



Reduced apelin levels in lone atrial fibrillation

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KEYWORDS

Atrial fibrillation;
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Aims Apelin is an endogenous peptide hormone that appears to have a physiological role in counter-regulation of the angiotensin and vasopressin systems. This peptide has been reported to be down-regulated in subjects with acute heart failure, but has not been studied in other cardiovascular conditions. We studied apelin levels in 73 subjects with lone atrial fibrillation (AF).

Methods and results Study subjects had electrocardiographic evidence of paroxysmal or chronic AF and a structurally normal heart on echocardiography. Subjects were excluded if they had a history of coronary artery disease, rheumatic heart disease, cardiomyopathy, significant valvular disease, hyperthyroidism, or antecedent hypertension. Controls were recruited from a healthy outpatient population. Plasma apelin levels were determined using a commercially available immunoassay. Seventy-three subjects with lone AF and 73 healthy controls were enrolled and studied. Mean levels of apelin were significantly lower in subjects with lone AF when compared with controls (307 vs. 648 pg/mL, $P < 0.00005$).

Conclusion Reduced apelin levels were observed in this homogenous population of lone AF subjects and may represent an underlying diathesis predisposing to this common arrhythmia.

Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia and is associated with significant morbidity and mortality.¹ AF is also a major independent risk factor for the subsequent development of left ventricular dysfunction, with up to one-third of those affected by the arrhythmia developing congestive heart failure (CHF) within 10 years.² Dissection of the aetiology of AF is likely to shed light not only on the mechanisms of this arrhythmia, but also may offer insights into the early pathobiology of CHF. Recent work has identified a substantial heritable contribution to AF, particularly in subjects with lone AF where as many as 30% may have a family history.^{3–5} These data raise the possibility that substantial intrinsic differences in the individual threshold for this arrhythmia may exist.

To understand the mechanisms of AF, we are studying biomarkers that might identify those individuals with an increased propensity to the arrhythmia. There is some evidence of a subtle underlying atrial or ventricular myopathy even in lone AF.^{6,7} However, the relationship between AF and myocardial abnormalities has been obscured to some extent by the rapid cellular remodelling seen with the arrhythmia.^{8–10} We hypothesized that there might be abnormalities in atrial endocrine function early in the course of primary forms of AF, and have therefore explored humoral axes involved in signalling to and from atrial cells. One such endocrine axis is the recently described apelin-angiotensin receptor-like 1 (APJ) pathway.¹¹

The APJ receptor belongs to a family of 7-transmembrane G-protein coupled receptors first cloned in 1993.¹² Orphaned for many years, the endogenous ligand, apelin, was subsequently isolated.¹¹ Expression of both the APJ receptor and its ligand parallels that of the angiotensin receptor AT1 and angiotensinogen, respectively, suggesting a role in similar biological processes.^{13,14} Intraperitoneal administration of apelin in rats has been shown to result in short-term increase in drinking behaviour, a finding similar to the thirst-promoting effect of angiotensin II.¹⁵ However, administration of intravenous apelin lowered blood pressure in anaesthetized rats through a nitric oxide dependent pathway.¹⁶ Apelin also is one of the most potent endogenous positive inotropic substances yet identified.¹⁷ These results combined with recent evidence of increased pressor responses in mice null at the APJ receptor suggest that the apelin-APJ axis plays an important counter-regulatory role to the effects of angiotensin.¹⁸

Circulating apelin levels are elevated early in the natural history of heart failure, but ultimately are depressed in overt CHF.¹⁹ Interestingly, following ventricular offloading in severe heart failure using a left ventricular assist device, expression of the APJ gene is markedly up-regulated, whereas the natriuretic peptide genes are down-regulated.²⁰ In a homogeneous group of patients with lone AF, we found a highly significant reduction of plasma apelin levels when compared with a matched control population. Apelin-12 levels also were inversely correlated with levels of nt-proBNP. These findings confirm a subtle but definite perturbation of the cardiac humoral axis in individuals with a history of AF but without overt structural heart disease.

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Methods

Study population

All studies were performed with Institutional Review Board approval at the Massachusetts General Hospital. Prior to any study procedures, written informed consent was obtained from each study subject. Individuals were considered eligible for enrolment if they had at least one documented EKG with AF and had a structurally normal heart on echocardiography. Subjects were excluded if they had a history of coronary artery disease, rheumatic heart disease, cardiomyopathy, significant valvular disease, hyperthyroidism, or hypertension.

Seventy-three subjects with lone AF were enrolled between 5 July 2001 and 17 April 2002. These subjects were matched on the basis of age, sex, race, and ethnicity to 73 control subjects recruited from a healthy outpatient population (Genomics Collaborative Inc.).

