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Association of Plasma Levels of F11 Receptor/Junctional Adhesion Molecule-A (F11R/JAM-A) With Human Atherosclerosis

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Objectives The purpose of this study was to determine the association of the F11 receptor (F11R) with human vascular disease.

Background A molecule identified as critical for platelet adhesion to a cytokine-inflamed endothelial surface in vitro is F11R. The F11R is known to be expressed in platelets and endothelium and reported recently to be overexpressed in atherosclerotic plaques.

Methods A novel enzyme-linked immunosorbent assay was developed for the measurement of soluble F11R in human plasma. The F11R levels, along with a number of other biomarkers, were measured in 389 male patients with known or suspected coronary artery disease (CAD) undergoing coronary angiography at a Veterans Administration Medical Center.

Results Patients with normal or nonobstructive disease (CAD angiographic score of 0), mild-to-moderate disease (score of 1 to 3), and severe disease (score of 4 to 6) had median F11R plasma levels of 38.6 pg/ml (mean 260 ± 509.6 pg/ml), 45.2 pg/ml (mean 395.3 ± 752.7 pg/ml), and 105.8 pg/ml (mean 629 ± 831.7 pg/ml), respectively ($p = 0.03$). By multivariate analysis, the variables independently associated with CAD score were age, hyperlipidemia, chronic renal insufficiency, left ventricular function, and plasma F11R levels. The F11R was the only biomarker that was independently associated with CAD score. Consistent with the previously reported effects of tumor necrosis factor (TNF)-alpha on F11R expression in cultured endothelial cells, F11R levels correlated strongly with plasma TNF-alpha levels ($r = 0.84$; $p < 0.0001$).

Conclusions Plasma F11R is independently associated with the presence and severity of angiographically defined CAD. By virtue of its strong correlation to plasma TNF-alpha, F11R may be an important mediator of the effects of inflammation on the vessel wall. Strategies that block F11R may represent a novel approach to the treatment of human atherosclerosis. (J Am Coll Cardiol 2007;50:1768–76) © 2007 by the American College of Cardiology Foundation

There is a growing body of evidence showing that the interaction between platelets and endothelial cells contributes significantly to the pathogenesis of atherosclerosis (1). Platelet adhesion to the inflamed endothelium triggers the formation of thrombi leading to atherosclerotic lesions (2). A key molecule identified as critical for platelet adhesion to an activated endothelial surface is the F11 receptor (F11R) (3). The F11R is a novel cell adhesion molecule that is a member of the immunoglobulin superfamily and a human orthologue of a murine cell adhesion molecule found in endothelial cells known as junctional adhesion molecule A, or JAM-A (4,5). We have identified, sequenced, and cloned the human gene for F11R/JAM-A and have demonstrated

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that F11R plays a critical role in the adhesion of platelets to cytokine-inflamed endothelial cells (3,5–7). We have also recently demonstrated very high levels of F11R/JAM-A protein and mRNA in the atherosclerotic plaques of patients with advanced aortic and peripheral vascular disease as well as in an animal model of atherosclerosis and reported data demonstrating a critical role for F11R in triggering atherogenesis (8).

Although these investigations point to a role for F11R in the pathogenesis of atherosclerosis, there have been no clinical studies to establish a link between circulating plasma F11R and vascular disease in humans. The purpose of the present study was to determine whether plasma levels of soluble F11R correlate with the presence or extent of angiographically defined coronary artery disease (CAD). To this end, we developed a novel F11R enzyme-linked immunosorbent assay (ELISA) and report for the first time the detection, quantification, and characterization of F11R in humans. The results have implications for the development of novel therapeutic strategies targeting F11R and platelet adhesion and for the use of F11R as a predictor/marker for the early diagnosis of CAD.

Abbreviations and Acronyms

BSA = bovine serum albumin
CAD = coronary artery disease
CRI = chronic renal insufficiency
ELISA = enzyme-linked immunosorbent assay
ESR = erythrocyte sedimentation rate
F11R = F11 receptor
HRP = horseradish peroxidase
hs-CRP = high-sensitivity C-reactive protein
JAM-A = junctional adhesion molecule-A
LV = left ventricular
MI = myocardial infarction
MMP = matrix metalloproteinase
PAI = plasminogen activator inhibitor
PBS = phosphate-buffered saline
RANTES = regulated upon activation, normal T-cell expressed and secreted
TIMP = tissue inhibitor of metalloproteinase
TNF = tumor necrosis factor

Methods

Study design. This study was conducted at an urban Veterans Administration (VA) medical center and was approved by the local institutional review board. Written informed consent was obtained from all patients. Between January 1999 and October 2002, 389 male patients with known or suspected CAD undergoing diagnostic coronary angiography were enrolled in the study. The only exclusion criteria for participation in the study were active gastrointestinal bleeding or the presence of anemia, which was defined as a hemoglobin concentration <8.0 g/dl. Clinical and demographic information was obtained by interview as well as review of computerized medical records. Fasting blood was obtained from all patients at the time of angiography for subsequent analysis.

Blood sampling. After an overnight fast of at least 12 h, blood was obtained from all patients enrolled in the study. Blood was collected from the arterial sheath (after a 5-ml discard) at the time of angiography but before the injection of contrast material. Blood was immediately placed in vacutainer tubes, spun at 10 *g* for 20 min in a cold centrifuge, and the plasma aliquoted into multiple 1.5-ml Eppendorf tubes. The samples were subsequently stored at −70°C until analysis at a later date.

