Altered Expression of ADAMs (A Disintegrin And Metalloproteinase) in Fibrillating Human Atria

Marco Arndt, PhD*; Uwe Lendeckel, PhD*; Christoph Röcken, MD; Karen Nepple, MS; Carmen Wolke, PhD; Antje Spiess, BSc; Christof Huth, MD; Siegfried Ansorge, PhD; Helmut U. Klein, MD; Andreas Goette, MD

Background—ADAMs (A Disintegrin And Metalloproteinase) are ectoproteases that have recently been reported to be expressed in cardiac tissue. Although they are known to regulate cell-cell and cell-matrix interactions, their pathophysiological role in various cardiac diseases is unclear. The purpose of the present study was to determine whether structural remodeling of the atria during atrial fibrillation (AF) is associated with altered ADAM expression.

Methods and Results—Atrial tissue samples of 30 patients undergoing open-heart surgery were examined. Fifteen patients had persistent AF (≥6 months), and the remaining 15 patients had no history of AF. ADAM9, ADAM10, and ADAM15 expression was analyzed quantitatively at the mRNA and protein levels. ADAM expression was localized by immunohistochemistry. ADAM expression was correlated with amounts of integrins β1 and β3. The amount of ADAM10 protein more than doubled during AF (82±15 versus 36±8 U; \( P < 0.01 \)). Amounts of ADAM15 protein (102±12 versus 40±6 U; \( P < 0.01 \)) and mRNA (24.0±5.6 versus 10.5±2.5 U; \( P < 0.05 \)) increased significantly during AF compared with sinus rhythm. ADAM9 protein was not detected in any sample. ADAM/integrin ratios showed an increase of 4- to 6-fold (\( P < 0.05 \)) in patients with AF who had significantly dilated atria (4.94±0.6 versus 4.3±0.7 cm; \( P < 0.05 \)). ADAM/integrin ratios correlated with atrial diameter.

Conclusions—AF is associated with an increase in the expression of ADAM10 and ADAM15. Enhanced ADAM-dependent disintegrin and metalloproteinase activity may be a molecular mechanism that contributes to the dilation of fibrillating human atria. (Circulation. 2002;105:720-725.)

Key Words: metalloproteinases ■ fibrillation ■ pathology ■ remodeling

Atrial fibrillation (AF) is the most common arrhythmia in clinical practice and is a potential cause of thromboembolic events. Several studies have shown that AF induces significant changes in the electrophysiological properties of atrial myocytes and causes alterations in the structure of the atrial tissue. Alterations in the atrial tissue architecture may contribute to the mechanical dysfunction and dilation of the atria. The molecular basis for the development of structural atrial remodeling of fibrillating human atria is still a matter of debate. Its understanding, however, could have an important therapeutic impact.

ADAMs (A Disintegrin And Metalloproteinase), also referred to as MDCs (metalloproteinase/disintegrin/cysteine-rich), form a large family of membrane-bound glycoproteins that function in proteolysis, signaling, adhesion, and fusion. Generally, they are involved in essential cellular processes such as fertilization, myoblast fusion, neuronal development, and cleavage-secretion of membrane-bound proteins. Because of their disintegrin and metalloproteinase activity, ADAMs are known to regulate cell-cell and cell-matrix interactions and may thereby influence the architecture of cardiac tissue. In addition, ADAMs may also be of importance for structural remodeling of cardiac tissue by performing cleavage-secretion of surface-bound proteins.

The purpose of the present study was to assess the role of cell surface members of the ADAM family (ADAMs9, 10, and 15) and their relationship to the expression of integrins β1 and β3 in atrial tissue of patients with and without AF.

Methods

Patients

Right atrial appendages were obtained from 30 patients undergoing cardiac bypass surgery or mitral/aortic valve replacement. Tissue samples were taken from 15 consecutive patients with chronic persistent AF (≥6 months) and from 15 patients with no history of AF (SR). The clinical characteristics are shown in Table 1. The study was approved by the local ethics committee, and all patients gave written informed consent to participate in the study.
RNA Isolation and Reverse Transcription

Samples of human atrial appendages were rapidly frozen in liquid nitrogen and stored at −192°C until further analysis. Total RNA was prepared as described recently by applying the method of Chomczynski and Sacchi. A quantity of total RNA was transcribed into cDNA by using avian myeloma virus reverse transcriptase (Promega, Mannheim, Germany) as described previously.

