APJ polymorphisms in coronary artery disease patients with and without hypertension

COLOMBA FALCONE[1,2,3], SARA BOZZINI[1], SANDRA SCHIRINZI[2], MARIA PAOLA BUZZI[1,2], CHIARA BOIOCCHI[1], ROSSANA TOTARO[2], MARIALISA BONDESAN[2] and GABRIELE PELISSERO[3]

[1]Interdepartimental Center for Research in Molecular Medicine (CIRMC), University of Pavia, Pavia; [2]Department of Cardiology, Istituto di Cura Città di Pavia University Hospital, Pavia; [3]IRCCS San Donato Hospital, San Donato Milanese, Milan, Italy

Received April 14, 2011; Accepted August 8, 2011

DOI: 10.3892/mmr.2011.685

Abstract. Apelin is an endogenous peptide that increases cardiac inotropism through its APJ receptor. Certain findings indicate that the apelinergic system may have a pathophysiological role in cardiovascular disease and there is evidence showing the role of the apelinergic system in blood pressure regulation *in vitro* and in animal models. The role of the apelin-APJ system in cardiovascular physiology and its interaction with other neuroendocrine pathways has not been fully elucidated. However, the small number of reported studies indicates that apelin signaling may be involved in the regulation of blood pressure, cardiac contractile function, fluid balance, angiogenesis and inhibition of apoptosis. We evaluated the possible relationship between the G212A and A445C APJ polymorphisms and coronary artery disease (CAD) in Italian patients and in healthy controls by RFLP-PCR. We analyzed the allelic and genotypic frequencies of APJ polymorphisms in 664 patients (378 with hypertension) and 143 controls. There were no differences between allelic and genotypic frequencies in patients in respect to the controls for both polymorphisms analyzed. In the CAD population, there was an increased frequency of the G212 allele in patients with hypertension in respect to patients without hypertension. No differences were present in the two subgroups for the A445C polymorphism. Although the functional role of the G212A polymorphism has not yet been identified, it is possible to hypothesize that the presence of the A allele may cause a gain in function of the apelin/APJ system associated with a lower risk of hypertension.

Introduction

Essential hypertension is one of the most common complex diseases resulting from genetic, environmental factors and their interaction. Genome-wide association studies have identified hypertension-susceptible genes, whereas the predicted genetic contribution has not been fully elucidated. The most studied genes are implicated in pathways related to the renin-angiotensin system as its activation and the production of angiotensin II play important roles in the progression of cardiac and renal disease (1,2).

Apelin is a novel peptide that acts through its G-protein-coupled receptor termed APJ. Apelin and APJ are both expressed extensively in body tissue (3-10). In particular, high concentrations of APJ are present in the cardiovascular system where this receptor is expressed on a number of cell types, including the endothelium, smooth muscle and myocytes (11). The role of the apelin-APJ system in cardiovascular physiology and its interaction with other neuroendocrine pathways has not been fully elucidated. However, the small number of reported studies indicates that apelin signaling may be involved in the regulation of blood pressure (12-14), cardiac contractile function (15), fluid balance (16), angiogenesis (17) and inhibition of apoptosis (18).

Tatemoto *et al* (12) suggested that apelin may be a new member of the family of vasoactive substances that include bradykinin, acetylcholine and serotonin, a group of agents that are known to stimulate NO production via activation of eNOS in endothelial cells. In addition, Ishida *et al* (13) demonstrated that APJ has a hypotensive effect *in vivo* and plays a regulatory role against the vasopressor action of angiotensin II. Recently, Li *et al* (14) identified apelin and APJ as elements of a new pathway with a role in counter regulation of the renin-angiotensin system which is implicated in blood pressure regulation.

Our study aimed to evaluate the G212A (rs11544374) and A445C (rs948847) polymorphisms of the APJ gene as predisposing factors in a population with coronary artery disease (CAD).

