表 10. 65 歳未満男女の将来の虚血性心疾患発症を予測する血液検査項目のカットオフ値（AUC の大きさ順）

	Cutoff	感度	特異度	AUC	AUC 95%信頼区間		p値	
クレアチニン	1	0.547	0.530	0.550	0.544	0.557	<0.001	***
LDH	309	0.501	0.569	0.547	0.539	0.556	<0.001	***
血糖	99	0.391	0.668	0.536	0.526	0.546	<0.001	***
Na	142	0.360	0.695	0.534	0.522	0.545	<0.001	***
膵アミラーゼ	52	0.804	0.273	0.531	0.520	0.541	<0.001	***
血色素量	14.1	0.652	0.399	0.531	0.524	0.537	<0.001	***
尿素窒素	13.5	0.541	0.499	0.528	0.521	0.534	<0.001	***
中性脂肪	75	0.743	0.297	0.527	0.521	0.533	<0.001	***
尿酸	4.9	0.651	0.393	0.525	0.519	0.532	<0.001	***
GOT	22	0.422	0.614	0.523	0.517	0.529	<0.001	***
Ca	4.7	0.415	0.617	0.522	0.510	0.533	<0.001	***
βリポ蛋白	462	0.419	0.617	0.521	0.515	0.528	<0.001	***
白血球数	6400	0.381	0.652	0.520	0.514	0.526	<0.001	***
γGTP	13	0.695	0.338	0.519	0.513	0.525	<0.001	***
HDL コレステロール	66	0.320	0.712	0.519	0.512	0.525	<0.001	***
アルブミン	4.3	0.712	0.316	0.515	0.509	0.521	<0.001	***
LDL コレステロール	108.4	0.594	0.434	0.514	0.508	0.520	<0.001	***
総コレステロール	224	0.260	0.770	0.513	0.507	0.520	<0.001	***
GPT	19	0.493	0.525	0.510	0.504	0.516	0.001	***
Cl	106	0.227	0.799	0.509	0.497	0.520	0.131	NS
血液像リンパ球	32.5	0.619	0.398	0.507	0.500	0.513	0.036	*
血液像好中球	54	0.675	0.339	0.506	0.500	0.512	0.053	NS
血小板数	22.3	0.715	0.300	0.506	0.500	0.512	0.061	NS
HbA1c	4.4	0.817	0.208	0.505	0.494	0.516	0.331	NS
総蛋白	7.7	0.187	0.822	0.505	0.499	0.511	0.096	NS
K	3.9	0.902	0.110	0.505	0.493	0.516	0.412	NS
血沈1時間	4	0.609	0.405	0.500	0.494	0.506	0.924	NS

表 11. 65 歳未満男性の将来の虚血性心疾患発症を予測する血液検査項目のカットオフ値（AUC の大きさ順）

	Cutoff	感度	特異度	AUC	AUC 95%信頼区間		p値	
LDH	253	0.773	0.291	0.543	0.533	0.553	<0.001	***
膵アミラーゼ	51	0.814	0.273	0.537	0.525	0.550	<0.001	***
クレアチニン	1	0.730	0.316	0.531	0.523	0.538	<0.001	***
アルブミン	4.3	0.756	0.284	0.524	0.517	0.532	<0.001	***
尿素窒素	12.8	0.683	0.352	0.524	0.517	0.531	<0.001	***
K	4.3	0.439	0.596	0.521	0.507	0.534	0.003	**
GPT	22	0.515	0.518	0.519	0.512	0.526	<0.001	***
血沈1時間	7	0.228	0.801	0.518	0.511	0.526	<0.001	***
HbA1c	4.8	0.478	0.563	0.518	0.505	0.531	0.006	**
血糖	108	0.202	0.841	0.517	0.505	0.529	0.006	**
Cl	106	0.235	0.798	0.511	0.498	0.525	0.099	NS
白血球数	6400	0.441	0.580	0.511	0.504	0.518	0.003	**
Na	142	0.399	0.631	0.511	0.497	0.525	0.108	NS
血液像好中球	55.9	0.586	0.434	0.509	0.502	0.517	0.017	*
Ca	4.8	0.266	0.758	0.507	0.493	0.521	0.307	NS
尿酸	5.2	0.774	0.246	0.506	0.498	0.513	0.134	NS
γGTP	20	0.623	0.393	0.504	0.497	0.512	0.231	NS
βリポ蛋白	420	0.617	0.395	0.504	0.496	0.512	0.306	NS
血色素量	14.2	0.863	0.154	0.504	0.497	0.511	0.306	NS
血液像リンパ球	32.5	0.610	0.403	0.503	0.496	0.511	0.406	NS
GOT	26	0.290	0.726	0.503	0.496	0.510	0.450	NS
総蛋白	7.6	0.227	0.779	0.502	0.495	0.510	0.521	NS
HDLコレステロール	57	0.410	0.605	0.501	0.494	0.508	0.791	NS
中性脂肪	87	0.735	0.281	0.501	0.494	0.508	0.807	NS
総コレステロール	224	0.251	0.766	0.501	0.494	0.508	0.832	NS
血小板数	35.1	0.042	0.966	0.500	0.493	0.508	0.952	NS
LDLコレステロール	84.2	0.869	0.149	0.500	0.493	0.507	0.985	NS