Quantitative Polymerase Chain Reaction

Quantitative polymerase chain reaction (PCR) was performed using the Lightcycler LC24 (Idaho Technology). All samples were analyzed in triplicate. A 10-μL reaction mixture consisted of 1× reaction mixture with BSA (Idaho Technology); 3 mmol/L MgCl₂; 200 μmol/L dNTPs; 0.4 U InViTaq-polymerase (InViTek); 0.2 μL of a 1:1000 dilution of SYBR-Green I (Molecular Probes); 1 μL cDNA; and 0.5 μmol/L of the specific primers for ADAM9 (5′-GCTAGTTGGACTGGAGATTGG-3′ and 5′-TTATTAC-CACAGGGAGGGAGC-3′), ADAM10 (5′-TAACCTCCGG-CAGGTGTTG-3′ and 5′-GCCACCGACTCCATCGAGG-3′), and ADAM15 (5′-CAAATATAGGGAGGACTG-3′ and 5′-TAGCAGCAGTTCTCAAAGTGTG-3′).

Initial denaturation at 95°C for 10 seconds was followed by 40 cycles with denaturation at 95°C for 0.1 second, annealing at 65°C for 3 seconds, and elongation at 72°C for 16 seconds. Quantities of 18S-mRNA were determined with the reverse transcriptase primer pair available from Ambion and used to normalize cDNA contents. The fluorescence intensity of the double-strand-specific SYBR-Green I, reflecting the amount of PCR product actually formed, was read in real time at the end of each elongation step. Then, amounts of specific initial template mRNA were calculated by determining the time point at which the linear increase of sample PCR product started relative to the corresponding points of a standard curve; these are given as arbitrary units.

Patient Characteristics

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<th>HMI</th>
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AF (n=15)

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<td>52.33±14.94</td>
<td>4.94±0.6*</td>
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SR indicates sinus rhythm; AF, chronic persistent atrial fibrillation; f, female; m, male; CAD, number of diseased coronary arteries; HMI, history of myocardial infarction, present (1), absent (0); VD, valve disease requiring valve replacement; MR, mitral regurgitation; AS, aortic stenosis; AR, aortic regurgitation; MS, mitral stenosis; A, ACE inhibitors; B, β-blockers; C, calcium-channel blockers; D, digitoxin; Di, diuretics; N, nitrates; and NA, data not available.

*P<0.05 vs sinus rhythm.
Western Blotting
Sample preparation and blotting were performed exactly as described previously. Monoclonal anti-integrins $\beta_1$ and $\beta_2$ and polyclonal rabbit anti-ADAM10, anti-ADAM9 (Chemicon), and anti-ADAM15 antibodies as well as goat-anti-mouse horse-radish peroxidase (New England BioLabs) and Supersignal West Dura Extended Duration Substrate (Pierce) were used for immunochromoluminescence detection. The resulting images were quantified densitometrically using RFLP-Scan software (MWG Biotech). The mean relative absorption units of the group with sinus rhythm (SR) were set as controls, and these were compared with the corresponding means of the AF groups.

To determine the amounts of membrane-bound or soluble ADAM15, tissue samples were homogenized in 5 volumes of 20 mmol/L HEPES buffer and 0.25 mol/L sucrose, pH 7.0. The supernatant obtained at 100,000g was regarded as the soluble fraction and the resuspended pellet as the membrane fraction.

Densitometric quantification for comparison of the different groups was performed only on blots processed equally and exposed on the same x-ray film.

