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

Running title: Ueberham et al.; ACE polymorphism – outcome after catheter ablation

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Journal Subject Codes: [22] Ablation/ICD/surgery, [109] Clinical genetics
Abstract

Background - The 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 anti-AF drug treatment. This study tested the hypothesis that the angiotensin-converting enzyme gene (ACE) insertion/deletion (I/D) polymorphism associates with atrial fibrillation (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 [I/D] polymorphism was genotyped using polymerase chain reaction. After a blanking period of 3 months, AF recurrence (defined as any atrial arrhythmia lasting at least 30s) 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 (OR 1.111, 95 % CI 1.040 – 1.187, p=.002) and ACE DD genotype (OR 2.251, 95 % CI 1.056 – 4.798, p=.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.

Key words: atrial fibrillation, genetics, genetic polymorphism, catheter ablation, ACE polymorphism, ACE I/D genotype
Introduction

Current atrial fibrillation (AF) guidelines\textsuperscript{1, 2} 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 \%\textsuperscript{3} to 89 \%\textsuperscript{4} depending on patient characteristics, ablation strategies and follow-up. Prediction of rhythm outcome remains challenging, although a recent meta-analysis identified non-paroxysmal AF, valvular AF and increased left atrial diameter as independent clinical predictors for recurring AF.\textsuperscript{5}

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.\textsuperscript{6, 7} However, there is only limited data correlating genotypes with outcomes of AF therapies\textsuperscript{8-10} and in particular with rhythm outcome after AF ablation.\textsuperscript{11-13} Consequently, this study tested the hypothesis that the angiotensin-converting enzyme gene (\textit{ACE}) I/D polymorphism associates with AF recurrence after catheter ablation. Using a candidate gene approach, this polymorphism was selected since the \textit{ACE} deletion allele, \textit{ACE} D, is associated with increased ACE serum levels, higher cardiac ACE activity and increased cardiac fibrosis.\textsuperscript{14-16} The \textit{ACE} D allele was also demonstrated to associate with electrical remodeling in patients with lone AF and those with heart disease\textsuperscript{17} and has been linked with failure of anti-AF drug therapy and AF recurrence in Chinese-Han.\textsuperscript{8, 18}

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 left atrial diameter, 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. Non-fluoroscopic 3D catheter orientation, CT image integration, and tagging of the ablation sites were performed using Ensite NavX, Ensite Velocity (St. Jude Medical, St. Paul, MN, USA) or CARTO 3 (Biosense Webster, Diamond Bar, CA, USA). Trans-septal access and catheter navigation were performed with a steerable sheath (Agilis, St. Jude Medical., St. Paul, MN, USA). Patients presenting with AF at the beginning of the procedure were electrically cardioverted and ablation was performed during sinus rhythm (i.e. 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, pre-selected 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 endpoint.

**Follow-up**

After ablation procedure, patients were followed 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 at least 3 months and according to the CHA2DS2-VASc-Score thereafter and proton pump inhibitors were prescribed for 4 weeks. Recurrence of AF was detected using serial 7-day Holter ECG recordings after a blanking period of 3 months at 3, 6 and 12 months after CA. When patients’ symptoms referred to AF, supplementary electrocardiograms and Holter recordings were arranged. AF recurrence was defined as a documented episode of AF or any other atrial tachycardia lasting at least 30 sec. In case of sustained early recurring AF, patients underwent direct-current cardioversion. Further antiarrhythmic drug therapy was left to the assessment of the treating physician.

**Genotyping**

DNA extraction was performed using a commercial isolation kit (peqGOLD Blood DNA Mini Kit, PeqLab, Erlangen, Germany) according to the manufacturer instructions. ACE I/D polymorphism was detected using polymerase chain reaction. A set of commercially synthesized primers (TibMolBiol, Berlin, Germany, sense primer 5’ CTGGAGACCACCTCCCATCTTCT 3’ and antisense primer 5’ GATGTGGCCATCACATTGTCAGAT 3’) were used for PCR. DNA amplification contained between 35 and 40 cycles. Agarose gel electrophoresis and ethidium bromide staining were used to identify the PCR 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 values ± one standard deviation and categorical variables as frequencies. Comparison of continuous variables was performed using the unpaired Student’s t-test and of categorical variables using the Pearson chi-square 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 <.15 in the multivariable logistic regression analysis, along with variables found to be significantly associated with each genotype. A two-sided p-value <.05 was considered as statistically significant.

