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Association of polymorphisms of leptin, leptin receptor and apelin receptor genes with susceptibility to coronary artery disease and hypertension

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ABSTRACT

Aims: Apelin and leptin are factors which have a potential physiological and pathological role in cardiovascular homoeostasis. Apelin receptor (APLNR), leptin receptor (LEPR) and leptin variants may affect the vascular tone in heart or peripheral circulation, thereby predisposing patients to hypertension and coronary artery disease (CAD). The aim of the present study was to evaluate four single nucleotide polymorphisms (SNPs) of APLNR genes (rs11544374 and rs948847), LEPR (rs1137101) and leptin (rs7799039) gene in patients with CAD and hypertension.

Materials and methods: This case-control study was carried out on 286 CAD-suspected patients. The participants were divided into four subgroups including: CAD patients with no hypertension (H^-CAD^+), hypertensive patients with no CAD (H^+CAD^-), CAD patients with hypertension (H^+CAD^+) and non-hypertensive non-CAD subjects as control group (H^-CAD^-). Genomic DNA from whole blood was extracted and four SNPs were assessed using PCR-RFLP.

Key findings: A significant difference was found in the genotype frequency of APLNR rs11544374 gene in H^+CAD^+ and H^-CAD^+ groups compared to control subjects ($P < 0.001$ for both comparisons). Regarding the rs1137101, the prevalence of A allele compared to G allele was significantly different among the four groups ($P = 0.02$). Results of multinomial regression analysis indicated that G allele carriers in the recessive genetic model (AA vs. AG + GG) of rs11544374 had a significantly protective effect compared to H^-CAD^+ and H^+CAD^+after adjustment (OR = 0.12; 95% CI = 0.02–0.61; $P = 0.01$ and OR = 0.40; 95% CI = 0.17–0.98; $P = 0.04$, respectively).

Significance: The findings of present study revealed that the APLNR rs11544374 gene polymorphism might serve as predisposing factor in CAD.

1. Introduction

Coronary artery disease (CAD) along with other complications including hypertension and myocardial infarction (MI) account for a vast number of morbidity and mortality rates around the world [1]. In addition to environmental factors, genetic ones can affect the development and progression of CAD as 40% to 60% of the risk for CAD is reported to be due to the latter [2]. Of the genetic factors, single nucleotide polymorphisms (SNPs) assume great importance to the extent that previous studies have shown a multitude of SNPs which are associated with CAD and hypertension [3]. One of the most significant health challenges in the world capable of leading to other fatal diseases as well is hypertension which is regarded as one of the CAD risk factors [4]. It should be noted that nearly 12 million 25–64-year old Iranians are suffering from hypertension [5].

Apelin is an endogenous ligand for apelin receptor (APLNR) coupled with G protein that could be detected in plasma and expressed on the surface of cells such as heart, lung, kidney, adipose tissue, gastrointestinal tract, brain, adrenal glands and endothelium [6, 7]. Located in the X chromosome (Xq25–26.1) and containing 3 exons with a

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coding region ranging from exons 1 to 2, apelin gene encodes proprotein 77 amino acids, which are later broken into smaller pieces and processed into a number of regulatory hormones [8]. APLNR expression and its activation contribute to controlling blood pressure and angiogenesis [9]. It is worth noting that the activation of APLNRs leads to secretion of nitric oxide (NO) which in turn brings about vascular wall smooth muscle cells [10]. Moreover, triggering intracellular signaling cascades by phosphorylation of extracellular signal–regulated kinases (ERKs), Akt and p70S6 kinases, apelin stimulates endothelial proliferation and angiogenesis [11].

It has been proven that genetic polymorphisms can play an important role in development of hypertension and CVDs. In a Turkish population-based study, it was found that the APLNR gene (rs948847-A445C) polymorphism was not associated with coronary artery disease (CAD), but it was shown to be related to weight and blood pressure [12]. It is reported that G212A allele (rs11544374) of the APLNR is effective in preventing hypertension [13]. In another study on idiopathic dilated cardiomyopathy patients, it was found that individuals with the 212A variant of the apelin gene were significantly less likely to develop heart disease than those with homozygous 212G variants [14].

Leptin is a hormone made by adipocyte which regulates energy balance by controlling hunger. Despite the well-known function of leptin on appetite [15], its effect on reduction of blood pressure was shown [16] and the defect in the receptor signaling in mice has improved the response of regulatory T cells and protection against atherosclerosis [17]. Elevated leptin level positively correlates with CVD, infusion of leptin increases arterial pressure in rats and leptin-deficient mice could not develop atherosclerosis [18]. It has been reported that serum leptin levels are associated with central arterial stiffness in CAD [19]. A broad range of various studies has given way to results about the association between leptin gene polymorphisms and obesity, as well as apelin gene polymorphisms and hypertension and CVDs. For example, Gupta et al. demonstrated the role of rs3761581 polymorphism in acute coronary syndrome [20].

