Genetic ACE I/D Polymorphism and Recurrence of Atrial Fibrillation After Catheter Ablation

Laura Ueberham; Andreas Bollmann, MD, PhD; Moore Benjamin Shoemaker, MD; Arash Arya, MD; Volker Adams, PhD; Gerhard Hindricks, MD; Daniela Husser, MD

**Background**—The angiotensin-converting enzyme (ACE) deletion allele, ACE D, is associated with increased cardiac ACE activity, cardiac fibrosis, and adverse outcomes in cardiovascular disease and has been linked with failure of antiatrial fibrillation (anti-AF) drug treatment. This study tested the hypothesis that the ACE gene insertion/deletion polymorphism associates with AF recurrence after catheter ablation.

**Methods and Results**—In 238 consecutive patients (69% male; mean age, 58±11 years) undergoing catheter ablation of paroxysmal (59%) or persistent (41%) AF, the ACE insertion/deletion polymorphism was genotyped using polymerase chain reaction. After a blanking period of 3 months, AF recurrence (defined as any atrial arrhythmia lasting ≥30 s) was detected using serial 7-day Holter ECG recordings after 3, 6, and 12 months. AF recurrence was observed in 39% and was associated with persistent AF, longer history of AF, previous antiarrhythmic drug use, previous use of diuretics, increased left atrial diameter, increased left ventricular end-diastolic diameter, additional linear ablation lesions, and ACE DD polymorphism. In multivariable analysis, left atrial diameter (odds ratio, 1.111; 95% confidence interval, 1.040–1.187; P=0.002) and ACE DD genotype (odds ratio, 2.251; 95% confidence interval, 1.056–4.798; P=0.036) remained predictors for AF recurrence.

**Conclusions**—Left atrial enlargement and the ACE DD polymorphism are predictors for AF recurrence after catheter ablation. The association between the ACE DD polymorphism and AF recidivism supports the use of genetic data for predicting response to AF therapies and highlights the role of fibrosis in AF development. *(Circ Arrhythm Electrophysiol. 2013;6:732-737.)*

**Key Words:** ACE I/D genotype ■ ACE polymorphism ■ atrial fibrillation ■ catheter ablation ■ genetics ■ polymorphism, genetic

**Clinical Perspective on p 737**

Current atrial fibrillation (AF) guidelines recommend catheter ablation for patients with symptomatic, antiarrhythmic drug refractory AF or as first-line therapy if no or minimal heart disease is present. Single-procedure ablation achieves freedom from AF in 57% to 89%, depending on patient characteristics, ablation strategies, and follow-up. Prediction of rhythm outcome remains challenging, although a recent meta-analysis identified nonparoxysmal AF, valvular AF, and increased left atrial diameter (LAD) as independent clinical predictors for recurring AF.

In the past 15 years, increasing interest in the genetic background of AF resulted in the identification of common gene variants that associate with AF. However, there is only limited data correlating genotypes with outcomes of AF therapies and in particular with rhythm outcome after AF ablation. Consequently, this study tested the hypothesis that the angiotensin-converting enzyme gene (ACE) insertion/deletion polymorphism associates with AF recurrence after catheter ablation. Using a candidate gene approach, this polymorphism was selected because the ACE deletion allele, ACE D, is associated with increased ACE serum levels, higher cardiac ACE activity, and increased cardiac fibrosis. The ACE D allele was also demonstrated to associate with electric remodeling in patients with lone AF and those with heart disease and has been linked with failure of anti-AF drug therapy and AF recurrence in Chinese-Han.

**Methods**

**Study Population**

The study included a total of 238 consecutive patients, enrolled in the Leipzig Heart Center AF ablation registry, who underwent left atrial radiofrequency catheter ablation for symptomatic, drug-refractory paroxysmal or persistent AF. All patients received a transthoracic and transesophageal echocardiography before ablation procedure to exclude left atrial thrombus, and standardized measurements of LAD, left ventricular ejection fraction, interventricular septal end-diastolic dimension, and left ventricular end-diastolic diameter were...
performed. Antiarrhythmic medication (class I and III antiarrhythmic drugs) was stopped before ablation procedure. The study was approved by the local ethics committee (Medical Faculty, University of Leipzig), and all patients gave their written informed consent.

