as well as the periphery.

REFERENCE

1. World Health Organization (WHO). (2015).Cardiovascular diseases.
2. Benjamin EJ, Blaha MJ, Chiuve SE, et al. Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association. Circulation 2017; 135:46.
3. Bhatia, Sujata K. *Biomaterials for clinical applications* .2010; p. 23.
4.Coronary Artery Disease (CAD);2015. by Michael Jay Katz, MD, Ph.D.; Sheryl M. Ness, Ma, BSB, AND, RN, OCN.
5.Wong, ND. "Epidemiological studies of CHD and the evolution of preventive cardiology.". Nature Reviews. Cardiology. 2014;11(5):276-89.
6.Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA. "The hormone resistin links obesity to diabetes". Nature .2001;409:307-312.
7. Nogueiras R, Novelle MG, Vazquez MJ, et al. Resistin: regulation of food intake, Q6glucose homeostasis and lipid metabolism.Endocr 2010;17:175-84.
8. Filkova M, Haluzik M, Gay S, Senolt L. The role of resistin as a regulator of inflammation: Implications for various human pathologies. Clin Immunol. 2009;133:157-170.
9.Rongvaux A, Shea RJ, Mulks MH, Gigot D, Urbain J, Leo O, Andris F. Pre-B-cell colony-enhancing factor, whose expression is up-regulated in activated lymphocytes, is a nicotinamide phosphoribosyltransferase, a cytosolic enzyme involved in NAD biosynthesis. Eur J Immunol. 2002;32:3225-3234.
10. Chen C, Jiang J, Lü JM, Chai H, Wang X, et al. Resistin decreases expression of endothelial nitric oxide synthase through oxidative stress in human coronary artery endothelial cells. Am J Physiol. 2010; 299:93-201.