Materials and methods

Study population. A total of 664 Italian patients (530 males, 134 females, mean age 63.2±8 years) were consecutively recruited among stable subjects who presented with a previous event of CAD and referred to the Department of Cardiology of the University Hospital. The presence of previous CAD was

Correspondence to: Professor Colomba Falcone, Department of Cardiology, Istituto di Cura Città di Pavia University Hospital, Via Parco Vecchio 27, Pavia, Italy
E-mail: colomba.falcone@unipv.it

Key words: APJ polymorphism, coronary artery disease, hypertension

defined as evidence upon coronary angiography of a luminal stenosis >75% in at least one major coronary vessel.

Individuals with valvular heart disease, cardiomyopathy, chronic kidney disease and inflammatory disease were excluded.

At study entry, all patients underwent a comprehensive clinical examination and biochemical evaluation. Clinical data included age, gender, BMI, hypertension, hyperlipidemia, diabetes mellitus, smoking habits and family history of CAD.

The control subjects visited our affiliated hospitals or clinics for a physical check-up. Controls were characterized by no history of angina and other heart disease, a normal resting ECG and normal exercise ECG stress testing. They were matched with CAD patients according to age, gender and ethnicity.

The study protocol was approved by our local ethics committee, and written informed consent to participate was obtained from all participants.

Definition of cardiovascular risk factors. Blood pressure was measured three times after a 10-min rest in a sitting position using a calibrate mercury sphygmomanometer. Hypertension was diagnosed when the average of three blood pressure readings was at least 140 mmHg systolic or 90 mmHg diastolic, or in the event the individuals were taking antihypertensive medication. Diabetes mellitus was defined as a prior diagnosis of the disease, a history of antidiabetic medication or plasma fasting glucose levels ≥126 mg/dl on two or more occasions. In this study, all diabetic patients had type 2 diabetes. Cigarette smokers were categorized as ever smokers (including current and former smokers who quit smoking for at least 6 months before the study entry) or non-smokers. A positive family history of coronary disease was defined as documented CAD in parents or siblings before the age of 60 years in men and 70 years in women. Each of these risk factors was coded as a categorical variable as either present or absent.

Assay of the APJ genotype. Genomic DNA was extracted from EDTA-treated blood using the QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany). Both polymorphisms of the APJ gene were analyzed by polymerase chain reaction-restriction fragment length polymorphism (RFLP-PCR). In brief, a section of the APJ gene that contains the G212A and A445C polymorphisms was amplified by PCR using 5'-GGAGGTGGGAGGAGGAG-3' 5'-CCGTTGCCCGTGGTGCCC-3' and 5'-CAGCATGGA GGAAGGTGG-3' 5'-GACCCGCAGCCTCAGCCG-3' primers, respectively. The conditions of amplification were as follows: 94˚C for 2 min; 30 cycles at 94˚C for 30 sec, 58˚C for 30 sec, 72˚C for 1 min and finally, 10 min at 72˚C. The PCR products were then subjected to *Dde*I and *Mwo*I (New England Biolabs, Beverly, MA, USA) digestion for 16 h at 37˚C and resolved by electrophoresis on 3% agarose gels.

A second randomized genotyping was performed for quality assurance and 100 (15%) patients were included.

Statistical analysis. All statistical analyses were conducted with use of the SPSS statistical package, version 11.0 (SPSS, Chicago, IL, USA). Kolmogorov-Smirnov test of normality was performed to verify the distribution of continuous data. Normally distributed data in the groups were expressed as the mean values ± SD. For continuous variables, the differences between the groups were evaluated with an unpaired t-test. A χ^2 test for goodness of fit was used to verify whether the observed allelic frequencies agreed with those expected under Hardy-Weinberg equilibrium. Allelic and genotypic distributions were estimated by allele counting and compared in the CAD and control groups by the χ^2 test. Crude odds ratios (ORs) are reported with their 95% confidence intervals (CIs). The study power was calculated using the StatMate software, version 2.0 (GraphPad, San Diego, CA, USA). Two-tailed p-values <0.05 were deemed to be significant.

Results

The general characteristics of the study participants are depicted in Table I. As expected, the prevalence of common cardiac risk factors was significantly higher in the CAD patients than in the controls.