Laboratory methods. Aliquoted plasma samples stored at −70°C were thawed and, using commercially available ELISA kits, the levels of the following biomarkers were measured: tissue inhibitor of metalloproteinase (TIMP)-1 (EMD Biosciences, San Diego, Califorinia), matrix metalloproteinase (MMP)-9 (EMD Biosciences), high-sensitivity C-reactive protein (hs-CRP) (Life Diagnostics, West Chester, Pennsylvania), plasminogen activator inhibitor (PAI)-1 (Diapharma, West Chester, Ohio), regulated upon activation, normal T-cell expressed and secreted (RANTES) (R&D Systems, Minneapolis, Minnesota), tumor necrosis factor (TNF)-alpha (R&D Systems), insulin (Linco Diagnostics, St. Charles, Missouri), and F11R (R&D Systems; and BD Biosciences, San Jose, California). The sensitivities of the TIMP-1, MMP-9, hs-CRP, PAI-1, RANTES, TNF-alpha, insulin, and F11R assays were 0.0096 ng/ml, 0.1 ng/ml, 0.1 mg/l, 0.5 ng/ml, 15.6 pg/ml, 15.6 pg/ml, 2 μU/ml, and 15.6 pg/ml, respectively. The intra-assay coefficients of variation for TIMP-1, MMP-9, hs-CRP, PAI-1, RANTES, TNF-alpha, insulin, and F11R were <5.2%, <10%, <7.6%, <3.0%, <5.0%, <10%, <7.0%, and <10%, respectively.

Development of ELISA for F11R. ANTIBODIES AND REAGENTS. Biotinylated antihuman JAM-A antibody, recombinant human JAM-A/Fc chimera, streptavidin-horseradish peroxidase (HRP) conjugate, buffers, HRP substrate and stop solution were purchased from R&D Systems. Bovine serum albumin (BSA) was obtained from Sigma (St. Louis, Missouri), Nunc-Immuno Maxi Absorp 96-well plates were obtained from VWR (Bridgeport, New Jersey), and M.Ab.F11, an F11R/JAM-A monoclonal antibody developed and characterized in our laboratory (3), was purchased from BD PharMingen Biosciences (Palo Alto, California).

PREPARATION OF REAGENTS FOR THE F11R ELISA. Aliquots of recombinant human JAM-A/Fc chimera (50 μg) were reconstituted with sterile 0.1% BSA–phosphate-buffered saline (PBS) to a final concentration of 100 μg/ml and stored at −70°C. Freshly-prepared M.Ab.F11 was diluted with sterile PBS to a final concentration of 4 μg/ml, and biotinylated antihuman JAM-A antibody was diluted to a final concentration of 200 ng/ml with 0.1% BSA-PBS. The stock solution of streptavidin-HRP conjugate was diluted 400× in 0.1% BSA-PBS. The substrate solution was prepared according to the manufacturer's instructions 15 min before use, and the wash buffer was diluted 25× with distilled H_2O. Freshly prepared blocking solution (1% BSA in PBS) and stock solutions were stored at −70°C.

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Table 1 Baseline Characteristics Grouped by CAD Score