11.Momiyama Y, Ohmori R, Uto-Kondo, et al. Serum resistin levels and cardiovascular events in patients undergoing percutaneous coronary intervention. JAtheroscler Thromb 2010.
12.Fehmann H & Heyn J Plasma resistin levels in patients with type 1 and type 2 diabetes mellitus and in healthy controls. Horm .2002 ; Res 34:671-673.
13.Silha JV, Krsek M, Skrha JV, Sucharda P, Nyomba BLG & Murphy LJ. Plasma resistin, adiponectin and leptin levels in lean and obese subjects: correlations with insulin resistance. Eur J Endocrinol .2003;149: 331-335.
14.Stejskal D, Adamovská S, Bartek J, Juráková R & Prosková J . Resistin - concentrations in persons with type 2 diabetes mellitus and in individuals with acute inflammatory disease.Biomed Pap Fac Univ Palacky Olomouc Czech Repub.2003;147:63-69.
15.Burnett MS, Devaney JM, Adenika RJ, Lindsay R & Howard BV Cross-sectional associations of resistin, coronary heart disease, and insulin resistance. J Clin Endocrinol.2006; 91: 64–68.
16.On YK, Park HK, Hyon MS & Jeon E . Serum resistin as a biological marker for coronary artery disease and restenosis in type 2 diabetic patients. Circ J.2007;71:868-873.
17. Yang R, Huang Q, Xu A, McLenithan JC, Eisen JA, Shuldiner AR, Alkan S & Gong D. Comparative studies of resistin expression and phylogenomics in human and mouse. Biochem Biophys Res Commun.2003;310:927-935.
18. Holcomb IN, Kabakoff RC, Chan B, et al. A novel cysteine-rich secreted protein associated with pulmonary inflammation, defines a new gene family. EMBO J 2000;19:4046-55.
19. Schork NJ, Fallin D & Lanchbury JS. Single nucleotide polymorphisms and the future of genetic epidemiology.Clin Genet .2000; 58: 250-264.
20. Hiroshi Onuma, Yasuharu Tabara, Ryoichi Kawamura, Takashi Tanaka, Jun Ohashi, Wataru Nishida, Yasunon Takata, Masaaki Ochi, Kazuya Yamada, Rruichi Kawamoto, Katsuhiko Kohara, Tetsuro Miki, Hideichi Makino, Haruhiko Osawa. A at Single Nucleotide Polymorphism -358 Is Required for G at -420 to confer the Highest Plasma Resistin in the General Japanese Population. Journal .pone .2010.
21. Hiroshi Onuma, Yasuharu Tabara, Ryoichi Kawamura, Jun Ohashi, Wataru Nishida, Yasunon Takata, Masaaki Ochi, Tatsuya Nishimiya, R Yuichi Kawamoto, Katsuhiko Kohara, Tetsuro Miki and Haruhiko Osawa. Plasma Resistin is Associated With Single Nucleotide Polymorphisms of a Possible Resistin Receptor, the Gene, in the General Japanese Population. 2013; 62(2): 649-652.
22.Theerawut Thammakun, Wongsa Laohasiriwong ,Ratthaphol Kraiklang ,and Nittaya Saengraik. Association of +62 G>A Polymorphism in the Resistin Gene with Type 2Diabetes Mellitus among Thais: Case-Control Study. Journal of Clinical and Diagnostic Research. 2017; 11(2):15-20.
23.Hivert MF, Manning AK, McAteer JB, Dupuis J, Fox CS, Cupples LA, et al. Association of variants in RETN with plasma resistin levels and diabetes related traits in the framingham offspring study.2009;58(3):750-6.
24.Cho YM, Youn BS, Chung SS, Kim KW, Lee HK, Yu KY, et al. Common genetic polymorphisms in the promoter of resistin gene are major determinants of plasma resistin concentrations in humans. Diabetologia.2004;47(3):559-65.
25.Osawa H, Yamada K, Onuma H, Murakami A, Ochi M, Kawata H, Nishimiya T, Niiya T, Shimizu I, Nishida W, Hashiramoto M, Kanatsuka A. The G/G genotype of a resistin single-nucleotide polymorphism at -420 increases type 2 diabetes mellitus susceptibility by inducing promoter activity through specific binding of Sp1/3.Am J Hum Genet.2004;75:678-686.
26. Ukkola O, Kunnari A, Kesaniemi YA. Genetic variants at the resistin locus are associated with the plasma resistin concentration and cardiovascular risk factors. Regul Pept. 2008; 149(1-3):56-9.
27.Kunnari A, Ukkola O, Kesaniemi YA. Resistin polymorphisms are associated with cerebrovascular disea,se in Finnish type 2 diabetic patients. Diabetic Medicine. 2005; 22:583-589.
28. Chung SS, Choi HH, Kim KW, Cho YM, Lee HK & Park KS Regulation of human resistin gene expression in cell systems: an important role of stimulatory protein 1 interaction with a common promoter polymorphic site.Diabetologia .2005; 48:1150-1158.
29.Sandeep Kumara, Vani Gupta,∗, Nidhi Srivastava, Vandana Gupta, Sameeksha Mishra, Supriya Mishra, Madhav Natu Shankar, Uma Roy, Abhijit Chandra, M.P.S. Negi, Sandeep Kumar. .Resistin 420C/G gene

polymorphism on circulating resistin, metabolic risk factors and insulin resistance in adult women. Journal homepage; 2014.
30.www.osse.bii.a-star.edu.sg
31.osse.bii.a-star.edu.sg
32.Menzaghi C, Coco A, Salvemini L, et al. Heritability of serum resistin and its genetic correlation with insulin resistance-related features in nondiabetic Caucasians. Journal of Clinical Endocrinology and Metabolism.2006; 91:2792-2795.
33.Lee JH, Chan JL, Yiannakouris N, et al. Circulating resistin levels are not associated with obesity or insulin resistance in humans and are not regulated by fasting or leptin administration: crosssectional and interventional studies in normal, insulin-resistant, and diabetic subjects. Journal of Clinical Endocrinology and Metabolism. 2003,88:4848-4856.
34.Utzschneider KM, Carr DB, Tong J, et al. Resistin is not associated with insulin sensitivity or the metabolic syndrome in humans. Diabetologia. 2005;48:2330-2333.
35.Norate GD, Ongari M, Garlaschelli K, et al. Effect of the -420C\G variant of the resistin gene promoter on metabolic syndrome, obesity, myocardial infarction and kidney dysfunction.Journal of Medicine.2007;(262):104-112.