The distribution of the A445C and G212A polymorphisms is shown in Table II. Genotypes were in Hardy-Weinberg equilibrium both in the patients and in the controls. There were no significant differences in the genotypic or allelic frequencies of the two evaluated polymorphisms between the patients and controls. Considering the role of the apelin system in the renin-angiotensin system which is implicated in blood pressure regulation, our CAD patients were also analyzed accordingly to the presence of hypertension, as reported in Table III. Dividing CAD patients in two subgroups on the basis of the presence of high blood pressure, statistical significant differences were found in the genotypic and allelic frequency distribution of the G212A polymorphism of the APJ receptor. There was a higher frequency of the homozygous GG genotype in patients with hypertension (p=0.0005), while in patients without hypertension there was a significant increase (p=0.0121) in the frequency of the homozygous AA genotype. Furthermore, the distribution of alleles was significantly different in the two subgroups; for patients with hypertension there was a higher G allelic frequency and a lower frequency of the A allele (p=0.0001). The crude OR for the presence of hypertension in subjects bearing the homozygous GG genotype was 2.00 (95% CI 1.45-2.76).

In the control group, the G212A polymorphism was not associated with the presence/absence of hypertension. No difference was present for A445C in patients with and without hypertension.

Discussion

This study investigated the role of the APJ receptor gene as both a susceptibility and modifier candidate gene for CAD. Subsequently, the genotypic and allelic frequencies of the G212A and A445C polymorphisms were evaluated in CAD patients with and without hypertension.

The human APJ gene is located on the long arm of chromosome 11 and encodes a 377 amino acid G protein-coupled receptor with seven transmembrane-spanning domains (19) for which apelin is the only known ligand. In view of the structural properties that the apelin receptor shares with chemokine receptors, it may be preferentially coupled to

Table I. Clinical characteristics of the 664 CAD patients and 143 healthy controls.

	CAD patients (n=664)	Controls (n=143)	p-value
Age (years)	63.2±8.00	63.8±11.60	NS
Males, n (%)	530 (80)	121 (85)	NS
BMI (kg/m^2)	26.1±2.11	25.7±2.30	NS
Total cholesterol (mmol/l)	5.2±0.90	4.9±1.16	NS
Triglycerides (mmol/l)	2.89 (0.99-12.8)	1.5 (1.2-2.1)	<0.0001
HDL cholesterol (mmol/l)	1.28±0.52	1.37±0.57	NS
LDL cholesterol (mmol/l)	3.11±0.57	3.14±1.50	NS
Hypertension, n (%)	378 (57)	27 (19)	<0.0100
Ever-smoking, n (%)	252 (38)	22 (15)	<0.0010
Familiarity, n (%)	143 (21)	15 (10)	<0.0010

NS, not significant.

Table II. Genotypic and allelic frequencies of the APJ G212A and A445C polymorphisms in the CAD subjects and healthy controls.

	CAD patients (n=664)	Controls (n=143)	p-value
G212A genotype, n (%)			
GG	291 (44)	76 (53)	0.053
GA	306 (46)	56 (39)	0.156
AA	67 (10)	11 (8)	0.469
G212A allele, n (%)			
G	888 (67)	208 (73)	0.063
A	440 (33)	78 (28)	
A445C genotype, n (%)			
AA	232 (35)	51(36)	0.065
AC	319 (48)	70 (49)	0.807
CC	113 (17)	22 (15)	0.803
A445C allele, n (%)			
A	783 (59)	172 (60)	0.663
C	545 (41)	114 (40)	

Table III. Clinical characteristics, genotypic and allelic frequency of the G212A polymorphism in the CAD patients with and without hypertension.