Characteristic	CAD Score 0 (Normal or Nonobstructive Disease) n = 74	CAD Score 1 to 3 (Mild to Moderate Disease) n = 276	CAD Score 4 to 6 (Severe Disease) n = 39	Total Population n = 389	p Value
Age, yrs, mean (SD)	61.7 (10.1)	66.0 (9.8)	67.4 (10.0)	67.7 (9.6)	0.0010
Family history of premature CAD, n (%)	15 (20.3)	66 (23.9)	15 (38.5)	96 (24.7)	0.0886
Diabetes mellitus, n (%)	29 (39.2)	119 (43.1)	21 (53.9)	169 (43.4)	0.3206
Hypertension, n (%)	57 (77.0)	231 (83.7)	37 (94.9)	325 (83.6)	0.0515
History of tobacco use, n (%)	59 (79.7)	226 (81.9)	31 (79.5)	316 (81.2)	0.8762
Active tobacco use, n (%)	27 (36.5)	84 (30.4)	9 (23.1)	120 (30.9)	0.3281
Hyperlipidemia, n (%)	29 (39.2)	154 (55.8)	30 (76.9)	213 (54.8)	0.0005
LDL, mean (SD)	107.6 (37.8)	106.6 (33.5)	103.9 (43.0)	106.6 (35.3)	0.4912
HDL, mean (SD)	39.8 (11.4)	38.8 (10.9)	36.4 (9.8)	38.8 (10.9)	0.5488
Total cholesterol, mean (SD)	172.5 (42.1)	174.6 (41.8)	168.8 (42.9)	173.6 (41.9)	0.9314
Triglycerides, mean (SD)	128.7 (97.9)	147.0 (97.8)	136.2 (82.7)	142.4 (96.4)	0.0958
BMI, mean (SD)	29.4 (5.9)	28.4 (5.5)	29.6 (6.4)	28.7 (5.7)	0.3584
Obesity, n (%)	24 (32.4)	97 (35.3)	15 (38.5)	136 (35.1)	0.8073
CHF on presentation, n (%)	27 (36.5)	63 (22.8)	12 (30.8)	102 (26.2)	0.0475
MI on presentation, n (%)	8 (10.8)	87 (31.5)	12 (30.8)	107 (27.5)	0.0017
CRI, n (%)	0 (0.0)	16 (5.8)	2 (5.1)	18 (4.6)	0.0609
ASA use, n (%)	60 (81.1)	237 (85.9)	34 (87.2)	331 (85.1)	0.5478
Beta-blocker use, n (%)	37 (50.0)	201 (72.8)	29 (74.4)	267 (68.6)	0.0006
ACE-I use, n (%)	42 (56.8)	167 (60.5)	27 (69.2)	236 (60.7)	0.4326
Statin use, n (%)	28 (37.8)	147 (53.3)	30 (76.9)	205 (52.7)	0.0004
Fibrate use, n (%)	1 (1.4)	13 (4.7)	2 (5.1)	16 (4.1)	0.4083
Prior CABG, n (%)	0 (0.0)	13 (4.7)	22 (56.4)	35 (9.0)	<0.0001
LV function, mean (SD)					0.8554
0, 1	41 (55.4)	162 (58.7)	18 (46.2)	221 (56.8)	
2, 3	25 (33.8)	106 (38.4)	14 (35.9)	145 (37.3)	
Not reported	8 (10.8)	8 (2.9)	7 (18.0)	23 (5.9)	
Troponin I*					0.0002
Mean (SD)	1.1 (3.6)	13.1 (55.3)	9.5 (23.1)	10.4 (47.3)	
Median (IQR)	0.2 (0.2, 0.3)	0.3 (0.2, 2.8)	0.3 (0.2, 8.0)	0.3 (0.2, 1.7)	
ESR*					0.1067
Mean (SD)	21.1 (18.5)	24.8 (24.0)	35.2 (33.2)	25.2 (24.4)	
Median (IQR)	13.5 (7.0, 30.0)	16.0 (8.0, 36.0)	28 (10.0, 45.0)	16.0 (8.0, 36.0)	
hs-CRP*					0.3392
Mean (SD)	17.4 (27.8)	24.4 (40.8)	32.3 (56.0)	23.8 (40.5)	
Median (IQR)	7.7 (3.8, 15.5)	7.8 (3.8, 19.5)	9.8 (3.2, 32.4)	9.2 (3.8, 18.8)	
PAI-1*					0.6227
Mean (SD)	40.3 (36.7)	43.3 (35.7)	45.6 (36.6)	42.9 (35.9)	
Median (IQR)	31.7 (18.0, 53.4)	34.9 (19.0, 56.9)	36.1 (24.0, 54.6)	33.4 (19.0, 56.3)	
MMP-9*					0.6597
Mean (SD)	26.9 (21.1)	27.4 (26.9)	23.0 (19.8)	26.9 (25.2)	
Median (IQR)	20.0 (12.5, 32.0)	17.1 (12.4, 32.2)	19.3 (10.5, 26.7)	18.1 (12.3, 31.4)	
TIMP-1*					0.3708
Mean (SD)	87.8 (40.4)	94.0 (47.5)	93.9 (41.9)	92.7 (45.6)	
Median (IQR)	77.4 (63.6, 92.2)	81.7 (66.7, 102.0)	81.1 (70.8, 98.6)	81.1 (66.5, 100.9)	
F11R*					0.0329
Mean (SD)	260.4 (509.6)	395.3 (752.7)	629.5 (831.7)	392.2 (725.2)	
Median (IQR)	38.6 (18.9, 203.7)	45.2 (15.3, 289.2)	105.8 (31.2, 1132.3)	46.1 (16.8, 303.4)	
TNF-alpha*					0.1292
Mean (SD)	134.4 (303.8)	175.8 (398.7)	326.9 (585.4)	182.6 (406.6)	
Median (IQR)	14.5 (7.8, 58.0)	15.0 (7.8, 61.0)	21.1 (12.6, 314.4)	16.3 (7.8, 75.0)	
Insulin*					0.3814
Mean (SD)	11.5 (8.2)	11.6 (2.1)	12.4 (10.4)	11.6 (11.3)	
Median (IQR)	8.9 (6.2, 13.4)	8.2 (5.6, 13.6)	9.7 (6.3, 12.9)	8.4 (5.8, 13.5)	
RANTES*					0.3555
Mean (SD)	3,935 (2,856)	4,878 (4,550)	4,074 (4,190)	4,620 (4,252)	
Median (IQR)	3,155 (1,273, 5,814)	3,186 (1,489, 7,477)	3,069 (1,145, 5,360)	3,184 (1,408, 6,622)	

*Skewed distribution.

ACE-I = angiotensin-converting enzyme inhibitor; ASA = aspirin; BMI = body mass index; CABG = coronary artery bypass graft; CAD = coronary artery disease; CHF = congestive heart failure; CRI = chronic renal insufficiency; ESR = erythrocyte sedimentation rate; F11R = F11 receptor; HDL = high-density lipoprotein; hs-CRP = high-sensitivity C-reactive protein; IQR = interquartile range; LDL = low-density lipoprotein; LV = left ventricular; MI = myocardial infarction; MMP = matrix metalloproteinase; PAI = plasminogen activator inhibitor; RANTES = regulated upon activation, normal T-cell expressed and secreted; SD = standard deviation; TIMP = tissue inhibitor of metalloproteinase; TNF = tumor necrosis factor.