Immunohistochemistry
Specimens from eight right atrial appendages were fixed in 10% buffered formalin and embedded in paraffin. Deparaffinized sections were stained with hematoxylin and eosin. Immunostaining with monoclonal anti-integrins $\beta_1$ and $\beta_2$ and polyclonal anti-ADAM10 (dilution 1:400) and anti-ADAM15 (1:100) was performed using a Vectastain ABC alkaline phosphatase kit (distributed by CAMON). Sections were deparaffinized and rehydrated in a graded alcohol series. Incubation was performed in a moist chamber at 37°C for 1 hour. Polyclonal anti-rabbit or anti-mouse IgG (30 minutes, room temperature; Immunotech) and the addition of its substrate Fast Red/Naphthol Mx (Immunotech). Washings between steps were performed using TBS. The specimens were counterstained with hematoxylin and embedded in gelatin. Specimens in which primary antibodies were omitted served as controls.

High-Resolution Immunofluorescence Analysis
Isolated human fibroblasts were grown for 24 hours on coverslips in RPMI 1640 (Life Technologies) supplemented with 10% FCS (BioWest). Cells were washed twice with PBS and subsequently fixed, permeabilized, and immunostained using the Fix&Perm cell permeabilization kit (An Der Grub BioResearch) according to the recommended protocol. Nonspecific binding was prevented by 30-minute incubation in a mixture of equal volumes of goat and mouse serum at room temperature. Subsequently, monoclonal anti-integrin-$\beta_1$, polyclonal rabbit anti-ADAM15, or a mixture of both was applied to the cells in 1:20 dilutions in PBS and incubated at room temperature for 15 minutes. After extensive washing in PBS, cells were incubated for 30 minutes with anti-rabbit IgG conjugated to FITC (Sigma; 1:200) and anti-mouse IgG conjugated to Texas Red (ICN Biomedicals; 1:200). For nuclear staining, 1 µg/mL 4',6-diamidino-2'-phenylindole dihydrochloride (DAPI, Roche) was in/H11032.

Results
The baseline patient characteristics are shown in Table 1. The severity of the underlying heart diseases and noncardiac diseases were similar for both groups. The diameter of the left atrium was increased in patients with AF (AF, 4.94±0.6 cm; SR, 4.3±0.7 cm; P<0.05).

Figure 1. Atrial expression of ADAM9, ADAM10, and ADAM15 mRNA in patients with SR and AF. ADAM15 mRNA expression was significantly increased in patients with AF. *P<0.05.

ADAM 9, 10, and 15 Gene Expression
Generally, the mean amounts of ADAM mRNAs were elevated in patients with AF compared with those with SR. However, for ADAM9 (11.4±3.4 versus 5.0±0.6 U; P=NS) and ADAM10 (6.3±2.0 versus 2.5±0.4 U; P=NS), the observed increase did not reach statistical significance. The mean amounts of ADAM15 mRNA were significantly increased in patients with AF (24.0±5.6 U) compared with SR (104.9±24.8 U; P<0.05) (Figure 1).

ADAM9, ADAM10, and ADAM15 Protein Expression
ADAM9 was not detectable by Western blotting techniques in the atrial tissue of patients with AF and SR. In patients with AF, there was a marked upregulation of ADAM10 (0.82±0.15 versus 0.36±0.08 U; P<0.01) and ADAM15 (1.02±0.12 versus 0.4±0.06 U; P<0.01) compared with those with SR (Figure 2A and 2B).

In patients with AF, the majority of ADAM15 protein was detected in the membrane fraction (78.8%). In contrast, 69.6% of ADAM15 immunoreactivity was assigned to the cytosolic fraction in patients with SR (Figure 3). Similar to the overall results, differences in ADAM protein levels were also observed when sex-matched pairs (n=11) were analyzed. Sex per se did not influence ADAM expression.

Integrin $\beta_1$ and $\beta_2$ Protein Expression
In patients with AF, there was a trend toward an increased expression of integrin $\beta_1$ only (109.7%±46.4%) and integrin $\beta_1$ (120.2%±58.5%) compared with SR (100%; P=NS).

Correlation of ADAM15 and Integrin $\beta_1$
Protein Expression
In patients with AF, the ADAM15/integrin $\beta_1$, and ADAM15/integrin $\beta_1$ protein ratios were significantly elevated com-
pared with patients with SR (β₁ ratio, 4.46 ± 2.6 versus 0.65 ± 0.14 U, and β₃ ratio, 1.51 ± 0.64 versus 0.37 ± 0.17 U; \( P < 0.05 \)) (Figure 4). Analysis of individual expression values showed a significant positive correlation of ADAM15 with integrin \( \beta₁ \) protein levels (\( r = 0.76 \)). Importantly, the ADAM15/integrin \( \beta₁ \) ratio correlated significantly with the left atrial diameter (\( r = 0.75; \ P < 0.05 \)) and the duration of fibrillation (\( r = 0.80; \ P < 0.05 \)).

