Original Paper

Association of Adiponectin Gene Promoter Polymprphism (rs266729) With Ischemic Heart Diseases

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Abstract

Background: Ischemic Heart Disease is a group of diseases of the blood vessels supplying the heart muscle. Adiponectin is a protein secreted by adipocyte with insulin-sensitizing, Anti-inflammatory and anti-atherogenic properties. Several studies have shown that polymorphisms within the adiponectin gene can be associated with Ischemic heart disease.
Aim: The aim of the present study was to evaluate the impact of (rs266729) SNP in the promoter region of the *ADIPOQ* gene on the occurrence of Ischemic heart disease (IHD).
Methods: The study included 150 patients with IHD randomly selected based on World Health Organization (WHO) guideline and 150 as controls group. DNA was extracted from blood and genotyped by PCR-RFLP by using (Hha1) enzyme.
Result: The frequency of G allele of rs266729 (C/G) polymorphism was significantly (p=0.0001) in IHD (19.6%) compared with control (13.3%). The homozygous genotype (GG) significantly (0R=1.71, CI 95%=0.65-4.96, P= 0.0001) increased the risk of Ischemic Heart Disease compared with wild type (CC) after adjustment age, sex, and BMI, furthermore the heterozygous (CG) genotype significantly (0R=1.61, CI 95%=0.96-2.87, P= 0.0001) raised the risk of Ischemic Heart Disease.
Conclusion: Adiponectin gene polymorphism rs266729 is involved in the pathogenesis Ischemic heart disease.
Keywords: Adiponectin, Ischemic heart disease, Single nucleotide polymorphism

Introduction

Ischemic heart disease (IHD) is a group of diseases of the blood vessels supplying the heart muscle that includes: angina, myocardial infarction, and sudden cardiac death[(1)] IHD is a major cause of death and disability in world[(2,3)] and responsible for about one-third or more of all deaths in people order over age 35[(3,4)]. In the Iraq, the death rate of Ischemic heart diseases accounted for 187.65 deaths per 100.000 people according to the World Health Organization (WHO) data in 2014. The most common risk factors include both genetic and environmental factors [(5)]. On the genetic side there are many genes that associated with IHD risk, adiponectin is one of this genes [(6)].

Adiponectin, a 30-kDa peptide hormone, protein secreted by adipose tissue, has been well recognized to exhibit insulin-sensitizing, anti-inflammatory and anti-atherosclerotic properties [(7)]. Plays an important role in the regulation of lipid metabolism, increased hepatic and skeletal muscle β- oxidation of fatty acids, increased lactate production of skeletal muscle, and glucose metabolism gluconeogenesis, increased glucose uptake by the cells and inhibition of oxidative stress and inflammation [(8,9)] Low adiponectin levels have been shown in (IHD) in humans [(10)].

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Adiponctein gene (APM1) is located on chromosome 3q27, a region that has previously been identified as a susceptibility locus for the metabolic syndrome and CHD [11], containing three exons and two introns and encoding 244 amino acids [12] have several single-nucleotide polymorphisms more than 10 (SNP), such as (rs266729) is located in the promoter region of ADIPOQ, and consists of a C > G substitution in the – 11377 position rs266729 [6]. Our aim was to examine the association between Gene Promoter Polymprphism *rs266729* and IHD risk.

Methods

Study design

A case–control study was contained 150 IHD patients (The criteria for IHD were a ≥70% organic stenosis of at least one segment of a major coronary artery or their main branches confirmed by coronary angiography) and 150 healthy person as control randomly selected was conducted to assess the association of SNP rs266729 of adiponectin (adipoQ) gene. All patients were diagnose by specialist physicians as having CHD, were based on WHO guidelines.

Genetic Analysis

Genomic DNA was extracted from whole blood EDTA using a DNA extraction kit (promega, U.K). Genotyping was performed by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) for adiponectin gene using thermocycler (Biometra, Germany). The primer sequences were obtained from Fang 2011[13]: forward5′-GGTGGACTTGACTTTACTGG-3′andreverse5′-TAGAAGCAGCCTGGAGAA -3′.

Amplification was performed in a total volume of 25 µl which contained 5 µl of DNA template, 1.5 µl of each primer (1 Mm final concentration) (OneAlpha, U.S.A.), 12.5 µl of GoTaq Green Master Mix (Promega), and 4.5 µl of nuclease free water. Cycling conditions were 94 °C for 4 min followed by 35 cycles of 94°C for 45s, 58 °C for 1min, 72 °C for 1min, and a final extension of 72 °C for 5 min. Amplification product of adiponectin gene was 334 bp. The product was digested with 10 u of restriction enzyme (HhaI) (Promega) and ran on 2% agarose gels.