PERFORMANCE OF F11R ELISA. The Nunc-Immuno Maxi Absorp 96-well plates were coated with M.Ab.F11 (4 μg/ml), incubated at 23°C overnight, washed 3 times with wash buffer, and incubated with 1% BSA-PBS for 2 h at 23°C. For preparation of the F11R standard curve, a series of dilutions of the recombinant human JAM-A/Fc protein in 0.1% BSA-PBS were prepared. Aliquots of these dilutions and aliquots of undiluted patient plasma samples (100 μl) were added to wells in triplicate. After a 2-h incubation period, biotinylated antihuman JAM-A antibody (200 ng/ml) was added and the plates incubated for 1 h 45 min at 23°C. After washings, diluted streptavidin-HRP (1:400, 100 μl/well) was added and the plates incubated for 30 min. After each step, the plates were washed 3 times with wash buffer. The freshly prepared substrate solution (100 μl/well) was then added and the plates incubated for an additional 30 minutes. Absorption was measured at 450 nm within 30 min. Reagent controls were subtracted from the sample values.

DETERMINATION OF SERUM F11R LEVELS IN HEALTHY CONTROLS. Fifteen control samples were obtained for the determination of basal levels of F11R from healthy adult male volunteers. These control donors were free of any known medical diseases or acute illnesses, and were not on any medications. Whole blood (10 ml) was obtained by venipuncture of the anticubital vein. After clot formation and retraction, samples were centrifuged at 223 *g* for 10 min at 22°C, and serum samples were collected and frozen at −20°C. The median serum level for this healthy control group was 36.0 pg/ml (mean 33.71 ± 20.9 pg/ml).

Definition of risk factors and clinical syndromes. Diabetes was defined as clinically known and treated diabetes mellitus. Patients were diagnosed as hypertensive if they were documented to have a blood pressure >140/90 mm Hg on more than 2 occasions or were already on antihypertensive therapy. Hyperlipidemia was diagnosed in patients who had been given lipid-lowering medication or had a history of total cholesterol levels >240 mg/dl (9). Smoking was defined as the inhaled use of cigarettes, cigars, or pipes in any quantity. Smokers were classified as former only if they had not smoked at all in the 6 months preceding the date of angiography. Obesity was defined as a body mass index ≥30 kg/m^2. Chronic renal insufficiency (CRI) was defined as a serum creatinine ≥2.0 mg/dl. Congestive heart failure on presentation was defined as the presence of either radiographic or clinical evidence of pulmonary venous congestion within the preceding 24 h of angiography. Myocardial infarction (MI) on presentation was diagnosed by a history of chest discomfort and troponin I >1.0 ng/ml.

Angiographic scoring system. A previously described and validated angiographic scoring system was used for the present analysis (10). The scoring system was specifically designed to take into account both native vessel (including left main coronary artery) and bypass graft disease. It was based on the traditional definition of an angiographically significant stenosis as one with a >50% obstruction and the anatomic territory subtended by the diseased artery or graft. Using visual estimation of stenosis severity, angiograms were scored as follows. Any obstructive lesion ≥50% in 1 of the 3 coronary arteries or their major (≥2.5 mm) branches received a score of 1. Multiple obstructive lesions ≥50% in a single artery or one of its major branches still only received a score of 1, except in the case of a left dominant system. In the case of a left-dominant system, an obstruction ≥50% in the proximal portion of the left circumflex artery received a score of 2. Similarly, in a left-dominant system, an obstruction ≥50% in both a large obtuse marginal branch and a left posterior descending artery (PDA) also received a score of 2. On the other hand, an obstruction ≥50% in a left-dominant system that was isolated to either a left PDA or an obtuse marginal (but not both) received a score of 1. An obstruction of ≥50% in the left main coronary artery received a score of 2 in a right-dominant system and 3 in a left-dominant system. A bypass graft with an obstructive lesion ≥50% received a score of 1. Patent grafts or those with an obstructive lesion <50% received a score of 0.

Angiograms were scored by 2 angiographers working independently. Any differences in interpretation (7%) were subsequently reconciled. The operators scoring the angiograms were blinded to the results of any subsequent laboratory analysis.

Left ventricular (LV) systolic function was assessed by contrast ventriculography and categorized as normal (ejection fraction [EF] ≥55%) or mildly (EF 45% to 54%), moderately (EF 31% to 44%), or severely (EF ≤30%) reduced. These 4 categories of LV systolic function were scored as 0, 1, 2, and 3, respectively.

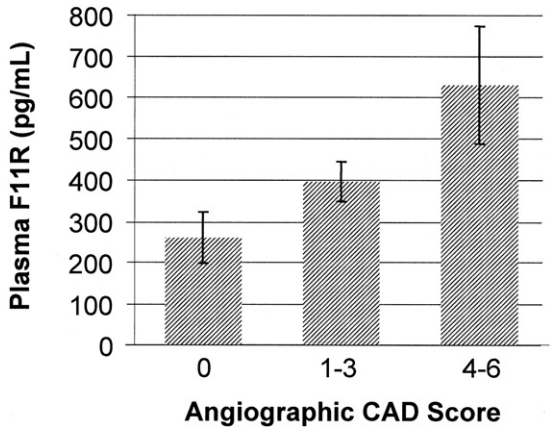

Figure 1 Plasma F11R Levels as a Function of CAD Score

Results are presented as mean ± SEM. There was a progressive and significant increase in plasma F11 receptor (F11R) levels with an increasing coronary artery disease (CAD) score when patients were grouped as having normal or nonobstructive disease (score of 0), mild to moderate disease (score of 1 to 3), and severe disease (score of 4 to 6). p = 0.0329 by the Wilcoxon rank-sum test.