Catheter Ablation

Left atrial catheter ablation was performed according to a previously described approach. In brief, patients were studied under deep propofol sedation with continuous invasive monitoring of arterial blood pressure and oxygen saturation. Nonfluoroscopic 3-dimensional catheter orientation, computed tomographic image integration, and tagging of the ablation sites were performed using Ensite NavX, Ensite Velocity (St. Jude Medical, St. Paul, MN) or CARTO 3 (Biosense Webster, Diamond Bar, CA). Trans-septal access and catheter navigation were performed with a steerable sheath (Agilis, St. Jude Medical, St. Paul, MN). Patients presenting with AF at the beginning of the procedure were electrically cardioverted, and ablation was performed during sinus rhythm (ie, AF termination with ablation was not attempted). In all patients, circumferential left atrial ablation lines were placed around the antrum of the ipsilateral pulmonary veins (irrigated tip catheter, preselected tip temperature of 48°C, and maximum power of 30–50 W). In patients with persistent AF, additional linear lesions were added at the left atrial roof, the basal posterior wall, and the left atrial isthmus. Ablation of complex fractionated electrograms was not performed.

After circumferential line placement, voltage and pace mapping along the ablation line were used to identify and close gaps. The isolation of all pulmonary veins with bidirectional block was verified with a multipolar circular mapping catheter and was defined as the procedural end point.

Follow-Up

After ablation procedure, patients were followed up in the outpatient clinic for 12 months. There was no reinitiation of antiarrhythmic drug class I or III therapy after ablation. Anticoagulation therapy was prescribed for 4 weeks. Proton pump inhibitors were prescribed for 3 months and according to the CHA2DS2-VASc-Score thereafter. Patients with persistent AF, additional linear lesions were added at the left atrial roof, the basal posterior wall, and the left atrial isthmus. Ablation of complex fractionated electrograms was not performed. After circumferential line placement, voltage and pace mapping along the ablation line were used to identify and close gaps. The isolation of all pulmonary veins with bidirectional block was verified with a multipolar circular mapping catheter and was defined as the procedural end point.

Genotyping

DNA extraction was performed using a commercial isolation kit (peqGOLD Blood DNA Mini Kit, PeqLab, Erlangen, Germany) according to manufacturer instructions. ACE insertion/deletion polymorphism was detected using polymerase chain reaction. A set of commercially synthesized primers (TibMolBio, Berlin, Germany, sense primer 5′ CTGGAGACATTCCCATCTTCT 3′ and antisense primer 5′ GATGGTGCCATACCATCTGCAGAT 3′) were used for polymerase chain reaction. DNA amplification contained between 35 and 40 cycles. Agarose gel electrophoresis and ethidium bromide staining were used to identify the polymerase chain reaction products with lengths of 190 bp (D allele) and 490 bp (I allele) as illustrated in Figure 1.

Statistical Analysis

Continuous variables are presented as mean±1SD, and categorical variables are presented as frequencies. Comparison of continuous variables was performed using the unpaired Student t test and of categorical variables using the Pearson χ² test. To identify predictors of AF recurrence, a multivariable logistic regression was performed. We adjusted for all variables found in univariate analysis with a P value <0.15 in the multivariable logistic regression analysis, along with variables found to be significantly associated with each genotype. A 2-sided P value <0.05 was considered as statistically significant.

Results

Patient Characteristics

Patient characteristics of the total study population and stratified by the ACE genotype are summarized in Table 1. Genotype frequencies were 23%, 44%, and 33% for the ACE II, ID, and DD genotype, respectively, and did not differ significantly from those expected by Hardy–Weinberg equilibrium calculations. DD carriers were more often men and users of inhibitors of the renin–angiotensin–aldosterone system, whereas other clinical, echocardiographic, and procedural variables were comparable among different ACE genotypes.

Predictors for AF Recurrence

Complete pulmonary vein isolation as a procedural end point was achieved in 98% of cases. AF recurrence was observed in 39% between 3 and 12 months. Patients with AF recurrence are compared with patients without AF recurrence in Table 2. In univariate analysis, recurring AF was associated with persistent AF, longer history of AF, previous antiarrhythmic drug use, previous use of diuretics, increased LAD, increased left ventricular end-diastolic diameter, additional linear ablation lesions, and the ACE DD genotype. Both, unadjusted and adjusted multivariable analyses identified LAD and ACE DD genotype as
predictors for AF recurrence (Table 3). AF recurrence rates according to LAD and genotype are depicted in Figure 2.