Statistical analysis

Mean and standard deviation (M ± SD) are described. Student T test and ANOVA test were used to compare phenotypic data between control and IHD groups using SPSS windows software (SPSS Inc., Chicago, IL). Genotype frequencies were tested for Hardy–Weinberg equilibrium by X2 test using online softwareweb-Assotest (www.ekstoem.com). 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

The patients included (90 male and 60 female), ages with mean ± SD (57.41±6.38) and BMI (28±3). The control group (74 male and 76 female) ages with mean ± SD (49.16±4.6) and BMI (22.76±2.91). Results of digestion with restriction enzyme (Hha1) for adiponectin gene rs266729 included 334 bp band for wild type (CC) genotype, for the heterozygous genotype (CG) three bands 334, 212 and 122 bp and for homozygous genotype (GG) two bands 212 and 122 bp. Genotype and allele frequencies of adiponectin gene are shown in (Table 1).

Genotype frequencies of rs266729 were consistent with Hardy-Weinberg equilibrium in both IHD individuals (p=0.604) and Control (p=0.236). The power of this study to detect a significant difference at level of 0.05 was 91.2%.

Table 1. Genotype and allele frequency of rs266729 polymorphism of adiponectin gene and association of this variant in IHD and Control group in the study individuals.

Genotypes	Control n=150	IHD n=150	Unadjusted OR(95% CI)	Pvalue	Adjusted OR(95%CI)	P value
CC(Reference)	116	100				
CG	28	41	1.67(0.98-2.95)	0.001	1.61(0.96-2.87)	0.0001
GG	6	9	1.74(0.60-5.06)	0.001	1.71(0.65-4.96)	0.0001
Frequency of G allele	40(13.3%)	59(19.6%)		0.0001		

The results shown that adiponectin gene polymorphism rs266729 (homozygous GG and heterozygous CG genotype) was significantly associated with IHD patients and the frequency of G allele was higher in IHD patients.

Discussion

The results of the assessment of genotype distribution of the rs266729 SNP under different inheritance models showed a significant increase of the G allele in IHD patients when compared with those of the control group. However, the minor G allele frequency in the IHD was illustrated to be significantly higher than those of the control group.

To illustrate the probable cause of the involvement of rs266729 SNP of adiponectin gene in IHD development, we should know the functions of adiponectin in particular those related to the anti-atherogenic and the CAD protective effects. It suppresses the expression of end-othelial adhesion molecules, the pro-liferation of vascular smooth muscle cell, and the transformation of macrophage to foam cell. Moreover, the production of tumor necrosis factor-alpha (TNF-a) and interleukin-6(IL-6) is inhibited by macrophages in vitro [(14,15)]. It has been reported that the management of recombinant adenovirus expressing human adiponectin to apoE-deficient animals showed a Thirty percent (30%) reduction in atherosclerotic lesions in the absence of any effects on metabolic traits [(16)].

It is reasonable to consider the effect of the rs266729 SNP in promoter adiponectin gene on the level of adiponectin. Sp1 was associated with the gene promoter and Sp1 over-expression enhanced gene promoter activity [(17,18)]. Studies have shown that the G allele of rs266729 SNP directs the DNA-binding activity, When the DNA sequence changes to the SP1 binding site of transcriptional elements, resulting in a decrease in the transcriptional activity of the ADIPOQ gene promoter [(19)] and the expression of the ADIPOQ gene is negatively regulated [(20)] Moreover, it has been reported that the intended SNP may be involved in the destruction of the binding site of the transcriptional stimulatory protein, Sp1 [(19)]. It is seemed that the G allele may be associated with low adiponectin concentration resulting in the consequent sequels of such deficiency [(21-24)].

The current results are consistent with those of Hoeffel et al [(25)] and (Zhang et al [(26)]. On the other hand, the present study is inconsistent with those of Zhong[(27)] and Chen[(28)].

Conclusions

Adiponectin gene polymorphism rs266729 was associated with IHD. Carriers of the homozygous genotype (GG) and heterozygous (CG) genotype of rs266729 have strong association and increased risk of development of IHD.

References

1. World Heart Federation 2015.
2. Lozano R, Naghavi M, Foreman K. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet.2010; 380:2095–2128.
3. Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, et al.. Heart Disease and