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Statistical methods. The study population was divided into 3 groups based on the CAD score as follows: 1) normal or nonobstructive disease (CAD score 0); 2) mild to moderate disease (CAD score 1 to 3); and 3) severe disease (CAD score 4 to 6).

Summary statistics for the continuous variables were presented both as mean (with standard deviation) and as median (with interquartile range), and comparisons between the 3 groups were performed with the nonparametric Kruskall-Wallis test. Data for the biomarkers were presented as median values, and as 25th and 75th percentile values. The associations between the biomarkers and both F11R and angiographic score were assessed with the Spearman rank correlation. Categoric data were summarized as frequencies and percentages, and the comparisons between the 3 groups were performed with the Pearson chi-square test or the Fisher exact test. The relations between plasma F11R levels and the cardiovascular risk factors such family

Table 2 Association of F11R With Cardiovascular Risk Factors and Medication Use

Risk Factor	Present	Absent	p Value
Family history of premature CAD			0.1148
n	87	263	
Mean (SD)	305.1 (606.7)	421 (759.2)	
Median (IQR)	31.8 (15.6, 186.4)	52.3 (18.8, 367.1)	
Diabetes mellitus			0.8851
n	152	198	
Mean (SD)	328.8 (609.8)	440.9 (800.8)	
Median (IQR)	46.9 (17.2, 285.0)	45.9 (16.3, 476.2)	
Hypertension			0.4786
n	291	59	
Mean (SD)	408.0 (754.6)	314.4 (556.9)	
Median (IQR)	46.1 (17.1, 344.8)	46.2 (13.1, 234.6)	
History of tobacco use			0.0344
n	289	61	
Mean (SD)	353.8 (609.8)	574.3 (922.6)	
Median (IQR)	43.3 (16.3, 277.3)	97.6 (27.7, 636.9)	
Active tobacco use			0.0284
n	113	237	
Mean (SD)	310.9 (684.5)	431.0 (742.1)	
Median (IQR)	31.1 (14.7, 194.9)	56.6 (18.5, 387.2)	
Obesity			0.7954
n	122	227	
Mean (SD)	377.3 (724.7)	402.0 (728.1)	
Median (IQR)	45.9 (17.4, 284.5)	47.2 (16.2, 357.6)	
Hyperlipidemia			0.2196
n	187	163	
Mean (SD)	440.0 (761.5)	337.5 (679.4)	
Median (IQR)	58.9 (16.8, 388.5)	40.3 (16.2, 203.7)	
ASA use			0.4114
n	296	54	
Mean (SD)	370.3 (708.1)	512.5 (809.2)	
Median (IQR)	45.6 (17.9, 287.6)	66.5 (15.3, 948.7)	
Beta-blocker use			0.0449
n	242	108	
Mean (SD)	332.9 (630.2)	525.2 (891.7)	
Median (IQR)	40.3 (16.1, 269.0)	83.7 (19.6, 665.5)	
ACE-I use			0.5838
n	213	137	
Mean (SD)	356.4 (738.8)	448.0 (702.6)	
Median (IQR)	45.4 (17.4, 248.6)	54.8 (15.3, 684.4)	
Statin use			0.2647
n	180	170	
Mean (SD)	443.8 (772.0)	337.7 (670.2)	
Median (IQR)	58.4 (16.9, 387.9)	42.8 (16.2, 234.6)	

Abbreviations as in Table 1.

history, diabetes, hypertension, history of tobacco, active tobacco use, obesity, and hyperlipidemia were established with the Wilcoxon rank sum test.

The variables associated with CAD score were identified with univariate linear regression. Multivariate linear regression with backward selection of variables was used to identify those variables that were independently associated with CAD score. For the linear regression analyses, all biomarkers (except troponin I) were log-transformed to reduce the skewness and kurtosis of the data.

All analyses used 2-sided tests with an overall significance level of alpha = 0.05.

Results

Baseline characteristics. A total of 389 male patients were enrolled in the study. The baseline clinical and laboratory characteristics of the study population stratified by angiographic score are shown in Table 1.

More advanced levels of CAD were seen in association with age, hyperlipidemia, CHF on presentation, MI on presentation, prior coronary artery bypass graft surgery, statin use, beta-blocker use, higher troponin I levels, and hypertension (borderline significance). Of the biomarkers assayed, only F11R levels were significantly associated with increasing CAD score (Table 1). Specifically, patients with normal or nonobstructive disease (CAD score of 0), mild to moderate disease (CAD score of 1 to 3), and severe disease (CAD score of 4 to 6) had median F11R plasma levels of 38.6 pg/ml (mean 260 ± 509.6 pg/ml), 45.2 pg/ml (mean 395.3 ± 752.7 pg/ml), and 105.8 pg/ml (mean 629 ± 831.7 pg/ml), respectively ($p = 0.03$) (Fig. 1).

Association of F11R with clinical characteristics and other biomarkers. There was no association between plasma F11R and any of the traditional cardiovascular risk factors, with the exception of former and active tobacco use (Table 2). With respect to medication use, patients on beta-blocker therapy had lower median F11R levels compared with those not on such therapy (40.3 vs. 83.7 pg/ml, respectively; $p < 0.05$).