Histochemistry and Immunohistochemistry
Immunohistochemical analysis showed that the distribution pattern of ADAM10 and ADAM15 was similar in the atria of patients with (n = 4) or without (n = 4) AF. Both ADAMs were expressed by vascular smooth muscle cells; endocardial cells; fibroblasts; vascular endothelial cells; and occasionally, epicardial cells. In addition, immunostaining for ADAM15 was found in cardiac myocytes; in the lumens of blood vessels; and, also occasionally, extracellularly within the interstitial matrix (Figure 5).

The pattern of integrin \( \beta₁ \) and \( \beta₃ \) expression was also similar in patients with and without AF. Integrin \( \beta₁ \) was expressed mainly by myocytes and only occasionally by fibroblasts, whereas integrin \( \beta₃ \) was found in myocytes, fibroblasts, endothelial cells, and epicardial cells.

High-resolution immunofluorescence analysis revealed a colocalization of ADAM15 and integrin \( \beta₁ \) in isolated human fibroblasts (Figure 6).

Discussion
Main Findings
The present study describes profound changes in ADAM expression in fibrillating atrial tissue and suggests a molecular mechanism that seems to contribute to atrial dilation during AF. Atrial tissue of patients with AF shows increased

Figure 2. A, Representative Western blots of ADAM9, ADAM10, and ADAM15 from patients with SR and AF. B, Densitometric quantification of Western blots from patients with SR and AF. *\( P < 0.01 \).

Figure 3. ADAM15 protein amounts in cytosolic and membrane fractions of atrial tissue from patients with SR and AF. *\( P < 0.05 \) cytosol vs membrane; †\( P < 0.05 \) AF vs SR.

Figure 4. Ratios of protein amounts of ADAM15 and integrin \( \beta₁ \) or \( \beta₃ \) are significantly elevated in patients with AF (gray boxes) as compared with SR (white boxes). *\( P < 0.05 \).

Figure 5. Immunostaining of atrial tissue with ADAM10 (left lane) and ADAM15 (right lane) antibody (red staining). Fibroblasts showed moderate to strong immunostaining for ADAM10 (arrowheads). ADAM15 was found in fibroblasts (arrowheads), in cardiac myocytes and extracellularly in the interstitium (†). AF did not change the staining pattern of these ADAMs. Original magnification ×40.
levels of ADAM10 and ADAM15 expression at both the mRNA and protein levels. Membrane expression of ADAM15 is significantly upregulated during AF, whereas most ADAM15 is retarded in the cytoplasm during SR. The ADAM15/integrin $\beta_i$ ratio is significantly increased in fibrillating tissue and correlates with the left atrial diameter and the duration of fibrillation.

**Effect of Increased ADAM Expression**

The purpose of the present study was (1) to determine whether ADAMs 9, 10, and 15 are expressed in human atrial tissue and (2) to investigate whether the expression pattern changes at mRNA and/or protein levels during AF. AF is known to be associated with progressive structural changes of the atria resulting in atrial dilation and loss of transport function. Some of these changes may be a consequence of the activity of ADAMs. The expression of ADAMs has been described in cardiac tissue. However, the present study is the first to show an alteration in cardiac ADAM expression in response to a specific pathological condition.