表 12. 65 歳未満女性の将来の虚血性心疾患発症を予測する血液検査項目のカットオフ値（AUC の大きさ順）

	Cutoff	感度	特異度	AUC	AUC 95%信頼区間		p値	
LDH	319	0.450	0.636	0.556	0.540	0.572	<0.001	***
Na	141	0.464	0.623	0.544	0.523	0.566	<0.001	***
血沈1時間	9	0.573	0.492	0.542	0.530	0.553	<0.001	***
中性脂肪	69	0.638	0.432	0.540	0.529	0.552	<0.001	***
血糖	90	0.597	0.464	0.539	0.521	0.558	<0.001	***
総コレステロール	210	0.411	0.657	0.538	0.527	0.550	<0.001	***
βリポ蛋白	438	0.351	0.714	0.537	0.525	0.549	<0.001	***
膵アミラーゼ	73	0.695	0.371	0.535	0.516	0.554	<0.001	***
LDL コレステロール	127.2	0.364	0.704	0.535	0.523	0.546	<0.001	***
Ca	4.5	0.756	0.300	0.534	0.513	0.556	0.001	**
GOT	17	0.677	0.380	0.534	0.523	0.545	<0.001	***
クレアチニン	0.8	0.697	0.351	0.534	0.522	0.545	<0.001	***
総蛋白	7.3	0.643	0.395	0.527	0.516	0.538	<0.001	***
血小板数	25.9	0.475	0.566	0.521	0.510	0.532	<0.001	***
GPT	18	0.278	0.751	0.519	0.508	0.530	0.001	***
HbA1c	4.6	0.670	0.368	0.518	0.497	0.539	0.084	NS
血液像リンパ球	29.3	0.790	0.247	0.518	0.506	0.529	0.002	**
アルブミン	4.3	0.635	0.391	0.517	0.506	0.528	0.003	**
尿酸	4.6	0.330	0.698	0.516	0.505	0.527	0.003	**
尿素窒素	11.6	0.684	0.344	0.514	0.503	0.525	0.012	*
血色素量	14.3	0.097	0.926	0.508	0.497	0.519	0.150	NS
γGTP	13	0.417	0.601	0.508	0.497	0.519	0.165	NS
白血球数	7000	0.155	0.865	0.507	0.496	0.518	0.212	NS
HDL コレステロール	70	0.435	0.589	0.506	0.495	0.517	0.263	NS
Cl	104	0.645	0.371	0.506	0.485	0.527	0.576	NS
K	4.4	0.190	0.823	0.502	0.481	0.524	0.821	NS
血液像好中球	54.2	0.669	0.345	0.502	0.490	0.513	0.753	NS

Ⅲ. 研究成果の刊行に関する一覧表

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Ⅳ. 研究成果の刊行物・別刷

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Circulating cathepsin K as a potential novel biomarker of coronary artery disease

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ABSTRACT

Background: Cathepsin K (CatK) is one of the most potent mammalian collagenases involved in atherosclerosis-based vascular disease. We investigated whether circulating CatK is associated with the prevalence of coronary artery disease (CAD).

Methods: Two-hundred fifty-two consecutive subjects were enrolled from among patients who underwent coronary angiography and intravascular ultrasound analyses. One-hundred thirty-two age-matched subjects served as controls. Plasma CatK, intact procollagen type I N-terminal propeptide (I-PINP), and linked carboxy-terminal telopeptide of collagen type I (ICTP) were measured.

Results: Patients with CAD had higher CatK levels (44.0 ± 31.2 versus 15.5 ± 8.3 ng/mL, $P < 0.001$) and ICTP/I-PINP ratios (0.2 ± 0.1 versus 0.04 ± 0.03, $P < 0.001$) than the controls. Patients with acute coronary syndrome had higher CatK levels than those with stable angina pectoris. Overall, linear regression analysis showed that the CatK levels correlated positively with ICTP/I-PINP ratios ($r = 0.41$, $P < 0.001$). Multiple logistic regression analysis showed that CatK levels were independent predictors of CAD (odds ratio, 1.15; 95% CI, 1.07 to 1.23; $P < 0.01$). Furthermore, CatK levels were also correlated positively with percent plaque volumes and inversely with percent fibrous volumes by intravascular ultrasound.

Conclusions: These data indicated that high levels of CatK are closely linked with the presence of CAD and that CatK serves as a novel biomarker for CAD.

1. Introduction

In the initial years after their discovery, cysteinyl cathepsins were shown to localize in lysosomes and endosomes and to function there to degrade unwanted intracellular or endocytosed proteins [1]. Recent studies have discovered non-traditional roles for Cats in the extracellular space during the development and progression of cardiovascular disease [2–4]. Among cathepsins, cathepsin K (CatK), which is one of the most potent mammalian collagenases, was first identified in macrophages [5]. Previous studies have shown that CatK abounds in vascular smooth muscle cells and endothelial cells and infiltrated macrophages of human and animal atherosclerotic lesions [6–8]. Consistent with these biochemical observations, these vascular cells and macrophages can secrete CatK, which degrades type I collagen and elastin [9,10]. CatK deficiency has been shown to reduce diet-induced atherosclerotic lesion formation [11]. Recent studies have shown that the levels of serum CatS or CatL were increased in patients with diabetes and chronic kidney disease [12,13]. These data suggested that Cats levels are associated with atherosclerosis-based cardiovascular disease. However, nothing is known about the relationship between CatK and coronary artery disease (CAD). Here, we investigated whether plasma CatK levels are associated with the presence of CAD.