Stroke Statistics-2017 Update: A Report From the American Heart Association. Circulation. 2017; 135:146-603.
4. Nichols M, Townsend N, Scarborough P, Rayner M. Cardiovascular disease in Europe 2014: epidemiological update. European Heart Journal.2014;35:2950.
5. Prakash J, Mittal B, Awasthi S, Srivastava N. Association of adiponectin gene polymorphism with adiponectin levels and risk for insulin resistance syndrome. Int J Prev Med 2015;6:31.
6. Briefed, Jana, Stumvotl, Michael, Kovacs, Peter. Genetics of adiponectin. Biochimie 2012;94:2157-2163.
7. Sayols-Baixeras, S., Lluís-Ganella, C., Lucas, G., & Elosua, R. .Pathogenesis of coronary artery disease: focus on genetic risk factors and identification of genetic variants. The application of clinical genetics.2014; 7:15.
8. Li P, Jiang R, Li L, Liu C, Yang F, Qiu Y. Correlation of serum adiponectin and adiponectin gene polymorphism with metabolic syndrome in Chinese adolescents. Eur J Clin Nutr. 2015; 69:62-7.
9. Yamauchi T, Nio Y, Maki T, Kobayashi M, Takazawa T, Iwabu M,etal.Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. Nat Med. 2007; 13:332–339.
10. Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. JAMA. 2004; 291:1730 –7.
11. Francke S, Manraj M, Lacquemant C. A genomewide scan for coronary heart disease suggests in Indo-Mauritians a susceptibility locus on chromosome 16p13 and replicates linkage with the metabolic syndrome on 3q27. Hum Mol Genet. 2001; 10:2751– 65
12. Jee SH, Sull JW, Lee JE, Shin C, Park J, Kimm H, et al. Adiponectin concentrations: a genome-wide association study. Am J Hum Genet.2010;87:545–52.
13. Fang Liu, Zhiyi He, Shumin Deng, Hui Zhang, Nan Li, Jialiang Xu . Association of adiponectin gene polymorphisms with the risk of ischemic stroke in a Chinese Han population. Molecular Biology Reports.2011;38:1983–1988.
14. Ding M, Xie Y, Wagner RJ, Jin Y, Carrao AC, Liu LS, et al. Adiponectin induces vascular smooth muscle cell differentiation via repression of mammalian target of rapamycin complex 1 and FoxO4. Arteriosclerosis, Thrombosis, and Vascular Biology.2011; 31:1403–1410.
15. Ohashi K, Parker JL, Ouchi N, Higuchi A, Vita JA, Gokce N, et al. Adiponectin promotes macrophage polarization toward an anti-inflammatory phenotype. Journal of Biological Chemistry .2010;285:6153–6160
16. Yamauchi T, Kamon J, Waki H, Imai Y, Shimozawa N, Hioki K, et al. Globular adiponectin protected ob/ob mice from diabetes and ApoE-deficient mice from atherosclerosis. Journal of Biological Chemistry .2003;278:2461–2468.
17. Berg, A.H., Combs, T.P., Scherer, P.E. ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. Trends in Endocrinology and Metabolism.2002;13:84–89.
18. Liu M, Sun Z, Zhou A, Aidong Zhou, Hong Li, Liping Yang, Chang Zhou et al. Functional characterization of the promoter region of human TNFAIP1 gene. Molecular Biology Reports.2010;37:1699–1705
19. Zhang D, Ma J, Brismar K, Efendic S, Gu HF.A single nucleotide polymorphism alters the sequence of SP1 binding site in the adiponectin promoter region and is associated with diabetic nephropathy among type 1 diabetic patients in the Genetics of Kidneys in Diabetes Study. Journal of Diabetes Complications. 2009;23: 265–272.
20. Suriyaprom, Kanjana, Phonrat, Benjaluck, Tungtrongchitr, Rungsunn,. Association of adiponectin gene –11377CNG polymorphism with adiponectin levels and the metabolic syndrome in Thais. Asia Pacific Journal of Clinical Nutrition. 2014; 23:167–173.
21. Vasseur F, Helbecque N, Lobbens S, Vasseur Delannoy, C. Dina,K. Clément et al.Hypoadiponectinaemia and high risk of type 2 diabetes are associated with adiponectin-encoding (ACDC) gene promoter variants in morbid obesity: evidence for a role of ACDC in diabesity. Diabetologia.2005;48:892–899.
22. Schwarz PE, Towers GW, Fischer S, Govindarajalu, S., Schulze, J., Bornstein, S.R et al. Hypoadiponectinemia is associated with progression toward type 2 diabetes and genetic variation in the ADIPOQ gene promoter. DiabetesCare. 2006; 29:1645–1650.
23. Jiala, Ishwarlal, Adams-Huet, Beverley, Frank Duong, B.S., Smith, Gerred. Relationship between retinol-binding protein-4/adiponectin and leptin/adiponectin ratios with insulin resistance and inflammation. Metabolic Syndrome and Related Disorders.2014;12, 227–230.

gene and cardiovascular disease: a meta-analysis. Bio Medical Center Medical Genetics.2012;13:40.

27. Zhong C, Zhen D, Qi Q, Genshan M. A lack of association between adiponectin polymorphisms and coronary artery disease in a Chinese population. Genetics and Molecular Biology.2010; 33:428–433.
28. Chen G, Qin Q, Li Y, Ren B, Lu YR, Kou L, Yang N, Feng JP, Zhao BR. Association between plasma adiponectin and small, dense low density lipoprotein in patients with coronary artery disease. Zhonghua xin xue guan bing za zhi.2012; 40:752–756.
24. de Haan,Willeke, Bhattacharjee, Alpana, Ruddle, Piers, Kang, Martin H., Hayden, Michael R. ABCA1 in adipocytes regulates adipose tissue lipid content, glucose tolerance and insulin sensitivity. Journal of Lipid Research.2014; 55, 516–523.
25. Hoefle G, Muendlein A, Saely CH Risch L, Rein P, Koch L, Schmid F,et al . The -11377 C[G promote variant of the adiponectin gene, prevalence of coronary atherosclerosis, and incidence of vascular events in men.Thrombosis and Haemostasis. 2007; 97:451–457.
26. Zhang H, Mo X, Hao Y, Gu D. Association between polymorphisms in the adiponectin