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Biochemical and genetic role of apelin in essential hypertension and acute coronary syndrome☆

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ABSTRACT

Background: Apelin-APJ pathway has emerged as a potent regulator of blood pressure (BP) and blood flow in vasculature and heart. Variants in apelin gene may affect the vascular tone in peripheral circulation or heart, thereby predisposing to cardiovascular diseases. The aim of our study was to investigate the association of two apelin gene polymorphisms rs3761581 and rs2235312, and apelin levels in patients with essential hypertension (EH) and acute coronary syndrome (ACS).
Methods: The study comprised of three groups namely, (1) 118 healthy control subjects, (2) 92 EH patients, and (3) 60 ACS patients. DNA was extracted from peripheral blood leukocytes and genotyping was performed by SNaPshot method. Plasma apelin 13 levels were estimated using ELISA.
Results: EH and ACS patients had a significantly lower level of apelin 13, regardless of gender ($p = 0.003$, $p = 0.017$, respectively). Interestingly, the female EH and ACS patients had lower levels of apelin 13 than their male counterparts. The G allele of rs3761581 was more apparent in patients especially in ACS than the controls.
Conclusion: Reduced apelin levels may enhance vasoconstriction to influence high BP and heart's workload in EH and ACS. Genetic involvement of apelin needs to be established in well-defined larger sample size.

1. Introduction

Hypertension is directly responsible for 57% of stroke deaths and 24% of coronary artery disease (CAD) deaths in India [1]. Epidemiologic studies suggest that hypertension, cigarette smoking, diabetes, dyslipidemia and obesity are the major risk factors for CAD. However, these traditional risk factors have not been able to completely explain and predict the development of the atherosclerotic process.

Essential hypertension (EH) and acute coronary syndrome (ACS) are multifactorial diseases involving both environmental and genetic components. Numerous studies have investigated the genes of Renin-angiotensin-aldosterone system (RAAS), Kallikrein-kinin system (KKS) and nitric oxide (NO) signaling pathways in EH and cardiovascular diseases [2–5]. However, large fraction of etiology remains unexplained as these polymorphisms understandably cannot fully contribute to the underlying genetic risk for EH and cardiovascular diseases because of their multi-genetic nature.

The apelin and its receptor, APJ, constitute relatively new peptidic system with an emerging and important physiological and pathophysiological role. In-vitro and preclinical models have suggested that the apelin-APJ pathway has a role in cardiovascular homoeostasis and also in fluid balance and metabolism [6]. The apelin-APJ pathway regulates systemic perfusion and has effects on both the heart and vasculature. The APJ receptors are present on endothelial cells, vascular smooth muscle cells and cardiomyocytes [6,7]. Apelin is both arterial as well as venous dilator and these effects appear to be mediated through NO signaling. Although, numerous studies have identified role of exogenous apelin [8], role of endogenous apelin-APJ pathway is less clear. Evidence of genetic involvement of apelin-APJ pathway in susceptibility to hypertension is available [9,10]; however, their role in ACS is still unsure. We, hence, aimed to determine the plasma apelin 13 levels in EH and ACS. We also sought to investigate the distribution of apelin gene (*APLN*) polymorphisms, rs3761581 and rs2235312.

2. Materials and methods

2.1. Study participants

The study was approved by the ethics review committee of both institutions (IGIB and GBPH). Informed consent was obtained from each

☆ All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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Table 1
The primers and optimal conditions for PCR amplification.

Gene	SNP rs ID	Primer sequence	Cycling conditions	Product size (bp)
APLN	rs3761581	F 5′-AGA TCC AGA AGA GTG AGA AGA C-3′ R 5′-CAG GAT AAC ACT AAT CCA GCA G-3′	I 95 °C 5′, D 95 °C 30″, A 58 °C 40″, E 72 °C 45″, 35 cy, FE 72 °C 10′	347
	rs2235312	F 5′-CTC TAT TCA AGA CCT GAA CAC G-3′ R 5′-GTC TGA GTG GTT GTC TAT TGG-3′	I 95 °C 5′, D 95 °C 30″, A 66.8 °C 40″, E 72 °C 45″, 35 cy, FE 72 °C 10′	370

Representations used in table: PCR, Polymerase chain reaction; SNP, Single nucleotide polymorphism; bp, base pair; F, forward; R, reverse; I, initial denaturation; D, denaturation; A, annealing; E, extension; cy, cycles; FE, final extension;.

participant. Subjects, totaling 270, with age ≥25 and ≤60 years, were divided into three groups namely, (1) 118 healthy control subjects, (2) 92 subjects of EH, and (3) 60 subjects of ACS. Since both the selected SNPs were located on X-chromosome, it was imperative to check the gender-based allelic distribution hence the subjects were further divided into males and females to perform all the analyses.

Table 2
Demographic and clinical characteristics.