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2. Methods

2.1. Study population and definition

From January 2009 to May 2011, a total of 252 consecutive patients with CAD from Nagoya University Hospital (Nagoya, Japan) were enrolled into the study. The patients with CAD were subgrouped into stable angina pectoris (SAP; $n = 197$), unstable angina pectoris (UAP; $n = 33$), and acute myocardial infarction (AMI; $n = 22$) groups by symptoms and clinical examinations. SAP was diagnosed as invariable exertional chest pain over the 3 months before going to the hospital (with invariable meaning the same degree of exertion and excitation provocation and the same location, quality, and 3- to 5-min duration), and was relieved by rest or nitroglycerin. UAP was diagnosed by typical chest pain at rest in the 24 h before going to the hospital, depressed ST $\geq$ 0.1 mV, and/or T-wave inversion on electrocardiogram but normal creatine kinase-MB level. The diagnosis of AMI was based on elevation of cardiac biomarkers (at least 1 positive biomarker: creatine kinase-MB, or troponin T) and electrocardiogram indicative of new ischemia (new ST-T change or new left bundle branch block) and a history of prolonged chest pain [14]. Age-matched control subjects were selected from among apparent healthy subjects who had visited Nagoya Chunichi Hospital for a routine annual checkup. A total of 132 subjects who showed no evidence of cardiovascular disease—defined as no typical chest pain on exertion, no myocardial infarction by history or electrocardiogram, negative exercise test, and no current medications—were recruited as non-CAD controls. In addition, diabetes mellitus was confirmed if patients had hemoglobin A1c levels $\geq$6.5%, a fasting plasma glucose concentration >126 mg/dl, and/or a history of any anti-hyperglycemic medication or previous diagnosis of diabetes. Hypertension was defined as a systolic blood pressure >140 mmHg, a diastolic blood pressure >90 mmHg, and/or having received treatment for hypertension. Patients were excluded if they had prior evidence of cardiomyopathy, primary valvular disease, congenital heart disease, end-stage renal disease with maintenance hemodialysis, and secondary cardiac muscle disease caused by any known systemic condition. The study protocol was approved by the ethics committee of the Nagoya University School of Medicine and the Chunichi Hospital, and written informed consent was obtained from all patients and control subjects.

A blood sample was obtained prior to percutaneous coronary intervention, and various lipids, hemoglobin A1c (HbA1c), and inflammatory profiles were measured. The age, gender, smoking history, body mass index, systolic and diastolic blood pressures, and medication history were recorded for each subject.

3. Laboratory examination

Human plasma CatK levels were determined by using ELISA kits (Biomedica Gruppe, Biomedica Medizinprodukte, Vienna, Austria) in duplicate. Serum levels of intact procollagen type I N-terminal propeptide (I-PINP), carboxy-terminal telopeptide of collagen type I (ICTP), creatinine, low density lipoprotein (LDL), high density lipoprotein (HDL), high-sensitivity C-reactive protein (hs-CRP), and hemoglobin A1c were measured at a commercial laboratory (SRL, Tokyo, Japan). Plasma CatK values were expressed as ng/mL, and interassay and intraassay coefficients of variation were 4.6% and 6.1%, respectively.

4. Quantitative coronary angiogram (QCA)

Coronary angiography was obtained prior to percutaneous coronary intervention. Angiography showing the maximal degree of stenosis was adapted for QCA. QCA analysis was performed using a contour detection minimum cost algorithm (QCA-CMS Version 3.0; Medis, Leiden, The Netherlands). All CAD patients had coronary artery severe stenosis defined as the presence of $\geq$50% diameter stenosis of at least one major artery. The reference segment diameter was averaged from 5-mm long angiographically normal segments proximal to the lesion; if a normal proximal segment could not be identified, a distal angiographically normal segment was analyzed as previously described [15]. Coronary arterial plaque volumes and characteristics were analyzed by a conventional IVUS in conjunction with an integrated backscatter (IB)-IVUS.

5. Gray-scale intravascular ultrasound (IVUS)

Patients with CAD received IVUS imaging analysis prior to percutaneous coronary intervention for left anterior descending coronary artery, left circumflex coronary artery, or right coronary artery lesions with severe stenosis (defined as percent stenosis $\geq$50% diameter) as determined by QCA. Patients were excluded if they had severe stenotic left main coronary artery lesions for which bypass revascularization would be needed.

A commercially available imaging system (Clear View; Boston Scientific, Natick, MA) with a motorized pullback device (SciMed, Fremont, CA) and a 40 MHz IVUS catheter were used for the Gray-scale IVUS analysis. The external elastic membrane (EEM) and lumen were traced by manual planimetry according to the American College of Cardiology guidelines [15]. The cross-sectional area (CSA) of the EEM was measured by tracing the leading edge of the adventitia. Plaque plus media CSA was calculated as (EEM−lumen CSA). The percent plaque area was defined as: [(EEM area−lumen area)/EEM area] × 100. Grayscale 3-dimensional IVUS image analysis was performed to compute the vessel volume, lumen volume, and total plaque volume (sum of the EEM, lumen CSA, and plaque plus media CSA at 1-mm axial intervals for the analysis segments). The percent plaque volume (%) was calculated as (plaque volume/vessel volume) × 100.

6. IB-IVUS

IB signals were obtained with a commercially available system connected to the IVUS system (IB-IVUS; YD Co., Ltd., Nara, Japan). IB values for two histological categories (fibrous area and lipid area) were calculated as the average power of the ultrasound backscattered signal using a fast Fourier transform, measured in decibels. The lipid volume and fibrous volume were calculated from 3-dimensional IVUS images as the sum of the fibrous and lipid area in each CSA at 1-mm axis intervals, respectively. The percentages of the fibrous and lipid (volume) [fibrous and lipid (volume)/plaque area (volume) × 100] were calculated automatically.

7. Statistical analysis

Summary descriptive statistics for continuous parameters are presented as means ± SD. Categorical variables were compared among study groups by using the chi-square test. Student's *t*-test (for comparison of continuous parameters between two groups) or 1-way analysis of variance (for comparison of continuous parameters among 3 or more groups), followed by a Tukey post hoc test, was used to test significant differences. hs-CRP concentrations were logarithmically transformed because the data showed a skewed distribution. If the homogeneity of variance assumption was violated, the nonparametric Kruskal–Wallis test was used instead. The factors that were related at the $P < 0.05$ level were selected by univariable analyses as independent variable candidates for multiple logistic regression analysis, which were used to

evaluate the independent contribution of clinical parameters to CAD. Correlation coefficients were calculated using linear regression analysis. StatFlex (version 6.0; Artech, Osaka, Japan) was used for all statistical analyses. *P* values of less than 0.05 were considered statistically significant.