The APLNR, leptin and LEPR SNPs are believed to be candidate genes for CAD as well as hypertension because of their roles in regulation of lipid metabolism, regulating the blood pressure and angiogenesis [21]. Moreover, there is little and inconsistent data regarding the impact of the APLNR, LEPR and leptin polymorphisms with the risks of CAD and/or Hypertension in the sample of Iranian population. Therefore, the aim of the present study was to evaluate four SNPs within the genes encoding the APLNR (rs11544374 and rs948847), LEPR (rs1137101) and leptin (rs7799039) in patients with CAD and hypertension in a south eastern Iranian population.

2. Material and methods

2.1. Subjects

This case-control study was carried out on a number of 286 CAD-suspected subjects diagnosed as candidates of coronary angiography. Subjects were selected from Shafa Hospital of Kerman University of Medical Sciences, Kerman, Iran (July–December 2015). Subjects' selection process was performed as described elsewhere [22]. Briefly, all CAD-suspected participants were engaged in selective coronary angiography as they were symptomatic, had a history of hospitalization in CCU, or showed evidence of myocardial ischemia during noninvasive investigations such as exercise test or perfusion imaging. Additionally, luminal diameter with 50% or more narrowing in the main coronary artery vessels was conceived of as CAD suspects whereas those without any plaque were diagnosed as normal (control group). Coronary artery angiography was carried out and interpreted by a specialized cardiologist in all participants. Moreover, systolic pressure ≥ 140 mmHg and diastolic pressure ≥ 90 mmHg and/or reception of antihypertensive medication were regarded as the diagnostic criteria for hypertension. However, one should note that dissatisfied participants, patients with tumors, respiratory diseases, congenital heart disease, cerebral infarction, diabetes or chronic kidney diseases, and autoimmune diseases were excluded. The study was conducted according to the principles of the revised *Declaration of Helsinki*, a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects and approved by the ethics committee of Kerman University of Medical Sciences (IR.KMU.REC/96/26). The written informed consent was obtained from all participants.

2.2. Samples and data collection

Participants' clinical history and demographic data were collected using a questionnaire. Body mass index (BMI) was calculated as the ratio of weight to the square of height (kg/m^2). The average of two blood pressure (BP) measurements (10-minute intervals) from the right arm was used using a standard mercury sphygmomanometer. Blood samples were collected in EDTA containing tubes.

2.3. Genotyping

Genomic DNA was extracted from whole blood samples using “salting-out” method with slight modifications [19]. Isolated genomic DNA was stored at −20 °C until the polymerase chain reaction (PCR). Four SNPs (APLNR gene (rs11544374 and rs948847), LEPR (rs1137101) and leptin (rs7799039) genes) were assessed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The primers used for detection of polymorphisms are shown in Table 1. The PCR amplification was performed using a Biometra T advanced (Analytik Jena, Germany). The PCR conditions were set as follows: initial denaturation at 94 °C for 5 min followed by 30 cycles of amplification including denaturation at 94 °C for 30 s, annealing (60 s at 58 °C for rs1137101, 25 s at 58 °C for rs948847, 30 s at 64 °C for rs11544374, and 25 s at 60 °C for rs7799039), extension at 72 °C for 30 s, and the final extension at 72 °C for 5 min. PCR products

Table 1
The primers used for APLNR rs948847 and rs11544374, LEPR rs1137101, and Leptin rs7799039 polymorphisms genotyping.

Polymorphism	Sequence (5′ ≥ 3′)	Restriction enzyme	Product length
rs1137101-A > G	F: AAACTCAACGACACTCTCCTT R: TGAACTGACATTAGAGGTGA	*MspI*	AA: 80 AC: 80, 57, 23 GG: 57, 23
rs948847-A445C	F:CAGCATGGAGGAAGGTGG R: GACCCGCAGCCTCAGCCTCAGCCG	*MwoI*	AA: 215 AC: 135, 215 CC: 135
rs11544374-G212A	F: TTCTGCAGGAGACAGGCTTC R: ACTCAGACTGGTTGTCTGCC	*DdeI*	GG: 155, 81, 46 AG: 201, 155, 81, 46 AA: 201, 81
rs7799039-G2548A	F: TTTCCTGTAATTTTCCCGTGAG R: AGAGATTAAAGCAAAGACAGGC	*HhaI*	GG: 188, 61 AG: 249, 188, 61 AA: 249