Clinical characterization

Each subject underwent a physical examination and a structured interview to elicit details of symptoms, past medical history, medications, and possible triggers for AF. The medical history of all first-degree relatives was obtained using a standardized questionnaire. All subjects with lone AF underwent electrocardiography at enrolment and a standardized echocardiogram was also obtained from each individual. This study included a comprehensive 2D, M-mode, and Doppler evaluation. Ejection fraction was estimated using the modified Quinones method.²¹ Blood samples for serologic analyses were drawn at enrolment from each subject in the sitting position.

Blood sampling and apelin-12 hormone assay

Blood samples were obtained in EDTA containing tubes and centrifuged. Plasma was extracted, aliquoted, and stored at -80°C until analysis. Plasma apelin-12 levels were determined using a commercially available enzyme immunoassay without extraction (Phoenix Pharmaceuticals, Belmont, CA, USA) according to manufacturer's instructions. This assay employs an immunoaffinity purified rabbit antibody specific for apelin 1-12. The antibody has 100% cross-reactivity to apelin 1-12, 1-13, and 1-36; there is no cross-reactivity to ADM-52, BNP-32, CNP-22, ANP (25-56), ghrelin, ET-1, or bradykinin. Plasma proANP and nt-proBNP levels were determined using commercially available enzyme immunoassays without extraction (distributed in the United States by ALPCO diagnostics, Windham, NH, and manufactured by Biomedica Gruppe, Germany) according to manufacturer's instructions. All assays were performed in duplicate with intra-experimental standards using a Victor 3 plate reader (Perkin-Elmer, Wellesley, MA, USA). Values were normalized to a standard curve. The intra-assay and interassay variances for apelin-12 were 24 and 18%, respectively.

Statistical analysis

Plasma apelin-12 values were highly skewed and therefore were log transformed prior to analysis. Normally distributed values are displayed as means with 95% confidence intervals. For comparisons between lone AF and control populations, cases were paired with healthy controls matched for age, gender, race, and ethnicity. The means of normally distributed continuous variables were compared using paired *t*-tests. Differences between groups for categorical variables were compared using a χ^2 or Fisher's exact test.

In subjects with lone AF, a multivariate analysis was performed to determine the correlates of apelin-12 levels by regressing log-transformed apelin-12 values on clinical variables with a *P*-value of ≤ 0.1 on univariate analysis. Data were compiled and analysed in MS Excel 2000, MS Access 2000 (Microsoft Office 2000, Redmond, WA, USA), and Intercooled Stata 8.0 (Stata Corp., College Station, TX, USA).

Results

Baseline characteristics

During the study period, a total of seventy-three subjects with lone AF were enrolled. These subjects with lone AF were matched to 73 healthy controls based on age, gender, race, and ethnicity. Body mass index and systolic and diastolic blood pressures were similar between subjects and controls (*Table 1*).

The mean age at diagnosis with AF was 47.1 ± 11.3 years, and mean age at enrolment was 54.2 ± 10.1 years. As observed in other cohorts with lone AF, subjects were predominantly male (79.5%). Ninety-six per cent of subjects initially presented with paroxysmal AF, and 89% of subjects remained in paroxysmal AF at study enrolment (*Table 2*). The majority of subjects with lone AF had frequent, paroxysmal arrhythmias with 53% reporting more than 100 lifetime episodes. Although 27% of subjects in sinus rhythm had evidence of left atrial enlargement on EKG, there were no other electrocardiographic abnormalities.

Table 1 Baseline characteristics of subjects with lone AF and controls

Baseline characteristics	Lone AF	Controls
Number	73	73
Male	79.5 % (58)	79.5 % (58)
Age at enrolment	54.2 (CI 51.9–56.5)	54.3 (CI 51.8–56.7)
BMI	26.5 (CI 25.5–27.4)	26.9 (CI 25.9–28.0)
Systolic blood pressure (mmHg)	121.5 (CI 118.4–124.6)	126.6 (123.1–130.0)
Diastolic blood pressure (mmHg)	75.0 (CI 73.2–76.7)	77.7 (74.8–80.6)
Race and ethnicity		
Caucasian	97.3 % (71)	97.3 % (71)
African-American	1.4 % (1)	1.4 % (1)
Asian	1.4 % (1)	1.4 % (1)
<i>Medications</i>		
Beta-blocker	53.4 (39)	0
Digoxin	11.0 (8)	0
Calcium channel blocker	19.2 (14)	0
Lipid lowering agent	13.7 (10)	0
ACE-inhibitor or ARB	0	0
Antiarrhythmic agent	42 (58)	0
Amiodarone	9 (12)	
Sotalol	4 (5)	
Propafenone	11 (15)	
Flecainide	14 (19)	
Other	4 (5)	
<i>Personal history of AF</i>		
Age at first diagnosis of AF	47.1 (CI 44.5–49.7)	—
Over 100 episodes AF	53.1 (34)	—
Paroxysmal AF at initial presentation	95.9 (70)	—
Paroxysmal AF at study enrolment	89.0 (65)	—
History of an electrical cardioversion	34.3 (25)	—