8. Results

The clinical characteristics of the patients with CAD ($n = 252$) and controls ($n = 132$) are shown in Table 1. There were no significant differences in age, gender, or body mass index. Patients with CAD had a significantly higher prevalence of hypertension and diabetes; they were also more likely to have had myocardial infarction or cerebrovascular disease, or to have undergone a coronary bypass graft or angioplasty. The frequencies of patients with CAD under treatment with antihypertensive, anti-lipid, anti-platelet, and anti-diabetic drugs were higher in the control subjects.

Compared with the control group, the plasma CatK levels (44.0 ± 31.2 versus 15.5 ± 8.3 ng/mL, $P < 0.001$) and ICTP/I-PINP ratios (0.2 ± 0.1 versus 0.04 ± 0.03, $P < 0.001$) were significantly increased in patients with CAD (Table 1). The levels of hs-CRP and hemoglobin A1c were higher and the LDL cholesterol and HDL cholesterol levels were lower in the CAD group than in control group ($P < 0.01$ for all comparisons), but there was no significant difference in the creatinine levels.

Table 2 shows the baseline characteristics of the SAP and UAP-AMI (patients with UAP or AMI) groups. There were no significant differences in age, gender, or body mass index. With the exception of the prevalence of hypertension and use of insulin treatments, there were no significant differences in clinical histories and medications between the two groups. Patients with UAP or AMI had significantly higher LDL cholesterol, hs-CRP, and ICTP/I-PINP ratios than did SAP patients. However, there were no significant differences in the HDL, HbA1c, and creatinine levels between the two CAD subgroups.

There were no significant differences in the target lesion location or QCA of target lesions between the SAP and UAP-AMI groups (Table 2). The data by gray-scale and IB-IVUS analysis showed that the UAP-AMI group had a significantly greater ratio of plaque area to the minimum luminal area (10.2 ± 4.0 versus 8.0 ± 3.6 mm^2, $P < 0.05$), plaque volume (112.0 ± 60.4 versus 87.3 ± 47.9 mm^2, $P = 0.04$), percent plaque volume (69.8 ± 10.1 versus 63.6 ± 11.1%, $P = 0.02$), and percent lipid volume (46.7 ± 11.5 versus 40.7 ± 13.9%, $P = 0.04$), and lower ratio of the fibrous area to minimum luminal

Table 1
Demographic and clinical variables of control and CAD patients.

	CAD ($n = 252$)	Control ($n = 132$)	*P* value
Age, yrs	67.2 ± 9.2	65.1 ± 8.9	0.09
Female, %	23.0	24.2	0.83
Body mass index, kg/m2	23.8 ± 4.4	23.5 ± 2.4	0.51
Clinical histories			
Hypertension, %	64.6	10.6	<0.01
Diabetes mellitus, %	49.5	7.5	<0.01
Current smokers, %	25.1	19.0	0.12
Previous myocardial infarction, %	7.6	0	
Previous angioplasty, %	12.2	0	
Previous bypass surgery, %	4.5	0	
Previous cerebrovascular disease, %	2.0	0	
Blood Examination			
LDL, mg/dl	105.0 ± 31.9	125.4 ± 30.5	<0.01
HDL, mg/dl	46.1 ± 11.2	55.1 ± 14.6	<0.01
Hemoglobin A1c, %	6.0 ± 1.0	5.0 ± 0.6	<0.01
Creatinine, mg/dl	1.0 ± 1.0	0.9 ± 0.1	0.40
hs-CRP, mg/dl	0.47 ± 1.1	0.03 ± 0.1	<0.001
ICTP, ng/ml	5.4 ± 6.3	1.9 ± 2.0	<0.001
ICTP/I-PINP	0.2 ± 0.1	0.04 ± 0.03	<0.001
CatK, ng/ml	44.0 ± 31.2	15.5 ± 8.3	<0.001
Medications			
ARBs or ACEIs, %	55.6	0	
CCBs, %	40.0	0	
β-blockers, %	9.4	0	
Anti-lipids, %	77.4	0	
Aspirin, %	100	0	
Insulin, %	12	0	

LDL, low density lipoprotein; HDL, high density lipoprotein; hs-CRP, high-sensitive c-reactive protein; ICTP, carboxyterminal telopeptide of type I collagen; I-PINP, intact procollagen type I N-terminal propeptide; CatK, cathepsin K; ARBs = Angiotensin II receptor blockers; ACEI, angiotensin converting enzyme inhibitor; CBBs, calcium channel blockers. Values are expressed as mean ± SD or number (%).