In previous studies, we described profound changes in the angiotensin system during AF, including an upregulation in the expression of angiotensin-converting enzyme and loss of angiotensin II type 1 receptor expression. In these patients, the switch from dominant angiotensin II type 1 receptor expression to that of angiotensin II type 2 receptor seems to represent a compensatory mechanism aimed at the limitation of progressive atrial fibrosis. Some of these changes may be a consequence of the activity of ADAMs. In the present study, we found that the expression of individual ADAMs is increased during AF. Several studies have shown significant morphological changes (fibrosis/atrial dilation) in atrial tissue of patients with AF. Interestingly, atrial tissue from patients with AF shows structural and molecular abnormalities comparable with those demonstrated in failing human ventricles. Recently, Ding et al described altered $\beta_i$-integrin deposition associated with morphological changes in the failing heart. Integrin attachment to the extracellular matrix represents fixed anchorage points, and it is assumed that they need to be changed repeatedly as myocytes grow and change morphology. Shedding of integrins into the extracellular matrix is consistent with cell growth, and it is proposed by Ding et al that integrin shedding is a significant feature of hypertrophic growth and, furthermore, is associated with changes in tissue architecture and cavity geometry. One likely mechanism is the shedding of integrin $\beta_i$ by ADAMs, which are known to be associated with integrins in other tissues. In addition to shedding of integrins, ADAMs can also bind to integrins and, thereby, affect cell adhesion. As shown for ADAM9 and 15, their potential role in regulating cell adhesion results from binding to integrin $\alpha_i\beta_i$ or $\alpha_i\beta_i$, respectively. It is tempting to speculate, thus, that changes in ADAM expression and their effects on integrins $\beta_i$ and $\beta_i$ alter cell-matrix interactions, which contributes to the dilation of fibrillating atria.

Our data strongly suggest a role for ADAMs in the structural remodeling of fibrillating atria. We demonstrated that ADAM15 and integrin $\beta_i$ are largely colocalized on human fibroblasts. The increased atrial expression of ADAM10 and 15 during AF goes along with nonsignificantly elevated amounts of integrin $\beta_i$ and $\beta_i$ proteins. Integrin cleavage products, however, were not detected by Western blotting or immunohistochemistry. Thus, it seems unlikely that ADAM10 and 15 participate in the shedding of these integrins. In contrast to integrin shedding, binding of ADAM at integrin molecules may inhibit a regular integrin-matrix interaction, which may increase “cell motility” and result in sliding and slippage of atrial cells. Thus, the increased ADAM/integrin interaction appears to be a molecular mechanism that explains atrial dilation; this is further supported by the significant correlation between ADAM/integrin ratio and atrial diameter. Interestingly, the observed increase in total ADAM15 protein in patients with AF was due to selective induction of membrane-bound ADAM15, whereas in patients with SR a major fraction could be assigned to the cytosol. This further suggests a functional linkage between ADAMs and integrins during AF. Progressive dilation of fibrillating atria is a well-described clinical phenomenon. Increased atrial diameters are predictive of a poor outcome for cardiovascular and, in addition, correlate with increased rates of thromboembolic events during AF. Therefore, the molecular findings demonstrated here may have significant clinical relevance because characterization of the molecular mechanism involved in atrial dilation may help to define novel therapeutic strategies in patients with AF. In this context, the development of specific inhibitors of ADAM activity appears to be feasible. Whether ADAMs have other roles in cell adhesion or ectodomain shedding of factors that affect fibrillating atrial tissue remains to be established in future studies.

**Limitations**

Some potential limitations may have influenced our results. All patients included in our study underwent cardiac surgery because of coronary artery disease or valve diseases. Thus, no comment can be made about the possible impact of ADAMs on structural changes in other patient populations. Only right atrial samples were examined; therefore, our present findings...
may not be representative of other parts of the atra. Although a relatively small number of tissue samples were analyzed, the differences in ADAM expression were found to be highly significant. The apparent discordance between quantities of ADAM9 mRNA and protein requires further investigation. However, discordances between mRNA and protein levels due to posttranscriptional processing have been described previously.14,35; these discordances might have resulted in protein levels of ADAM9 being below the detection limits of our study. Further experiments in animal models may support the present data as well as previous experimental findings.

**Conclusions**

To the best of our knowledge, the present study is the very first report demonstrating the regulation of atrial ADAM expression in patients with and without AF. The increased ADAM expression seems to contribute to structural changes (dilation) of fibrillating atria. These findings may be of clinical importance in developing new therapeutic strategies to prevent atrial dilation and, thereby, the risk of thrombo-embolic events in patients with AF.

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**References**

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