Receiver-operator characteristics curve analysis showed LAD ≥45 mm as the best cutoff for AF recurrence prediction. A risk score based on left atrial size (LAD ≥45 mm) and genotype (ACE DD) was evaluated and allowed identification of groups at low (0 risk factors), intermediate (1 risk factor), or high (2 risk factors) recurrence risk. Odds of AF recurrence were increased 2.75-fold per risk factor (odds ratio, 2.75; 95% confidence interval, 1.657–4.577; P <0.001).

The AF recurrence rate was 21.6% in the low-risk group, 39.7% in the intermediate-risk group, and 70% in the high-risk group (Figure 3).

### Discussion

#### Main Findings

To the best of our knowledge, this study is the first to investigate the influence of the ACE insertion/deletion polymorphism on AF recurrence after catheter ablation in whites. Analysis of 238 subjects clearly demonstrated an association between ACE DD polymorphism and AF recurrence 3 to 12 months after ablation. In addition, left atrial enlargement contributed to AF recidivism, which is consistent with previous analyses.5,20

### Comparison With Previous Studies

In a recent meta-analysis4 that included 4357 patients with paroxysmal AF, 1083 with persistent AF, and 1777 patients with...
long-standing AF undergoing 1.23 procedures per patient, the AF recurrence rate was 31% after a follow-up of 22 months, which is comparable with our recurrence rate. Among the pre-procedural variables, AF recurrence was associated with persistent AF (odds ratio, 1.78), valvular AF (odds ratio, 5.20), and an LAD of >50 mm (odds ratio, 5.10). Both persistent AF and left atrial enlargement were also associated with AF recurrence, and the latter remained as a significant predictor in multivariable analysis of our population. Interestingly, our left atrial cutoff of 45 mm mirrors closely the findings of a previous study. In contrast, patients with valvar heart disease represented only a minority of our population.

Only limited data indicate the possibility of genotype-based AF rhythm control therapies. Recently, 2 single nucleotide polymorphisms on chromosome 4q25 (rs2200733, rs10033464), identified in a genome-wide association study to associate with AF, also predicted AF recurrence after catheter ablation. The current patient population is slightly larger, and the follow-up period was extended to 12 months. In addition, the ACE genotype distribution is rather equal, so that one third of the examined population is affected by the DD genotype. We could clearly demonstrate that the presence of ACE DD genotype increases the risk of AF recurrence after radiofrequency catheter ablation. These results are in accordance with previous findings on anti-AF drug treatment, where patients with D allele are reported to have poorer response to antiarrhythmic drug therapy.

**ACE Insertion/Deletion Polymorphism and Functionality in AF Recurrence**

It is assumed that higher ACE and consequently higher angiotensin II levels are responsible for increased structural remodeling. Left atrial structural remodeling can be classified as macroscopic, measured as left atrial dilation, or microscopic attributable to left atrial fibrosis. Consistent with previous findings, the ACE polymorphism did not correlate with left atrial enlargement, and in vivo assessment of left atrial fibrosis is technically challenging and therefore not assessed in our study. However, the ACE polymorphism has been found to correlate with abnormal cardiac conduction as manifest in prolongation of PR interval and P-wave duration, both of which are recognized markers of AF risk. Also, it has been shown that the DD genotype leads to increased ACE serum levels and higher cardiac ACE activity. Furthermore, atrial fibrosis is thought to be increased via an angiotensin II-mediated increase in transforming growth factor-β1 mRNA expression. Regions of atrial fibrosis are known to result in conduction heterogeneity and increased AF susceptibility. Thus, multiple lines of evidence suggest the theory that ACE DD compared with the II/DD genotype leads to higher cardiac ACE, angiotensin II, and transforming growth factor-β1 levels, which in turn promotes structural remodeling and abnormal cardiac electrophysiology attributable to fibrosis.

Several previous studies have demonstrated that the reversal of electric remodeling occurs early after restoration of sinus rhythm, whereas structural remodeling including fibrosis takes much longer and is the responsible substrate for recurring AF. The association of the ACE DD genotype with a higher rate of recurring AF found in this study supports this concept. Similarly, a very recent study demonstrated that higher transforming growth factor-β1 levels are associated with AF recurrence after catheter ablation. Although this and our study resemble identical concepts in AF prediction after catheter ablation with comparable results, genotyping offers possible advantages compared with fluctuating and nonstandardized laboratory measurements. Although research into the fundamentals of structural remodeling have not yet been exhausted, the use of biomarkers involved in atrial fibrosis turned out as a new promising approach.
Study Limitations
This study included a highly selected patient population with a high percentage of lone (58%) and paroxysmal (59%) AF. Furthermore, a standardized ablation approach was applied. Consequently, it is not clear whether these findings are comparable with different ablation approaches, such as ablation of complex fractionated electrograms or energy sources or different patient populations.