There was an inverse correlation between F11R levels and the presence of MI. Patients presenting with MI had lower median plasma F11R levels compared with those patients presenting with chest pain and no enzymatic evidence of myocardial damage (34.3 vs. 58.4 pg/ml, respectively; $p = 0.0076$).

Table 3 Correlation Analysis Between Plasma F11R and Other Biomarkers

Variable	Spearman r Correlation	p Value
TNF-alpha	0.84	<0.0001
RANTES	0.12	0.0222
Troponin I	−0.12	0.0408
PAI-1	0.15	0.0050
MMP-9	0.18	0.0006
TIMP-1	0.08	0.1401
hs-CRP	−0.02	0.7691
Insulin	0.14	0.0093
ESR	0.11	0.0408

Abbreviations as in Table 1.

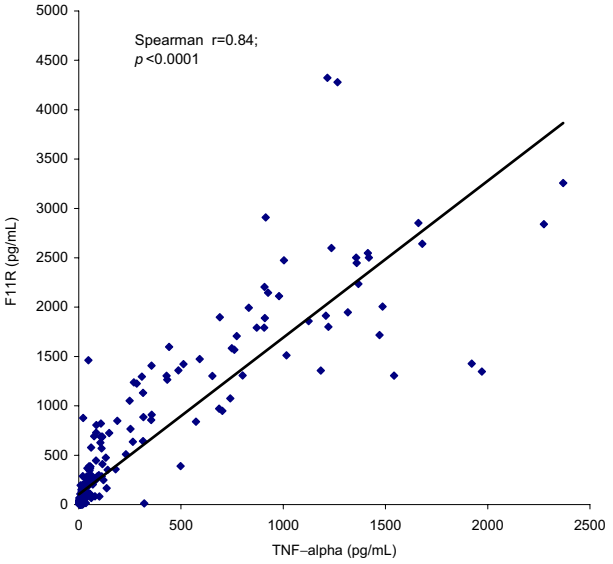

Figure 2 Correlation Between Plasma F11R and TNF-alpha Levels

There was a very strong and highly significant correlation ($r = 0.84$; $p < 0.0001$) between the plasma levels of tumor necrosis factor (TNF)-alpha and F11 receptor (F11R) in the entire cohort of patients, consistent with the in vitro observations that TNF-alpha increases the expression of F11R in cultured endotheial cells (6).

With respect to plasma biomarkers, plasma F11R levels were positively associated with TNF-alpha, RANTES, PAI-1, MMP-9, insulin, and the erythrocyte sedimentation rate (ESR) (Table 3). Of these positive correlations, the most significant was the correlation between F11R and TNF-alpha ($r = 0.84$; $p < 0.0001$), a prespecified analysis based on the basic science laboratory observation that TNF-alpha increases the expression of F11R/JAM-A in cultured endothelial cells (Fig. 2) (6).

Association of F11R with angiographic score. The following baseline clinical and laboratory characteristics were found to be associated with CAD score on univariate analysis: age, hypertension, active tobacco use, hyperlipidemia, CRI, plasma F11R, ESR, TIMP-1, hs-CRP, ratio of TIMP-1/MMP-9, LV function, beta-blocker use, and statin use (Table 4). By multivariate analysis, the variables that were independently associated with CAD score were age, hyperlipidemia, CRI, LV function, beta-blocker use, and plasma F11R (Table 5). Thus, F11R was the only biomarker that was independently associated with CAD score ($p = 0.0091$) (Table 5).

Discussion

The pathogenesis of atherosclerosis is multifactorial, but the initiating process considered to be most essential is the

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Table 4 Univariate Analyses for CAD Score

Predictors of CAD Score	p Value
Age/10 yrs	<0.0001
CHF on presentation	0.8927
Hypertension	0.0010
Diabetes mellitus	0.1694
Family history of premature CAD	0.0887
Active tobacco use	0.0464
History of tobacco use	0.5045
Hyperlipidemia	<0.0001
BMI	0.6301
Obesity	0.5315
Chronic renal insufficiency	0.0050
F11R	0.0470
ESR	0.0025
TIMP-1	0.0292
MMP-9	0.8798
PAI-1	0.2232
hs-CRP	0.0384
TIMP-1:MMP-9 ratio	0.0147
Troponin-I	0.2245
LV function	0.0035
TNF-alpha	0.1770
Insulin	0.9154
RANTES	0.4974
ASA use	0.5861
Beta-blocker use	0.0047
ACE-I use	0.0815
Statin use	<0.0001

Abbreviations as in Table 1.

deposition and oxidation of lipids in the vascular wall, with the subsequent generation of a dysfunctional endothelium (1). Through the expression of a variety of well-characterized adhesion molecules (such as members of the immunoglobulin gene superfamily, the integrin family, and the selectin family), dysfunctional endothelium attracts circulating inflammatory cells into the subendothelial space (11,12). This now inflamed and dysfunctional endothelium also attracts platelets, but the pathways through which this occurs have been less well characterized. A key molecule recently identified as critical for platelet adhesion to inflamed endothelium is F11R. The F11R is a cell adhesion molecule, a member of the immunoglobulin superfamily constitutively expressed on the surface of human platelets, that has been demonstrated to play a role in platelet aggregation, secretion, adhesion, and spreading (3,5–7). The same molecule is also present at tight junctions of endothelial cells, where it is known as JAM-A and acts as a cell adhesion molecule through homophilic interactions (4). We have previously demonstrated that F11R plays a critical role in atherogenesis, providing over 50% of the force of the adhesion of platelets to cytokine-inflamed endothelial cells (6). In addition, using molecular and immunofluorescence techniques, we have recently demonstrated that F11R expression (both protein and mRNA) is increased in the atherosclerotic plaques of surgically excised human specimens with advanced disease (8). However, to date there have been no clinical studies of circulating plasma F11R in human atherosclerosis.