Study subjects					
Parameters	Controls (n = 118)	EH patients (n = 92)		ACS patients (n = 60)	
Clinical characteristics	Mean ± SD	Mean ± SD	*p*-value	Mean ± SD	*p*-value
Age (years)	43.5 ± 9.6	45.4 ± 9.7	NS	47.8 ± 7.9	0.003
BMI (kg/m^2)	23.4 ± 4.2	22.9 ± 2.7	NS	24.4 ± 3.8	NS
SBP (mm of Hg)	115.5 ± 8.6	143.8 ± 7.3	<0.0001	122.0 ± 10.3	<0.0001
DBP (mm of Hg)	73.9 ± 5.5	91.2 ± 4.9	<0.0001	81.0 ± 6.1	<0.0001
Life style/history	n (percentage)	n (percentage)	*p*-value	n (percentage)	*p*-value
Alcohol history	25 (21.2%)	12 (13.0%)	NS	32 (53.3%)	<0.0001
Smoking history	28 (23.7%)	31 (33.7%)	NS	32 (53.3%)	<0.0001
Family history	None	None	–	None	–
Male study subjects					
Parameters	Controls (n = 60)	EH patients (n = 43)		ACS patients (n = 53)	
Clinical characteristics	Mean ± SD	Mean ± SD	*p*-value	Mean ± SD	*p*-value
Age (years)	47.1 ± 7.7	44.3 ± 9.8	NS	46.9 ± 7.8	NS
BMI (kg/m^2)	22.1 ± 2.8	24 ± 2.6	0.001	24.1 ± 3.6	0.001
SBP (mm of Hg)	115.8 ± 8.8	144.0 ± 7.0	<0.0001	120.9 ± 9.5	0.004
DBP (mm of Hg)	73.0 ± 5.1	91.2 ± 4.3	<0.0001	80.4 ± 5.2	<0.0001
Life style/history	n (percentage)	n (percentage)	*p*-value	n (percentage)	*p*-value
Alcohol history	25 (41.7%)	12(27.9%)	NS	31 (58.5%)	NS
Smoking history	27 (45%)	31 (72.1%)	0.003	31 (58.5%)	NS
Family history	None	None	–	None	–
Female study subjects					
Parameters	Controls (n = 58)	EH patients (n = 49)		ACS patients (n = 7)	
Clinical characteristics	Mean ± SD	Mean ± SD	p-value	Mean ± SD	*p*-value
Age (years)	39.9 ± 10	46.4 ± 9.6	0.001	54.1 ± 5.8	0.001
BMI (kg/m^2)	24.7 ± 4.9	21.8 ± 2.5	0.0002	26 ± 4.5	NS
SBP (mm of Hg)	115.2 ± 8.6	143.7 ± 7.3	<0.0001	130.3 ± 13.0	<0.0001
DBP (mm of Hg)	74.8 ± 5.7	91.2 ± 5.4	<0.0001	86.0 ± 9.8	NS
Life style/history	n (percentage)	n (percentage)	p-value	n (percentage)	p-value
Alcohol history	None	None	–	1 (14.3%)	–
Smoking history	1 (1.7%)	None	–	1 (14.3%)	<0.0001
Family history	None	None	–	None	–

Representations used in table: EH, essential hypertension; ACS, acute coronary syndrome; n, total no. of individuals in a group; SD, standard deviation; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; NS, non-significant data.
EPIINFO ver. 6 was used to determine p-values in comparison to healthy controls.
Clinical characteristics are represented as mean ± SD and Life style/history is represented as no. of individuals (percentage distribution).

Controls were recruited when they had systolic blood pressure (BP) ≤ 120 mm Hg and diastolic BP ≤80 mm Hg and had no evidence of CAD on history or ECG (Electrocardiogram), secondary hypertension, diabetes mellitus and any other common disease, had absence of antihypertensive medication and had no first degree relative having CAD. EH cases were defined as systolic BP ≥140 mm Hg and/or diastolic BP ≥90 mm Hg (according to JNC VII criteria) without any other diseases, and any complications. Patients with acute ST elevation myocardial infarction (MI), non ST elevation myocardial infarction and unstable angina were included in ACS cohort. Patients with chronic stable angina were not included in ACS group. Participants were not recruited if there was inability to give consent or do not comply with the study protocol. Age, height, weight, body mass index (BMI) and BP were recorded for the three groups.

2.2. Blood sample collection, DNA and plasma separation

Subjects with overnight fasting were rested for 30 min prior to drawing blood in supine position. Five milliliter of blood was drawn in acid citrate dextrose (ACD) anticoagulant. The collected blood sample was centrifuged (A-4-62, Eppendorf, Hamburg, Germany) for 10 min at 1500 rpm at 4 °C. Plasma was extracted and the peripheral blood leucocytes were used for DNA extraction. The DNA and plasma samples were labeled, aliquoted and stored at −80 °C if not used immediately.

2.3. Genotyping

DNA was extracted from peripheral blood leukocytes by salting out method. The PCR primers to amplify apelin loci were designed by Perl Primer software (Table 1). The reaction mixture, 20 μl, contained 50 ng of DNA, 3 pmol of each primer, 1× buffer, 0.33 U of Taq DNA polymerase and 0.2 mmol/l of deoxynucleotide triphosphates (dNTPs). The PCR cycling conditions are described in Table 1. The PCR products were purified by Polyethylene glycol (PEG) purification and were subjected to genotyping by SNaPshot method. SNaPshot primers were also designed by Perl Primer software. The primer sequence for rs3761581 and rs2235312 was 5′-TCCAGGGAACAAGAAAGGG-3′ (annealing at 57 °C) and 5′-CACCACAGTAAGAAGTGGG-3′ (annealing at 58 °C), respectively. Peak Scanner Software v1.0 was used for allele determination.

2.4. Biochemical analyses

Plasma Apelin 13 level was measured by competitive ELISA using Human Apelin 13, AP13 ELISA Kit (USCN Life Science Inc.).

2.5. Biostatistical analyses

SPSS 12 (SPSS Inc., Chicago, Illinois, USA) and EPIINFO ver.6 (Centers for Disease Control, Atlanta, Georgia, USA) software were used for statistical analysis. A goodness of fit test was used for testing the Hardy-Weinberg equilibrium (HWE) and a χ^2 test compared the genotype