Changes in matrix metalloproteinase-9 levels during progression of atrial fibrillation

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Abstract
Objectives: To observe levels of matrix metalloproteinase (MMP)-9 and evaluate their significance in various stages of idiopathic atrial fibrillation (AF).
Methods: Patients with idiopathic AF were recruited into this prospective study and classified into one of three groups according to stage of disease progression: paroxysmal AF; persistent AF; permanent AF. Healthy individuals were enrolled as control subjects. Serum levels of MMP-9 in all four groups were determined using a double-antibody sandwich enzyme-linked immunosorbent assay.
Results: Each AF group included 25 patients; 40 healthy individuals were included as controls. MMP-9 levels in the three AF groups were significantly higher than in the control group: 168.72 ± 25.970, 201.36 ± 31.26 and 253.20 ± 22.99 ng/ml for the paroxysmal, persistent and permanent AF groups respectively, versus 76.80 ± 14.90 ng/ml for the control group. MMP-9 levels increased with idiopathic AF disease progression (P < 0.05).
Conclusions: An elevated MMP-9 level appears to be associated with a diagnosis of AF. MMP-9 levels appear to increase in relation to the stage of idiopathic AF progression.

Keywords
Atrial fibrillation, concentration, disease progression, extracellular matrix, matrix metalloproteinase-9, pathological mechanism

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Introduction

Atrial fibrillation (AF) is a frequently occurring disease that is encountered in emergency medicine settings. Experimental and clinical research have shown that, whatever the initial cause or trigger, there is a relationship between AF and alterations in atrial electrical properties. However, the pathogenesis of AF is not yet fully understood. Atrial interstitial tissue fibrosis is an important mechanism of AF that could lead to atrium diameter expansion, atrial wall thinning and, thus, atrium structure reconstruction. Endogenous enzymes involved in extracellular matrix remodelling include the matrix metalloproteinases (MMPs), whose substrates are different types of collagen; consequently, the serum level of MMP-9 is considered to be a marker of extracellular collagen degradation. Studies have found that MMPs may be implicated in the development of myocardial fibrosis. For example, the MMP-9 level is an important index for myocardial fibrosis and also has a close relationship with other cardiovascular diseases. The present study observed MMP-9 levels in patients with idiopathic AF at different stages of disease progression, compared these levels with those found in healthy controls, and assessed the relationship between MMP-9 levels and idiopathic AF progression.

Patients and methods

Participants

This study recruited people with idiopathic AF who were outpatients or inpatients receiving care at Renmin Hospital of Wuhan University, Wuhan, Hubei, China between July 2010 and July 2012. Patients were recruited sequentially and were allocated to one of three study groups according to stage of idiopathic AF disease progression (paroxysmal AF, persistent AF and permanent AF), based on the American College of Cardiology/American Heart Association/European Society of Cardiology 2006 guidelines for AF treatment, until there were 25 patients in each group. Patients included in the study were free from organic cardiovascular disease, hyperthyroidism, pregnancy and infectious diseases, and had not undergone surgery within the previous 2 months. Echocardiography was performed to confirm that patients did not have atrial thrombus. None of the patients had undergone electrical or pharmacological cardioversion therapy in the previous month or had taken angiotensin-converting enzyme inhibitors, corticosteroids or statins in the previous week. Patients with AF who also had structural heart disease were excluded from the study.

The control group comprised people attending the hospital for routine physical examination who exhibited no special discomfort on examination or any abnormality on medical, blood biochemistry, electrocardiogram, echocardiographic (including liver and gallbladder echocardiographic), spleen or kidney ultrasonographic, or chest X-radiographic examinations.

The study protocol was approved by the Medical Ethics Committee of Renmin Hospital of Wuhan University, Wuhan, Hubei, China. All study participants provided written informed consent.

Blood sampling and analysis

Early in the morning after enrolment, 4-ml venous blood samples were taken from each patient in the fasting state. After treatment with 100 g/l sodium citrate as anticoagulant and centrifugation (4-16K, Sigma-Aldrich, Germany) at 1360 g for 10 min at 4°C, serum was dispensed into EP tubes (MCT-150-C, Axygen, America) and stored at −80°C until analysis. Blood samples were analysed in batches every 3 months. The serum concentration of MMP-9 was determined using a double-antibody sandwich enzyme-linked immunosorbent assay kit (Shanghai Senxiong Biotech, Shanghai,
China), according to the manufacturer’s instructions.