Table 2
Demographic and clinical variables of SAP and UAP-AMI

	SAP ($n = 197$)	UAP-AMI ($n = 55$)	*P* value
Age, yrs	67.4 ± 9.1	65.4 ± 9.7	0.16
Female, %	23.4	20.0	0.81
Body mass index, kg/m2	23.7 ± 4.1	24.1 ± 5.8	0.57
Clinical histories			
Hypertension, %	72.6	36.3	0.01
Diabetes mellitus, %	45.0	65.5	0.23
Current smokers, %	25.4	23.6	0.44
Previous myocardial infarction, %	3.6	1.8	0.28
Previous angioplasty, %	10.7	18.7	0.16
Previous bypass surgery, %	3.0	5.5	0.35
Previous cerebrovascular disease, %	1.5	3.6	0.21
Blood Examination			
LDL cholesterol, mg/dl	103.4 ± 29.5	114.4 ± 34.3	0.02
HDL cholesterol, mg/dl	46.3 ± 11.3	45.1 ± 10.6	0.47
Hemoglobin A1c, %	6.0 ± 0.9	6.1 ± 1.3	0.38
Creatinine, mg/dl	1.0 ± 1.0	0.7 ± 0.2	0.22
hs-CRP, mg/dl	0.4 ± 0.7	0.59 ± 1.0	0.04
ICTP, ng/ml	4.9 ± 5.4	6.6 ± 8.6	0.08
ICTP/I-PINP	0.15 ± 0.10	0.20 ± 0.2	0.02
Medications			
ARBs or ACEIs, %	59.9	40	0.10
CCBs, %	37.6	49.1	0.16
β-blockers, %	10.6	5.5	0.09
Anti-lipids, %	76.4	87.2	0.45
Aspirin, %	100	100	
Insulin, %	9.6	21.8	<0.01
Target lesion location			
Left anterior descending artery	79	25	0.19
Left circumflex artery	46	11	0.35
Right coronary artery	72	19	0.45
QCA of target lesions			
Reference vessel diameter, mm	2.4 ± 0.7	2.6 ± 0.6	0.45
Diameter stenosis, %	72.7 ± 13.4	78.8 ± 12.1	0.13
Lesion length, mm	12.0 ± 4.4	12.1 ± 4.0	0.63
Gray-scale IVUS			
MLA, mm^2	2.2 ± 1.0	1.9 ± 0.9	0.36
EEM area of MLA, mm^2	10.1 ± 3.9	12.4 ± 4.4	0.07
Plaque area of MLA, mm^2	8.0 ± 3.6	10.2 ± 4.0	<0.05
Lumen volume, mm^2	46.8 ± 25.0	46.4 ± 24.5	0.95
EEM volume, mm^2	11.7 ± 4.4	13.1 ± 3.3	0.19
Plaque volume, mm^2	87.3 ± 47.9	112.0 ± 60.4	0.04
Percent plaque volume, %	63.6 ± 11.1	69.8 ± 10.1	0.02
IB-IVUS			
Lipid area of MLA, %	41.6 ± 15.8	47.1 ± 18.4	0.09
Fibrous area of MLA, %	53.8 ± 14.8	42.0 ± 12.5	0.013
Percent lipid volume, %	40.7 ± 13.9	46.7 ± 11.5	0.04
Percent fibrous volume, %	53.6 ± 10.6	48.5 ± 10.1	<0.05

GCA, quantitative coronary angiography; IVUS, intravascular ultrasound; MLA, minimum luminal area; EEM, external elastic membrane; IB, integrated backscatter. Other abbreviations were as in Table 1. Values are expressed as mean ± SD or number (%).

area (42.0 ± 12.5 versus 53.8 ± 14.8 mm^2, $P = 0.013$) and percent fibrous volumes (48.5 ± 10.1 versus 53.6 ± 10.6%, $P < 0.05$) than did SAP patients. The quantitative data showed that patients with UAP or AMI had higher serum CatK levels than did SAP patients (Fig. 1), whereas there was no significant difference between the UAP and AMI groups (data not shown).

Linear regression analysis showed that the CatK levels correlated positively with the ICTP levels ($r = 0.3$, $P < 0.001$) and the ICTP/I-PINP ratios ($r = 0.4$, $P < 0.001$), while they correlated negatively with the HDL levels ($r = -0.24$, $P < 0.001$). There was also a correlation between CatK and hs-CRP levels ($r = 0.20$, $P < 0.001$). Interestingly, CatK levels were also correlated positively with plaque volumes ($r = 0.21$, $P = 0.002$) and inversely with percent fibrous volumes ($r = -0.16$, $P = 0.03$) by the IVUS in CAD patients.

The results of the logistic regression analysis for CAD are shown in Table 3. In single logistic regression analysis, body mass index, hypertension, diabetes mellitus, HDL cholesterol, LDL cholesterol, hs-CRP, ICTP/I-PINP, and CatK were significantly associated with CAD (Table 3). Multiple logistic regression analysis with age, body mass index, hypertension, diabetes mellitus, HDL cholesterol, LDL cholesterol, hs-CRP, ICTP/I-PINP, and CatK revealed that hypertension (odds ratio, 11.08; 95% CI, 2.33 to 52.65; $P < 0.01$), diabetes mellitus (odds ratio, 6.47; 95% CI, 1.34 to 31.37; $P = 0.02$), HDL (odds ratio, 0.92; 95% CI, 0.87 to 0.97; $P < 0.01$), hs-CRP (odds ratio, 2.43; 95% CI, 1.37 to 8.56; $P = 0.022$), ICTP/I-PINP (odds ratio, 1.72; 95% CI, 1.54 to 2.36; $P < 0.01$), and CatK (odds ratio, 1.15; 95% CI, 1.07 to 1.23; $P < 0.01$) levels were significantly correlated with CAD (Table 3).

9. Discussion

Previous reports showing that CatK deficiency alters wall remodeling and atherogenesis in mice [11] led us to hypothesize that cysteinyl CatK plays an important role in atherogenesis. In humans, limited information is available, with the exception that CatK expression has been shown to be increased in atherosclerotic plaques of the human aorta [6]. This study presented additional evidence to support the possible participation of CatK in atherosclerosis in humans.