	CAD patients with hypertension (n=378)	CAD patients without hypertension (n=286)	p-value
Age (years)	62.2±7.9	63.1±9.3	NS
Males, n (%)	294 (78)	236 (82)	NS
BMI (kg/m^2)	24.2±1.9	26.6±2.0	NS
Ever-smoking, n (%)	145 (38)	107 (37)	NS
Hyperlipidemia, n (%)	137 (36)	114 (40)	NS
Familiarity, n (%)	75 (20)	68 (24)	NS
G212A genotype, n (%)			
GG	188 (50)	103 (36)	0.0005
GA	162 (43)	144 (50)	0.0660
AA	28 (7)	39 (14)	0.0121
G212A allele, n (%)			
G	538 (71)	350 (61)	0.0001
A	218 (29)	222 (39)	

NS, not significant.

Gi protein and may consequently inhibit adenylyl cyclase and activate extracellular-regulated kinases. On the other hand, its homology with angiotensin receptor subtypes suggests that it could transduce its effects via a Gq protein (20).

In our CAD population, genotypic and allelic frequencies for both polymorphisms analyzed were similar to the control group.

The apelin-APJ signaling pathway has emerged as an important novel mediator of cardiovascular control and blood pressure homeostasis (13,21). The cardiovascular system is richly endowed with G protein-coupled receptors (GPCRs), which represent the largest group of transmembrane proteins responsible for the transduction of a diverse array of extracellular signals (15,22). Dysfunctional GPCR signaling transduction in the cardiovascular system may be an important contributor to the initiation, establishment and/or maintenance of hypertension (22-24). Genetic variation in apelin and its receptor likely contributes to essential hypertension, BMI and the onset age of hypertension (14). APJ and angiotensin receptor 1 as well as apelin and angiotensinogen show significant similarity in tissue distribution, suggesting that apelin and angiotensin II may be involved in the same biological process. Angiotensin is known to be a significant vasopressor, whereas apelin reduces blood pressure. In addition, apelin has not only been observed to alter arterial pressure, but the peptide also exhibits endothelium-dependent vasodilator actions *in vivo* and positive inotropic actions in the isolated heart (25). On the basis of these considerations, our study presents significant data with potential clinical implications.

Our results showed that in a subpopulation of patients with hypertension there was a significant increase in the frequency of the G allele than in patients with normal blood pressure. Moreover, a significant increase in the frequency of the homozygous AA genotype was noted in CAD patients without hypertension, when compared with patients with hypertension.

The APJ receptor gene was recently discovered, and the functional characterization of the apelinergic system began just after the identification of its endogenous ligand. In addition, the transcriptional regulation of the APJ gene appears to be complex and is currently poorly understood (26,27). For these reasons, the same hypothesis was formulated. The A allele, although its functional role has not yet been identified, may identify a functional recessive allele. We speculate that this polymorphism may not be a marker of CAD, but it may cause a gain in function of the apelin/APJ system and may be a genetic predisposing factor for hypertension. In particular, the APJ receptor gene is unlikely to be a gene causing hypertension, but it appears to be a modifier gene involved in the assessment of risk of hypertension.

A recent study also showed that patients carrying at least one copy of 212A had a significantly lower risk of heart failure (HF)-related events than those who were homozygous for the G212 variant (28). Our data confirm these results; in fact, the relative risk of HF among patients with hypertension, compared to that of the general population, was estimated to be 1.4 in an analysis from the First National Health and Nutrition Examination Survey (29).

In conclusion, our findings reveal an innovative line of research into the apelin/APJ system that involves a novel aspect of hypertension development. Greater understanding is also required of the molecular mechanisms governing apelin and APJ gene expression, as well as the processes involved in post-translational processing and metabolism of apelin peptides. Further investigation into this signaling pathway may further enhance the understanding of the pathophysiology and therapy of hypertension.