Table 2 Electrocardiographic and echocardiographic characteristics of subjects with lone AF

Electrocardiogram	
Normal sinus rhythm	25 (34.3)
Sinus bradycardia	33 (45.2)
AF	8 (11.0)
Atrial flutter	3 (4.1)
Paced or other rhythm	4 (5.5)
Mean ventricular rate (b.p.m.)	62.6 (CI 58.8–66.4)
P–R interval (ms)	175 (CI 167–184)
QRS interval (ms)	93.9 (CI 91.2–96.5)
QTc interval (ms)	406 (CI 396–416)
Axis (°)	35.3 (CI 26–44.8)
Left atrial enlargement	16 (26.7)
Left ventricular hypertrophy	2 (2.9)
Echocardiogram	
Ejection fraction (%)	61.3 (CI 59.8–62.9)
Left atrial size (mm)	39.4 (CI 37.8–41.1)
Left ventricular internal dimension (mm)	49.9 (CI 48.7–51.2)
Aortic root (mm)	33.1 (CI 31.4–34.8)
Posterior wall thickness (mm)	9.8 (CI 9.4–10.0)
Interventricular septal wall thickness (mm)	10.2 (CI 9.7–10.6)

Values are presented as number (percentage) unless otherwise indicated.

Echocardiography was notable for a mean left atrial diameter at the upper limits of normal. The mean values of all other echocardiographic parameters, including chamber dimensions, wall thicknesses, and functional indices, were normal in the study cohort. Control subjects had no significant past medical history.

Apelin levels

Mean apelin-12 levels were significantly decreased in subjects with lone AF when compared with matched controls (187 vs. 304 pg/mL, $P < 0.00005$) (*Figure 1*). As previously reported, mean nt-proBNP levels were elevated [187 fmol/mL (CI 161–215) vs. 145 fmol/mL (CI 116–173), $P = 0.0016$], but mean proANP levels were unchanged [2421 fmol/mL (CI 1421–3421) vs. 2166 fmol/mL (CI 1658–2673), $P = 0.52$] in lone AF subjects when compared with controls. At the time of sampling, 11 subjects were in AF and 58 subjects were in sinus rhythm; there was no difference in the median apelin levels between these two groups (302 vs. 282 pg/mL, $P = 0.08$). A strong negative correlation was observed between apelin-12 and nt-proBNP levels in subjects with lone AF ($r = -0.32$, $P = 0.005$). A weak negative correlation was noted between systolic blood pressure and apelin-12 levels ($r = -0.21$, $P = 0.078$).

Multivariable correlates of apelin levels

The results of multivariable regression modelling using clinical variables are illustrated in *Table 3*. Clinical variables with $P \leq 0.1$ on univariate analysis included log (nt-proBNP) and systolic blood pressure. A significant association was noted only between log (apelin-12) and log (nt-proBNP) values ($\beta = -0.184$, $SE = 0.072$, $P = 0.012$). The proportion of variance in apelin-12 levels explained by the multivariable model was 0.36.

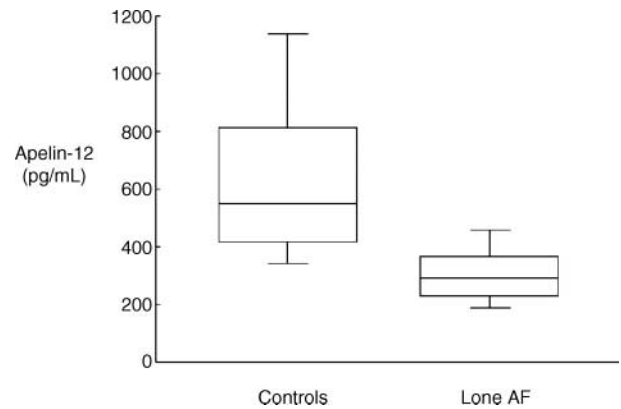


Figure 1 Box plots illustrating the median levels of apelin in subjects with LAF and in healthy controls. Boxes show interquartile ranges, and the bars represent the 10th and 90th percentiles.

Table 3 Predictors of log (apelin) from multivariable linear regression among subjects with lone AF

Clinical variable	β	SE	P -value
Systolic blood pressure	-0.002	0.001	0.107
Log (nt-proBNP)	-0.184	0.072	0.012

To assess the utility of apelin levels in discriminating between those with lone AF and normal controls, we plotted the receiver operating characteristic (ROC) curve for the reciprocal of apelin-12 in this context (*Figure 2*). The area under the curve was 0.89 (CI 0.84–0.94).