10. CatK, collagen, and inflammation biomarkers and atherosclerotic plaque characterization

Multiple lines of evidence indicate that CatK is the most abundant and important cysteinyl enzyme synthesized by the cardiovascular system, and that it is relevant to atherosclerosis-based

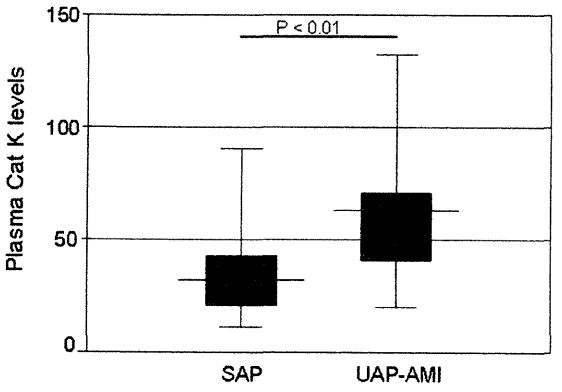

Fig. 1. Box plots of the CatK in the SAP and UAP-AMI groups. Boxes show interquartile ranges; the lower and upper boundaries of the boxes indicate the 25th and 75th percentile levels, respectively; and the horizontal lines within the boxes indicate the median levels.

vascular disease and its implications [8]. However, no previous study has evaluated circulating levels of CatK in humans with or without CAD. Our data show that patients with CAD had higher plasma CatK levels and higher ICTP:I-PINP ratios than did control subjects. Multivariable logistic regression analysis showed that plasma CatK as well as traditional risk factors of hypertension and diabetes mellitus were independently associated with CAD. Several recent studies have reported increased serum levels of several Cats (S and L) in association with CAD [8,12]. Collectively, our findings indicate that elevated plasma levels of CatK serve as a novel biomarker for CAD. It should be noted that the association between CatK and CAD may be a weak one. We must note that, in the present work, the correlations between CatK and plaque volume and percent fibrous volume were statistically significant but the related Pearson correlation coefficient was less than 0.3. It is well established that inhibition of 3-hydroxy-3-methyl-glutaryl-CoA reductase or angiotensin has an atherosclerotic regression effect in animals and humans [16,17]. Recent studies have reported that long-term treatment with statins or angiotensin antagonists not only reduced plasma and tissues CatK levels but also prevented cardiovascular and renal injury in animal models [18,19]. Here, the frequencies of patients with CAD under treatment with antihypertensive (angiotensin II type 1 receptor antagonists or angiotensin-converting enzyme inhibitors) and lipid-lowering drugs (statins) were 55.6% or 77.4%, respectively. Surrogate markers have recently come under scrutiny since a few of the intermediate endpoints (LDL-cholesterol, HDL-cholesterol and hemoglobin A1c) generally considered to be reliable have failed to predict clinical benefit following pharmacological intervention in the causal pathway [20]. Thus, the weak correlation coefficient might be in part due to pharmacological interventions-mediated expectative influence. Further investigations will be needed to study this issue.

Atherosclerotic plaque instability and rupture induced by inflammation are the major mechanisms of acute coronary syndrome or an acute clinical event [21,22]. In our analysis of the CAD subgroup of patients, we detected higher CatK levels in patients with UAP or AMI than in those with SAP, likely due to the active matrix degeneration plaque destabilization and consequent thrombosis during the pathogenesis of acute coronary syndrome. Moreover, patients with UAP or AMI had higher serum hs-CRP than did SAP subjects. Several recent reviews of the literature have reported that CRP is one of the acute-phase reactants indicating underlying systemic inflammation, and that CRP has been shown to have a predictive value for cardiovascular disease or risk factors in healthy subjects [22,23]. Plasma CRP is also able to discriminate between stable and unstable coronary artery disease; CRP levels are significantly higher in patients with unstable coronary artery disease compared to stable patients [22,24]. Thus, the significant positive correlation between CatK and hs-CRP supports the hypothesis that CatK production by activated inflammatory cells and its release into the extracellular milieu and the circulation are strongly linked to local inflammatory processes within the arterial wall. Here, we have shown that patients with UAP or AMI had significantly lower fibrous volume and higher CatK levels than did patients with SAP. Furthermore, our data showed that CatK was positively correlated with the plasma ICTP/I-PINP ratio (a collagen metabolism-related index) and plaque volume and inversely correlated with the percentage of fibrous volume. Matrix metalloproteinase has been implicated in atherosclerotic plaque growth and plaque rupture in humans and animal model [16,25]. The data from IVUS and laboratory examinations demonstrated that high levels of metalloproteinase-9 in patients who have AMI or UAP are related to the presence of plaque rupture in the culprit lesion [26]. Therefore, both the evaluation of the CatK concentration and the

Table 3
Independent predictors of CAD according to multivariable logistic regression analysis.

	Single			Multiple		
	Odds ratio estimate	95% CI	*P* value	Odds ratio estimate	95% CI	*P* value
Age(year)	1.04	0.92–1.18	0.52	1.01	0.92–1.01	0.77
Gender	0.85	0.18–4.07	0.84			
BMI(kg/m^2)	0.76	0.62–0.93	< 0.01	0.62	0.87–0.97	0.07
Hypertension, %	9.96	1.29–76.85	0.03	11.08	2.33–52.65	<0.01
Diabetes mellitus, %	8.28	1.02–66.96	< 0.05	6.47	1.34–31.37	0.02
HDL cholesterol(mg/dl)	0.88	0.81–0.97	< 0.01	0.92	0.87–0.97	<0.01
LDL cholesterol(mg/dl)	0.98	0.96–1.01	0.26			
hs-CRP	2.06	1.01–5.28	0.02	2.43	1.37–8.56	0.02
ICTP	1.09	0.45–2.62	0.84			
ICTP/I-PINP	1.86	1.29–4.78	< 0.001	1.72	1.54–2.36	<0.01
CatK	1.08	1.09–1.19	< 0.01	1.15	1.07–1.23	<0.01

Multiple regression model includes all variables at baseline with $P < 0.05$ by univariable analysis. Abbreviations are as in Table 1. CI = confidence interval.

evaluation of the matrix metalloproteinase concentration might serve as a biomarker for monitoring coronary atherosclerotic plaque vulnerability during acute coronary syndrome and myocardial infarction.