In the present study, we show for the first time that significantly higher levels of plasma F11R are found in patients with more advanced angiographically documented coronary artery disease. Furthermore, on multivariate analysis this association is independent of other factors that correlated with atherosclerosis. Our findings are in accord with the growing body of evidence that the adhesion of platelets to the vessel wall, with their subsequent activation and aggregation, contributes directly to the development and progression of atherosclerosis (1,13–16). Although there is incontrovertible evidence supporting the role of platelets in the acute thrombotic complications of atherosclerosis (17,18), the concept that platelet adhesion to an atherosclerotic lesion may influence plaque progression and stability has only recently received attention. For example, concurrent inhibition of cyclooxygenase (COX)-1 and -2, but not selective inhibition of COX-2, has been shown to cause a significant reduction in experimental atherosclerotic lesions in the mouse (13). Huo et al (15). have also recently demonstrated that circulating activated platelets promote monocyte recruitment to atherosclerotic arteries and accelerate the formation of atherosclerotic lesions in mice deficient in apolipoprotein E.

The findings of the present study are also in accord with our previously reported observation that TNF-alpha increases the endothelial expression of F11R/JAM-A, leading to increased adhesion of platelets which can in turn be blocked by specific inhibitory peptides of the action of F11R (6). In the present study, the correlation of F11R with TNF-alpha was highly significant and is the first clinical data to supports the experimental observations. Furthermore, the absence of a correlation between TNF-alpha and the other tested biomarkers supports the specificity of the TNF-alpha/F11R relationship. In total, these findings solidify the linkage between TNF-alpha, F11R expression, and the inflammatory process in human atherosclerosis.

The finding of a soluble fragment of the extracellular domain of F11R, a cell surface-bound transmembrane

Table 5 Multivariate Analysis for CAD Score*

Predictors of CAD Score	p Value
Age/10 yrs	<0.0001
Hyperlipidemia	0.0009
Chronic renal insufficiency	0.0088
LV function	0.0013
Beta-blocker use	0.0203
F11R	0.0091

*Multivariate linear regression model involves backward stepwise elimination. Twenty-seven clinical and laboratory variables were initially studied (Table 4) by univariate analysis. Only those predictors with $p < 0.05$ were subsequently entered into a multivariate model, the results of which are displayed here.

Abbreviations as in Table 1.

receptor, in a circulating soluble form is not unique. One potential mechanism is that F11R is shed from endothelial cells and/or platelets through a pathway involving enzymatic cleavage at a site in close proximity to the membrane, resulting in the release of the extracellular domain of F11R into the circulation. Such cleavage mechanisms have been reported for L-selectin (19,20) and for the beta-integrin glycoprotein IIIa (21), resulting in the shedding of fragments of these molecules. Whether the platelets or the endothelium are the source of the circulating F11R is not known. The direct demonstration of soluble F11R shedding during or after adhesion of human platelets to cytokine-inflamed endothelial cells is an area of continued basic research on F11R.

Finally, our findings of an inverse correlation between plasma F11R levels and the presence of MI may at first seem counterintuitive. However, it is conceivable that the pool of unbound circulating soluble F11R is diminished in the context of acute coronary syndromes by virtue of its binding to activated platelets, inflamed endothelium, and leukocytes (22–24). Alternatively, the decreased levels of soluble F11R protein may relate to the release of proteases from activated platelets that degrade F11R to smaller fragments, a mechanism that has been reported for other platelet proteins (21,25,26). It is also noteworthy that in its active conformation, the F11R molecule is phosphorylated in its extracellular domain (27). It is possible that in the setting of acute MI (i.e., activated platelets, local release of adenosine triphosphate from dense granules, elevated levels of proteases such as thrombin) changes in phosphorylation/dephosphorylation of the F11R molecule may result in enhanced proteolysis of F11R with a subsequent decrease in plasma levels.

Study limitations. There are several limitations of this study. First, the study was conducted in an exclusively male population. Second, the study population was relatively small, and larger studies in both men and women are needed to determine the factors that influence plasma F11R levels in the absence of atherosclerotic disease. Third, the study involved the measurement of a single baseline determination of plasma F11R and it is unclear whether the results would differ substantially with repeated measurements that could be affected by such factors as diurnal variation. Fourth, the lack of intravascular ultrasound limits the ability to correlate plasma F11R levels with the pathobiology of the vessel wall. Finally, the effects of concurrent illness on plasma F11R levels are still unknown.

Conclusions

The observations derived from this study are consistent with the growing body of evidence demonstrating the importance of platelets in the early phases of atherosclerosis and the critical role of F11R/JAM-A in triggering atherogenesis. The F11R appears to play a significant role in the development or progression of CAD. In particular, by virtue of its high correlation with plasma TNF-alpha, F11R may be an important mediator of the effects of inflammation on the vessel wall. Strategies that block F11R-mediated adhesion of platelets to endothelial cells may represent a novel approach to the treatment and prevention of human atherosclerosis.