**Additional assessments**

Body mass index, left atrial diameter, left ventricular ejection fraction, systolic and diastolic blood pressure, and concentrations of serum Na\(^+\) and K\(^+\) were determined by standard methods after enrolment.

**Statistical analyses**

This study recruited the same number of patients into each group to help with statistical comparisons. Continuous data were presented as mean ± SD; categorical data were presented as \(n\). Statistical comparisons were undertaken using SPSS\textsuperscript{®} software, version 11.0 (SPSS Inc., Chicago, IL, USA). A \(P\)-value < 0.05 indicated statistical significance. Between-group comparisons of continuous variables were undertaken using independent-samples \(t\)-test.

**Results**

This study enrolled 75 patients with AF: 25 cases for each stage of idiopathic AF disease progression (paroxysmal AF, persistent AF, permanent AF) and 40 healthy control subjects. There were no significant between-group differences in age, sex, body mass index, left atrial diameter, left ventricular ejection fraction, blood pressure, or serum Na\(^+\) and K\(^+\) concentrations (Table 1).

Levels of MMP-9 in the three AF-groups were significantly higher than in the control group \((P < 0.01)\) and showed a significant gradual increase from paroxysmal AF through persistent AF to permanent AF \((P < 0.05)\) (Table 2).

**Discussion**

Under physiological conditions, the extracellular matrix is in homeostasis by being continuously produced and degraded, and an imbalance in its synthesis or degradation is related to the occurrence of cardiovascular disease.\textsuperscript{11,12} MMPs can degrade many proteins of the extracellular matrix, such as collagen, laminin, fibronectin, proteoglycans and elastin.\textsuperscript{13–15} Increases in expression and activity of MMPs are observed in the development of many cardiovascular diseases. Specifically, MMP-9 has been

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**Table 1.** Baseline characteristics of patients with different stages of idiopathic atrial fibrillation (AF) and healthy controls, enrolled in a study investigating matrix metalloproteinase-9 levels and their relationship with AF progression.

| Characteristic                  | Paroxysmal AF, \(n = 25\) | Persistent AF, \(n = 25\) | Permanent AF, \(n = 25\) | Controls, \(n = 40\) |
|--------------------------------|----------------------------|---------------------------|--------------------------|----------------------|
| Age, years                     | 56.1 ± 17.3                | 58.6 ± 15.1               | 59.1 ± 16.4              | 54.8 ± 14.9          |
| Sex, male/female               | 19/6                       | 20/5                      | 19/6                     | 31/9                 |
| BMI, kg/m\(^2\)                | 27 ± 3.6                   | 29 ± 4.6                  | 28 ± 5.1                 | 27 ± 5.7             |
| Left atrial diameter, mm       | 28.3 ± 6.7                 | 30.1 ± 5.8                | 26.4 ± 7.2               | 26.1 ± 8.7           |
| LVEF, %                        | 64 ± 11                    | 66 ± 11                   | 64 ± 13                  | 69 ± 9               |
| Systolic BP, mmHg              | 125.2 ± 12.1               | 126.3 ± 10.8              | 122.3 ± 12.4             | 117.6 ± 13.6         |
| Diastolic BP, mmHg             | 78.0 ± 11.9                | 76.0 ± 8.1                | 78.0 ± 10.1              | 77.2 ± 11.8          |
| Serum Na\(^+\), mmol/l         | 142.5 ± 7.4                | 140.5 ± 6.2               | 143.5 ± 8.9              | 143.1 ± 8.1          |
| Serum K\(^+\), mmol/l          | 4.2 ± 0.7                  | 4.3 ± 0.6                 | 4.3 ± 0.7                | 4.4 ± 0.8            |