As recommended previously and commonly applied, we used serial 7-day Holter ECG monitoring to detect AF. However, even with this strategy, asymptomatic AF may have been missed. Because this would affect the entire cohort, a systematic error seems unlikely. Moreover, the follow-up was limited to 12 months and thus possible very late recurrences could not be analyzed.

ACE genotype distribution was different between men and women patients with AF. Further and larger studies are required to replicate this finding and to explore mechanisms and consequences.

The use of renin-angiotensin-aldosterone system modulators such as ACE inhibitors, angiotensin-receptor blockers, or aldosterone antagonist for secondary AF prophylaxis, in general, and after AF ablation, in particular, is controversial. Whether or not genotype-based renin-angiotensin-aldosterone system modulation offers benefits is unknown and should be explored in the future.

Although this study sought to predict rhythm outcome of AF ablation that is applied to a minority of the AF population, identification of new molecular targets for improved drug therapy and the discovery of biomarkers for risk stratification remains a major goal in the management of the growing number of patients with AF.

Most importantly, as with all biomarkers, our findings need to be validated in different AF populations undergoing AF catheter ablation. Nevertheless, it is noteworthy that our study was hypothesis driven and based on a previous biological assumption, and so the need for replication is not as strict.

Conclusions
Left atrial enlargement and the ACE DD polymorphism are predictors for AF recurrence after catheter ablation. The association between the ACE DD polymorphism and AF recidivism further supports the use of genetic data for predicting response to AF therapies and highlights the dominant role of fibrosis in AF development.

Acknowledgments
We thank Angela Kricke, Tina Fischer, and Virginia Kootz, for their help with laboratory and administrative work, as well as the entire staff involved in patient care.

Disclosures
D. Husser was supported by the Volkswagen Foundation (# 84 901), Germany. L. Ueberham was supported by the German Cardiac Society (Otto-Hess Stipend). The other authors report no conflict.

References
Clinical Perspective

Genetic markers are under evaluation as predictors for outcomes of atrial fibrillation (AF) therapies. They offer the chance to implement a tool for decision support in clinical practice that is highly individualized and easy to interpret, and that only needs to be tested once. The angiotensin-converting enzyme (ACE) insertion/deletion polymorphism represents a 287-base-pair intronic DNA segment on chromosome 17q23 that is either present (I, insertion) or absent (D, deletion). Previous studies demonstrated that the ACE D allele associates with increased ACE serum levels, higher cardiac ACE activity, increased cardiac fibrosis, electric remodeling, and poorer response to drug therapy. In this study, we assessed the association of the ACE insertion/deletion polymorphism and rhythm outcome after AF catheter ablation in 238 consecutive patients. Both the ACE DD genotype and larger left atrial diameter were independently associated with AF recurrence between 3 and 12 months. Stratification based on the number of risk factors for AF recurrence, that is, left atrial diameter ≥45 mm and ACE DD genotype allowed identification of groups at low-, intermediate-, or high-recurrence risk with an OR of 2.754 per risk factor. In combination with established clinical risk variables, the ACE insertion/deletion polymorphism should be considered as a potential marker to predict rhythm outcome of AF ablation, which may be useful for patient selection and postablation management. Further studies are required to verify the present results.
Genetic ACE I/D Polymorphism and Recurrence of Atrial Fibrillation After Catheter Ablation
Laura Ueberham, Andreas Bollmann, Moore Benjamin Shoemaker, Arash Arya, Volker Adams, Gerhard Hindricks and Daniela Husser

*Circ Arrhythm Electrophysiol*. 2013;6:732-737; originally published online July 22, 2013; doi: 10.1161/CIRCEP.113.000253

Circulation: Arrhythmia and Electrophysiology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-3149. Online ISSN: 1941-3084

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ep.ahajournals.org/content/6/4/732

Data Supplement (unedited) at:
http://circ.ep.ahajournals.org/content/suppl/2013/07/22/CIRCEP.113.000253.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Arrhythmia and Electrophysiology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Arrhythmia and Electrophysiology is online at:
http://circ.ep.ahajournals.org//subscriptions/
Figure 1. Receiver operator characteristics (ROC) curve for determination of the best left atrial diameter cut-off (AUC 0.673).

Figure 2. AF recurrence in relation to ACE genotype. AF recurrence occurred in 29.1 %, 37.1 % and 48.1 % for ACE II, ID and DD, respectively.