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REFERENCES

1. Ruggeri ZM. Platelets in atherothrombosis. Nat Med 2002;8:1227–34.
2. Libby P. Inflammation in atherosclerosis. Nature 2002;420:868–74.
3. Kornecki E, Walkowiak B, Naik UP, Ehrlich YH. Activation of human platelets by a stimulatory monoclonal antibody. J Biol Chem 1990;265:10042–8.
4. Martin-Padura I, Lostaglio S, Schneemann M, et al. Junctional adhesion molecule, a novel member of the immunoglobulin superfamily that distributes at intercellular junctions and modulates monocyte transmigration. J Cell Biol 1998;142:117–27.
5. Sobocka MB, Sobocki T, Banerjee P, et al. Cloning of the human platelet F11 receptor: a cell adhesion molecule member of the immunoglobulin superfamily involved in platelet aggregation. Blood 2000;95:2600–9.
6. Babinska A, Kedees MH, Athar H, et al. F11-receptor (F11R/JAM) mediates platelet adhesion to endothelial cells: role in inflammatory thrombosis. Thromb Haemost 2002;88:843–50.
7. Babinska A, Kedees MH, Athar H, et al. Two regions of the human platelet F11-receptor (F11R) are critical for platelet aggregation, potentiation and adhesion. Thromb Haemost 2002;87:712–21.
8. Babinska A, Azari BM, Salifu MO, et al. The F11 receptor (F11R/JAM-A) in atherothrombosis: overexpression of F11R in atherosclerotic plaques. Thromb Haemost 2007;97:272–81.
9. Bickel C, Rupprecht HJ, Blankenberg S, et al. Relation of markers of inflammation (C-reactive protein, fibrinogen, von Willebrand factor, and leukocyte count) and statin therapy to long-term mortality in patients with angiographically proven coronary artery disease. Am J Cardiol 2002;89:901–8.
10. Cavusoglu E, Ruwende C, Chopra V, et al. Tissue inhibitor of metalloproteinase-1 (TIMP-1) is an independent predictor of all-cause mortality, cardiac mortality, and myocardial infarction. Am Heart J 2006;151:1101e1–8.
11. Parker C 3rd, Vita JA, Freedman JE. Soluble adhesion molecules and unstable coronary artery disease. Atherosclerosis 2001;156:417–24.
12. Krieglstein CF, Granger DN. Adhesion molecules and their role in vascular disease. Am J Hypertens 2001;14:44S–54S.
13. Pratico D, Tillmann C, Zhang ZB, Li H, FitzGerald GA. Acceleration of atherogenesis by COX-1–dependent prostanoid formation in low density lipoprotein receptor knockout mice. Proc Natl Acad Sci U S A 2001;98:3358–63.
14. De Meyer GR, De Cleen DM, Cooper S, et al. Platelet phagocytosis and processing of beta-amyloid precursor protein as a mechanism of macrophage activation in atherosclerosis. Circ Res 2002;90:1197–204.
15. Huo Y, Schober A, Forlow SB, et al. Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E. Nat Med 2003;9:61–7.
16. Sachais BS, Kuo A, Nassar T, et al. Platelet factor 4 binds to low-density lipoprotein receptors and disrupts the endocytic machinery, resulting in retention of low-density lipoprotein on the cell surface. Blood 2002;99:3613–22.
17. Bhatt DL, Topol EJ. Scientific and therapeutic advances in antiplatelet therapy. Nat Rev Drug Discov 2003;2:15–28.
18. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation 2002;105:1135–43.
19. Kahn J, Ingraham RH, Shirley F, Migaki GI, Kishimoto TK. Membrane proximal cleavage of L-selectin: identification of the

cleavage site and a 6-kD transmembrane peptide fragment of L-selectin. J Cell Biol 1994;125:461–70.
20. Chen A, Engel P, Tedder TF. Structural requirements regulate endoproteolytic release of the L-selectin (CD62L) adhesion receptor from the cell surface of leukocytes. J Exp Med 1995;182:519–30.
21. Kornecki E, Ehrlich YH, De Mars DD, Lenox RH. Exposure of fibrinogen receptors in human platelets by surface proteolysis with elastase. J Clin Invest 1986;77:750–6.
22. Coller BS. Leukocytosis and ischemic vascular disease morbidity and mortality: is it time to intervene? Arterioscler Thromb Vasc Biol 2005;25:658–70.
23. Chavakis T, Preissner KT, Santoso S. Leukocyte trans-endothelial migration: JAMs add new pieces to the puzzle. Thromb Haemost 2003;89:13–7.
24. Ostermann G, Weber KS, Zernecke A, Schroder A, Weber C. JAM-1 is a ligand of the beta(2) integrin LFA-1 involved in transendothelial migration of leukocytes. Nat Immunol 2002;3:151–8.
25. Kornecki E, Ehrlich YH, Egbring R, et al. Granulocyte-platelet interactions and platelet fibrinogen receptor exposure. Am J Physiol 1988;255:H651–8.
26. Niewiarowski S, Norton KJ, Eckardt A, Lukasiewicz H, Holt JC, Kornecki E. Structural and functional characterization of major platelet membrane components derived by limited proteolysis of glycoprotein IIIa. Biochim Biophys Acta 1989;983:91–9.
27. Kedees MH, Babinska A, Swiatkowska M, et al. Expression of a recombinant protein of the platelet F11 receptor (F11R) (JAM-1/JAM-A) in insect cells: F11R is naturally phosphorylated in the extracellular domain. Platelets 2005;16:99–109.