References

1. Hiura Y, Tabara Y, Kokubo Y, Okamura T, Miki T, Tomoike H and Iwai N: A genome-wide association study of hypertension-related phenotypes in a Japanese population. Circ J 74: 2353-2359, 2010.
2. Ehret GB: Genome-wide association studies: contribution of genomics to understanding blood pressure and essential hypertension. Curr Hypertens Rep 12: 17-25, 2010.
3. Tatemoto K, Hosoya M, Habata Y, Fujii R, Kakegawa T, Zou MX, Kawamata Y, Fukusumi S, Hinuma S, Kitada C, Kurokawa T, Onda H and Fujino M: Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. Biochem Biophys Res Commun 251: 471-476, 1998.
4. Lee DK, Cheng R, Nguyen T, Fan T, Kariyawasam AP, Liu Y, Osmond DH, George SR and O'Dowd BF: Characterization of apelin, the ligand for the APJ receptor. J Neurochem 74: 34-41, 2000.
5. Hosoya M, Kawamata Y, Fukusumi S, Fujii R, Habata Y, Hinuma S, Kitada C, Honda S, Kurokawa T, Onda H, Nishimura O and Fujino M: Molecular and functional characteristics of APJ. Tissue distribution of mRNA and interaction with the endogenous ligand apelin. J Biol Chem 275: 21061-21067, 2000.
6. Kawamata Y, Habata Y, Fukusumi S, Hosoya M, Fujii R, Hinuma S, Nishizawa N, Kitada C, Onda H, Nishimura O and Fujino M: Molecular properties of apelin: tissue distribution and receptor binding. Biochim Biophys Acta 1538: 162-171, 2000.
7. De Falco M, de Luca L, Onori N, Cavallotti I, Artigiano F, Esposito V, de Luca B, Laforgia V, Groeger AM and de Luca A: Apelin expression in normal human tissues. In Vivo 16: 333-336, 2002.
8. Medhurst AD, Jennings CA, Robbins MJ, Davis RP, Ellis C, Winborn KY, Lawrie KW, Hervieu G, Riley G, Bolaky JE, Herrity NC, Murdock P and Darker JG: Pharmacological and immunohistochemical characterization of the APJ receptor and its endogenous ligand apelin. J Neurochem 84: 1162-1172, 2003.
9. O'Carroll AM, Selby TL, Palkovits M and Lolait SJ: Distribution of mRNA encoding B78/apj, the rat homologue of the human APJ receptor, and its endogenous ligand apelin in brain and peripheral tissues. Biochim Biophys Acta 1492: 72-80, 2000.
10. Reaux-Le Goazigo A, Alvear-Perez R, Zizzari P, Epelbaum J, Bluet-Pajot MT and Llorens-Cortes C: Cellular localization of apelin and its receptor in the anterior pituitary: evidence for a direct stimulatory action of apelin on ACTH release. Am J Physiol Endocrinol Metab 292: E7-E15, 2007.
11. Kleinz MJ, Skepper JN and Davenport AP: Immunocytochemical localization of the apelin receptor, APJ, to human cardiomyocytes, vascular smooth muscle and endothelial cells. Regul Pept 126: 233-240, 2005.
12. Tatemoto K, Takayama K, Zou MX, Kumaki I, Zhang W, Kumano K and Fujimiya M: The novel peptide apelin lowers blood pressure via a nitric oxide-dependent mechanism. Regul Pept 99: 87-92, 2001.
13. Ishida J, Hashimoto T, Hashimoto Y, Nishiwaki S, Iguchi T, Harada S, Sugaya T, Matsuzaki H, Yamamoto R, Shiota N, Okunishi H, Kihara M, Umemura S, Sugiyama F, Yagami K, Kasuya Y, Mochizuki N and Fukamizu A: Regulatory roles for APJ, a seven-transmembrane receptor related to angiotensin-type 1 receptor in blood pressure in vivo. J Biol Chem 279: 26274-26279, 2004.
14. Li WW, Niu WQ, Zhang Y, Wu S, Gao PJ and Zhu DL: Family-based analysis of apelin and AGTRL1 gene polymorphisms with hypertension in Han Chinese. J Hypertens 27: 1194-1201, 2009.
15. Szokodi I, Tavi P, Földes G, Voutilainen-Myllylä S, Ilves M, Tokola H, Pikkarainen S, Piuhola J, Rysä J, Tóth M and Ruskoaho H: Apelin, the novel endogenous ligand of the orphan receptor APJ, regulates cardiac contractility. Circ Res 91: 434-440, 2002.