11. CatK and lipid metabolism

LDL is considered an important factor in the initiation and progression of atherosclerotic plaques [27]. Lipoprotein modification and uptake by atherosclerotic lesion cells, mainly macrophages, are important pathologic steps in atherogenesis [8.28]. Several cathepsins have been implicated in ApoB-100 proteolytic modification, which enhances extracellular LDL particle aggregation, lipid droplet formation, and LDL retention to arterial proteoglycans [28]. CatK deficiency showed an increase in cholesterol ester storage in ApoE$^{-/-}$ bone marrow-derived macrophages, which was localized in the large lysosomal compartments [11]. We have shown that the UAP-AMI group had not only significantly higher percent plaque lipid volumes and serum LDL cholesterol levels, but also significantly higher CatK levels than did the SAP group. We found a direct negative correlation between CatK and HDL in all subjects. Lindstedt et al. studied the extracellular capacity of CatK to reduce cholesterol efflux by degradation of preβ-HDL and apoA-1 [29]. These findings suggest that CatK may participate in cholesterol uptake and/or efflux to contribute to macrophage-derived foam cell formation and plaque growth. However, the unexpected question of why CAD patients had lower LDL cholesterol than the controls remains unanswered. This phenomenon might be associated with lipid-lowering therapy. Further study will be needed to investigate this issue.

12. Study limitations

Several limitations of the present study should be pointed out. First, the small sample size of participants with and without CAD limited the power to prove relationships and differences or to conduct subgroup analysis by SAP and UAP-AMI. It has generally been considered that a thinner fibrous cap is associated with greater plaque vulnerability. Atherosclerotic plaque morphology and distribution revealed using a catheter-based IVUS technique are analyzed by visual inspection of acoustic reflections, and the echogenicity of different tissues may appear very similar. Therefore, the capacity of IVUS for identification of the plaque component is limited. Secondly, although the relationship of circulating CatK to coronary plaque and fibrous volumes in CAD patients was significant, our study was not designed to determine causality in humans. Third, patients with cardiomyopathy or end-stage renal disease with maintenance hemodialysis were excluded. On the other hand, plasma markers of CatK and collagen turnover are not coronary-specific. It is too hard to separate CatK and collagen markers from different arteries (the carotid artery, peripheral artery, cerebral artery, etc.) and tissues (myocardium, bone, fat, etc.). It is unclear how their inclusion or exclusion would influence the present results. Fourth, the control subjects and CAD patients were recruited from two different hospitals, and the former had not undergone coronary angiography. In addition, for ethical reasons, we considered that it was too problematic to use a non-drug-intervention period in order to exclude the pharmacological interventions-related influence on plasma CatK levels in this study. A large-scale population-based study will be needed to investigate these issues.

In conclusion, the present study showed that an increased level of CatK was a novel determinant of CAD prevalence. Our observations suggest that CatK represents a molecular target for cardiovascular disease and that measurement of plasma CatK levels would be helpful for the assessment of cardiovascular risk. However, it should be considered examining the role of Cathepsin K in cardiovascular disease in setting of prospective cohort studies where many of the problems afflicting case control studies are no longer an issue.

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Disclosures

The authors have no conflicts of interest to declare.

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ORIGINAL RESEARCH

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Mechanisms With Clinical Implications for Atrial Fibrillation–Associated Remodeling: Cathepsin K Expression, Regulation, and Therapeutic Target and Biomarker

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Background—The cysteine protease cathepsin K (CatK) has been implicated in the pathogenesis of cardiovascular disease. We sought to determine the link between atrial fibrillation (AF) and plasma CatK levels and to investigate the expression of and therapeutic target for CatK in vivo and in vitro.

Methods and Results—Plasma CatK and extracellular matrix protein peptides (intact procollagen type I of N-terminal propeptide; carboxyl-terminal telopeptide of type I collagen [ICTP]) were measured in 209 consecutive patients with AF (paroxysmal AF, 146; persistent AF, 63) and 112 control subjects. In addition, the regulation of CatK expression was investigated in vivo and vitro. Patients with AF had higher plasma CatK and ICTP levels than did control subjects. Patients with persistent AF had higher levels of plasma CatK and ICTP than did patients with paroxysmal AF. CatK was correlated with ICTP concentration and left atrial diameter in all subjects. In rabbits, superoxide production, CatK activity, fibrosis, and the levels of atrial tissue angiotensin II, angiotensin type 1 receptor, gp91phox, phospho-p38 mitogen-activated protein kinase, and CatK were greater in those with tachypacing-induced AF than in controls, and these changes were reversed with angiotensin type 1 receptor antagonist. Olmesartan and mitogen-activated protein kinase inhibitor decreased the CatK expression induced by angiotensin II in rat neonatal myocytes.

Conclusions—These data indicated that increased plasma CatK levels are linked with the presence of AF. Angiotensin type 1 receptor antagonist appears to be effective in alleviating atrial fibrosis in a rabbit AF model, partly reducing angiotensin type 1 receptor-p38mitogen-activated protein kinase-dependent and -independent CatK activation, thus preventing AF. (***J Am Heart Assoc.* 2013;2:e000503 doi: 10.1161/JAHA.113.000503**)

Key Words: angiotensin type 1 receptor • atrial fibrillation • cathepsin K • extracellular matrix • mitogen-activated protein kinase