Discussion

We have demonstrated that apelin levels are significantly reduced in a cohort of well-characterized subjects with lone AF. These abnormalities of circulating apelin are present even when the study subjects are in sinus rhythm. In multivariable analysis, only nt-proBNP was significantly correlated with apelin levels. These data extend previous observations that lone AF is associated with abnormal natriuretic peptide profiles and suggest that atrial endocrine function may be disrupted in those who have had even a single episode of the arrhythmia.^{22,23}

Apelin was first identified as a native ligand of the orphan G-coupled receptor APJ.¹¹ This 13-amino acid peptide is cleaved from a pro-peptide by an unknown endopeptidase and is then active at a number of sites throughout the body. The biological roles of this peptide and others cleaved from the propeptide are only beginning to be explored.^{15,24,25} Expression of the apelin gene has been demonstrated in the hypothalamus, venous endothelium, and the atrium under physiological conditions in adult vertebrates.¹³ It is not known whether other tissues are capable of expressing the gene under stimulated conditions. The APJ receptor is expressed in the developing heart tubes and vasculature, as well as in adult endothelium, atrial endocardium, and central nervous system.¹⁴ There are significant parallels between the expression patterns of apelin and its receptor and those of cognate components

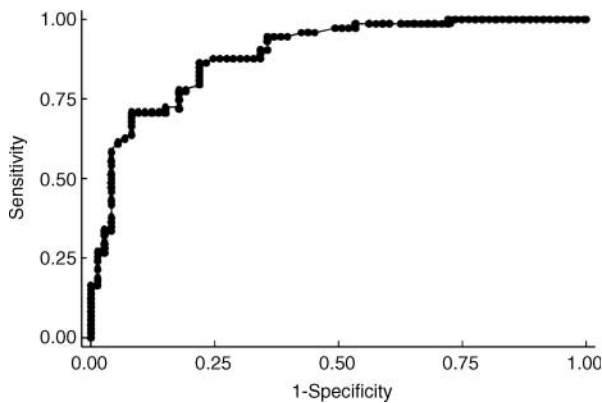


Figure 2 ROC curve comparing sensitivity and specificity of the reciprocal of apelin-12 levels differentiating between subjects with AF and healthy controls.

of the angiotensin system, suggesting that these two pathways might be involved in similar processes. Recent work has confirmed that apelin is a powerful vasodilator and positive inotrope, and appears to be counter-regulated, at least to some extent, with the angiotensinogen pathway.^{16,18} Apelin also may act centrally to counteract the vasopressin pathway in the regulation of extracellular fluid volume.²⁶

Our data broaden the association of AF with abnormal biomarker profiles which overlap substantially with those seen in CHF. Elevations in BNP, ANP, endothelin-1, and angiotensin II have all been described in AF cohorts, albeit with heterogeneous underlying pathologies.^{22,23,27-29} We have demonstrated that nt-proBNP and now apelin are abnormal even in lone AF patients in whom underlying structural heart disease has been rigorously excluded. AF has long been associated with myopathic remodelling, but these data and recent histological findings support a primary myocardial abnormality in predisposed individuals even when in sinus rhythm.^{6,7,30} Pathophysiological links between AF and CHF are gradually emerging. Epidemiological evidence of a reciprocal relationship between these two syndromes has been documented in several studies.^{2,31} In addition, blockade of the renin-angiotensin system has been shown to reduce the incidence of AF, strengthening the case for a pivotal role for this humoral axis in the arrhythmia.³²⁻³⁴ Although these similarities between AF and CHF may simply reflect shared reflex responses to reduced cardiac output, it is also conceivable that lone AF and CHF may share a common pathophysiological basis, at least in a subset of individuals.

This lone AF cohort is based in a tertiary care arrhythmia clinic, and may represent a selected subset of those with this form of arrhythmia. The demographics of the cohort suggest that they are comparable to previous studies of lone AF. Finally, it is not possible to completely eliminate the potential confounding influence of medication on biomarker levels. However, our study cohort is highly homogeneous and there was no discernable difference between apelin levels in subjects on any particular medication.

The sensitivity and specificity of depressed apelin levels in lone AF suggest that it may be possible to discriminate those with the underlying diathesis even when they are not in AF. Ultimately, it may be possible to use apelin levels as an index of subsequent risk of AF in individuals who have not

yet had a clinical arrhythmia. We have begun to explore this possibility in cohorts at increased risk of AF, including the relatives of AF probands, patients undergoing coronary artery bypass grafting, and prospectively collected cohorts of normal individuals. The ability to predict those who are susceptible to AF would allow those at risk to be treated prophylactically and may shed light on the early pathophysiology, not only of AF but also of CHF.

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