16. Taheri S, Murphy K, Cohen M, Sujkovic E, Kennedy A, Dhillo W, Dakin C, Sajedi A, Ghatei M and Bloom S: The effects of centrally administered apelin-13 on food intake, water intake and pituitary hormone release in rats. Biochem Biophys Res Commun 291: 1208-1212, 2002.
17. Kasai A, Shintani N, Oda M, Kakuda M, Hashimoto H, Matsuda T, Hinuma S and Baba A: Apelin is a novel angiogenic factor in retinal endothelial cells. Biochem Biophys Res Commun 325: 395-400, 2004.
18. Xie H, Yuan LQ, Luo XH, Huang J, Cui RR, Guo LJ, Zhou HD, Wu XP and Liao EY: Apelin suppresses apoptosis of human osteoblasts. Apoptosis 12: 247-254, 2007.
19. O'Dowd BF, Heiber M, Chan A, Heng HH, Tsui LC, Kennedy JL, Shi X, Petronis A, George SR and Nguyen T: A human gene that shows identity with the gene encoding the angiotensin receptor is located on chromosome 11. Gene 136: 355-360, 1993.
20. Masri B, Knibiehler B and Audigier Y: Apelin signalling: a promising pathway from cloning to pharmacology. Cell Signal 17: 415-426, 2005.
21. Chen MM, Ashley EA, Deng DX, Tsalenko A, Deng A, Tabibiazar R, Ben-Dor A, Fenster B, Yang E, King JY, Fowler M, Robbins R, Johnson FL, Bruhn L, McDonagh T, Dargie H, Yakhini Z, Tsao PS and Quertermous T: Novel role for the potent endogenous inotrope apelin in human cardiac dysfunction. Circulation 108: 1432-1439, 2003.
22. Heximer SP, Knutsen RH, Sun X, Kaltenbronn KM, Rhee MH, Peng N,Oliveira-dos-Santos A, Penninger JM, Muslin AJ, Steinberg TH, Wyss JM, Mecham RP and Blumer KJ: Hypertension and prolonged vasoconstrictor signaling in RGS2-deficient mice. J Clin Invest 111: 445-452, 2003.
23. Hao L, Du M and Lopez-Campistrous A: Agonist-induced activation of matrix metalloproteinase-7 promotes vasoconstriction through the epidermal growth factor-receptor pathway. Circ Res 94: 68-76, 2004.
24. Katugampola SD, Kuc RE, Maguire JJ and Davenport AP: G-protein-coupled receptors in human atherosclerosis: comparison of vasoconstrictors (endothelin and thromboxane) with recently de-orphanized (urotensin-II, apelin and ghrelin) receptors. Clin Sci 48: S171-S175, 2002.
25. Charles CJ: Putative role for apelin in pressure/volume homeostasis and cardiovascular disease. Cardiovasc Hematol Agents Med Chem 5: 1-10, 2007.
26. Lee DK, George SR and O'Dowd B: Unravelling the roles of the apelin system: prospective therapeutic applications in heart failure and obesity. Trends Pharmacol Sci 4: 190-194, 2006.
27. O'Carroll AM, Lolait SJ and Howell GM: Transcriptional regulation of the rat apelin receptor gene: promoter cloning and identification of an Sp1 site necessary for promoter activity. J Mol Endocrinol 36: 221-235, 2006.
28. Sarzani R, Forleo C, Pietrucci F, Capestro A, Soura E, Guida P, Sorrentino S, Iacoviello M, Romito R, Dessì-Fulgheri P, Pitzalis M and Rappelli A: The 212A variant of the APJ receptor gene for the endogenous inotrope apelin is associated with slower heart failure progression in idiopathic dilated cardiomyopathy. J Card Fail 13: 521-529, 2007.
29. He J, Ogden LG, Bazzano LA, Vupputuri S, Loria C and Whelton PK: Risk factors for congestive heart failure in US men and women: NHANES I epidemiologic follow-up study. Arch Intern Med 161: 996, 2001.