Atrial fibrillation (AF) is the most common cardiac arrhythmia in clinical practice. AF itself has been shown to cause changes in the function and structure of the atria, providing a possible explanation for the progressive nature of this arrhythmia.[1–3] In the atria, the extracellular matrix provides supportive scaffolding for cardiomyocytes, maintains the structural integrity of cardiac tissue, and is necessary for electrical conduction via cardiomyocytes.[4] Growing evidence supports the concept that structural remodeling of the extracellular matrix may be the key event leading to the development of AF and atrial mechanical dysfunction. Lysosmal protease cathepsins (Cats) traditionally have been known to degrade unwanted intracellular or endocytosed proteins.[5] However, the recent recognition of the inducible CatK and CatS has revealed their proteolytic functions in inflammatory disease, including atherosclerosis-based vascular disease processes.[6–8] More recently, several studies have reported that Cats play a functional role in intracellular and extracellular protein degradation in cardiac myocytes by contributing to matrix turnover, chamber dilation, and structural remodeling.[9–12] A few reports suggest that circulating Cats have a predictive value for proteolysis-associated disease, and related research has focused on vascular disease (including coronary artery diseases and aortic aneurysm).[13,14] To date, no studies have examined atrial Cat expression and plasma levels as potential biomarkers for atrial remodeling in atrial disease.

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Recently, activation of the renin–angiotensin system has been implicated as part of the mechanism of AF.[15] Cardiac-specific overexpression of angiotensin-converting enzyme in mice results in excessive levels of cardiac angiotensin II (Ang II), and the mice develop spontaneous AF.[16] Ang II has numerous cardiovascular effects that might lead to cardiac arrhythmia, including the induction of fibrosis and the proliferation of cardiac fibroblasts, the increased synthesis of collagen, and the promotion of reactive oxygen species generation.[17] Ang II has been shown to induce p38mitogen-activated protein kinase (p38MAPK)/extracellular signal-regulated kinase activation and atrial interstitial fibrosis.[18,19] Several recent studies have demonstrated that angiotensin inhibition prevents left ventricular fibrosis by decreasing the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase–dependent Cat activation.[11,20] Although the inhibition of Ang II through the use of Ang II type 1 receptor (AT1R) antagonists or angiotensin-converting enzyme inhibitors has prevented AF in animal models and a diverse human population with AF or at risk of developing AF,[21–24] the underlying mechanism is poorly understood.

In this study, we sought to determine whether circulating CatK levels are closely linked to the presence of AF and increased levels of the collagen type I degradation marker. In addition, we explored the possible mechanisms by which Ang II inhibition mitigates atrial remodeling and AF in a rabbit tachypacing model.

Methods

Study Population

We recruited 209 consecutive patients with paroxysmal AF (PAF; n=146) or persistent AF (PeAF; n=63) who were admitted to Nagoya University Hospital between March 2009 and December 2010 for scheduled radiofrequency catheter ablation with coronary angiography. AF in these patients had been diagnosed in light of symptoms, 12-lead electrocardiogram, and Holter electrocardiogram. As described previously,[25] PAF was defined on the basis of a history of 1 or more episodes of AF that self-resolved or were terminated medically within 7 days, and PeAF was defined according to a history of 1 or more episodes of AF over 7 days that required pharmacological or electrical cardioversion to establish normal sinus rhythm. None of the patients in this study had permanent AF. We excluded patients with dilated or hypertrophic cardiomyopathy, myocardial infarction, congenital heart disease, congestive heart failure, or valvular heart diseases and those receiving hemodialysis. AF patients were receiving standard therapy with antiarrhythmic drugs, β-blockers, angiotensin-converting enzyme inhibitors or AT1R blockers, and statins at the time they underwent ablation. We also assessed 112 subjects with and without paroxysmal atrial arrhythmia (no previously documented AF), who were considered to represent the control group. The study protocol was approved by the ethics committee of the Nagoya University School of Medicine, and written informed consent was obtained from all patients.

Laboratory Assay

Laboratory measurements were performed under blinded conditions. Human and rabbit plasma CatK levels were determined by using ELISA kits (Biomedica Gruppe, Biomedica Medizinprodukte). Serum interleukin-1β levels were measured by using commercially available kits. Serum levels of intact procollagen type I N-terminal propeptide (I-PINP), carboxyl-terminal telopeptide of collagen type I (ICTP), atrial natriuretic peptide, cystatin C, high-sensitivity C-reactive protein, hemoglobin A1c, and atrial tissue Ang II were measured at a commercial laboratory (SRL [Tokyo, Japan]). Plasma CatK values were expressed as ng/mL, and interassay and intraassay coefficients of variation were <8% (n=20).

Echocardiography

Two-dimensional and Doppler echocardiography was performed by an experienced sonographer using a Vivid4 System (GE Healthcare Bio-Sciences). The images were recorded on a DVD recorder and analyzed offline. Left atrial diameter (LAD) was obtained by using standard M-mode measurements, as recommended by the American Society of Echocardiography. The left ventricular ejection fraction was calculated using the modified Simpson's rule.

Animal Model and Treatment

A rabbit AF model was induced by ventricular tachypacing as described previously.[26] The study protocol was approved by the Institutional Animal Care and Use Committee of Nagoya University School of Medicine. Eleven-week-old male New Zealand White rabbits (Kitayama Labs) underwent surgery with or without the implantation of right ventricular pacemakers (Medtronic) under anesthesia with ketamine hydrochloride 35 mg/kg and xylazine 3 mg/kg. After the animals recovered from surgery, the pacemakers were programmed to pace at 380 beats/min for 4 weeks. Rabbits were divided into 3 groups as follows: nonpaced control rabbits (control rabbits; n=7), rabbits subjected to ventricular tachypacing treated with vehicle (0.5% carboxymethylcellulose, AF rabbits; n=7), and rabbits subjected to tachypacing with olmesartan treatment (Olm rabbits; n=5). Daily oral administration of olmesartan (1 mg/kg; Daichi-Sankyo) via gastric tube was initiated 1 week before surgery and continued throughout the study period. Electrocardiograms were monitored once per week to adjust