Data presented as mean ± SD or \(n\). BMI, body mass index; BP, blood pressure; LVEF, left ventricular ejection fraction. No statistically significant differences observed \((P \geq 0.05; \text{independent-samples} \, t\text{-test})\).
implicated in the process of myocardial fibrosis.\textsuperscript{16,17} In addition, MMP-9 was shown to be induced – and generated collagen-matrix fragments, such as endostatin and angiotatin – in patients undergoing coronary artery bypass grafting.\textsuperscript{18} In murine studies, Ducharme and colleagues\textsuperscript{19} found that MMP-9 played a key role in extracellular remodelling after myocardial infarction: following infarction, the loss of MMP-9 activity in mice with targeted deletion of \textit{mmp9} led to a significant reduction in collagen deposition in the infracted region. In addition, a reduction in inflammatory cell infiltration was observed in the \textit{mmp9}-knockout mice, and the authors concluded that left ventricular enlargement would ease after MI, thus providing evidence that MMP-9 is associated with cardiac remodelling.\textsuperscript{19} In the present study we observed the relationship between MMP-9 levels and the occurrence and progression of AF.

We selected patients with idiopathic AF as research participants to exclude the influence of organic diseases on MMP-9 levels. Close relationships between MMP levels and AF have been reported.\textsuperscript{20–22} Patients with both chronic rheumatic heart disease and AF showed abnormal expression of MMP-1,\textsuperscript{23} and MMP-9 has been associated with rapid atrial arrhythmia.\textsuperscript{24,25} In an animal model of rapid atrial pacing-induced atrial failure, MMP-9 activity in the atrial intramuscular matrix increased by 50% compared with sinus rhythm, while levels of specific tissue inhibitors decreased by 50%.\textsuperscript{26} Another study has shown that MMP inhibitors can decrease left ventricular dilatation and wall thinning of heart failure induced by overdrive pacing, to reduce atrial chamber wall stress.\textsuperscript{27} However, there is no report on whether the MMP-9 level is related to AF development and progression in humans.

We found that MMP-9 levels in patients with idiopathic AF were significantly higher than those in the healthy control population, indicating that MMP-9 may play a role in the occurrence and maintenance of AF. MMP-9 may be involved in degradation and reconstruction of the extracellular matrix under the influence of metallic zinc ions: it could, for example, cut off extracellular matrix components, regulate cell adhesion, act on extracellular components or other protein components and activate potential biological function, and participate directly or indirectly in tissue remodelling and wound repair.\textsuperscript{28} When MMP-9 levels are elevated, the above-mentioned functions are enhanced, which would result in excessive extracellular matrix degradation, increased tissue remodelling and myocardial fibrosis, and could contribute to micro re-entry and eventually lead to AF.\textsuperscript{29}

Our study also found that MMP-9 levels gradually increased from paroxysmal AF through persistent AF to permanent AF.

| Characteristic | Paroxysmal AF, n = 25 | Persistent AF, n = 25 | Permanent AF, n = 25 | Controls, n = 40 |
|---------------|------------------------|-----------------------|----------------------|-----------------|
| MMP-9 (ng/ml) | 168.72 ± 25.97\textsuperscript{a,b} | 201.36 ± 31.26\textsuperscript{a,b} | 253.20 ± 22.99\textsuperscript{a,b} | 76.80 ± 14.90 |

Data presented as mean ± SD.
\textsuperscript{a}\textit{P} < 0.05 for between-group treatment comparisons (independent-samples \textit{t}-test).
\textsuperscript{b}\textit{P} < 0.01 compared with control group (independent-samples \textit{t}-test).
In addition, we identified significant differences in MMP-9 levels between the three study groups. Other research has demonstrated that AF is characterized by self-sustaining and gradual progression, and that the majority of cases of paroxysmal AF will eventually evolve into persistent or even permanent fibrillation. The mechanism for this is not very clear. Other studies have mainly focused on the electrical remodelling of AF, believing that ion channel remodelling mainly causes AF progression. The results of the present study suggest that a gradual elevation in the MMP-9 level may worsen myocardial interstitial fibrosis, and that structural remodelling may also play an important role in AF progression, at least in part due to the known involvement of MMP-9 in the structural remodeling process.

The limitation of the present study is its sample size; a larger sample is needed for further research. Given that AF is a multifactorial disease of unclear aetiology, further research should also investigate factors in addition to MMP-9 that may have a bearing on its development and course.

In conclusion, our study found that MMP-9 levels are higher in patients with AF than in healthy controls, and that MMP-9 may therefore be associated with the development of AF. In addition, our findings provide indications of a relationship between the increase in the MMP-9 level and the progression of AF. Further research is necessary.

Declarations of conflict of interest

The authors declare that there are no conflicts of interest.

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