Selection of a recessive genetic model (II and ID vs. DD) was based on pathophysiologic considerations and previous studies showing (a) a significant association of the ACE DD genotype and AF recurrence in a population of lone AF subjects of Chinese Han origin,¹⁸ and (b) highest cardiac ACE activity in DD genotype while it was similar in II and ID genotypes.¹⁵

Expected and observed genotype frequencies were compared using chi-square test to evaluate Hardy-Weinberg-equilibrium.

The best left atrial diameter cut-off was determined by analysing the receiver operator characteristics (ROC) curve (ROC in supplemental materials).

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 male and users of inhibitors of
the renin-angiotensin-aldosterone system while 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 to 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 left atrial diameter, increased left ventricular end diastolic diameter, additional linear ablation lesions and the *ACE* DD genotype. Both, unadjusted and adjusted multivariable analysis identified left atrial diameter as predictors for AF recurrence (Table 3). AF recurrence rates according to left atrial diameter and genotype are depicted in Figure 2.

Receiver operator characteristics (ROC) curve analysis showed LAD ≥ 45mm as best cut-off for AF recurrence prediction. A risk score based on LA size (LAD ≥ 45mm) and *ACE* 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 (OR 2.75, 95 % CI 1.657 – 4.577, p<.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* I/D polymorphism on AF recurrence after catheter ablation in Caucasians. 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.\textsuperscript{5, 20}

**Comparison with previous studies**

In a recent meta-analysis\textsuperscript{5} that included 4,357 patients with paroxysmal AF, 1,083 with persistent AF and 1,777 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 to our recurrence rate. Among the pre-procedural variables, AF recurrence was associated with persistent AF (OR 1.78), valvular AF (OR 5.20) and a left atrial diameter of more than 50 mm (OR 5.10).\textsuperscript{5} 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 cut-off of 45 mm mirrors closely the findings of a previous study.\textsuperscript{20} In contrast, patients with valvular heart disease represented only a minority of our population.

Only limited data point to the possibility of genotype-based AF rhythm control therapies. Recently, two single nucleotide polymorphisms on chromosome 4q25 (rs2200733, rs10033464), identified in a genome wide association study to associate with AF,\textsuperscript{21} also predicted AF recurrence after catheter ablation.\textsuperscript{11} The current patient population is slightly larger and the follow-up period was extended to 12 months. In addition, the \textit{ACE} genotype distribution is rather equal, so that one third of the examined population is affected by the \textit{ACE} DD ‘risk’ genotype. We could clearly demonstrate that the presence of \textit{ACE} DD genotype increases the risk of AF recurrence following radiofrequency catheter ablation. These results are in accordance to previous findings on anti-AF drug treatment, where patients with D allele are reported to have poorer response to antiarrhythmic drug therapy.\textsuperscript{8}
ACE I/D polymorphism and functionality in AF recurrence

It is assumed that higher ACE and consequently higher angiotensin II levels are responsible for increased structural remodelling. Left atrial structural remodelling can be classified as macroscopic measured as left atrial dilation, or microscopic due 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 TGF-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/ID genotype leads to higher cardiac ACE, angiotensin II and TGF-β1 levels, which in turn promotes structural remodelling and abnormal cardiac electrophysiology due to fibrosis.

Several previous studies have demonstrated that the reversal of electrical remodelling occurs early after restoration of sinus rhythm while structural remodelling 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 TGF-β1 levels are associated with AF recurrence after catheter ablation. While this and our study resemble identical concepts in AF prediction after catheter ablation with comparable results, genotyping offers possible advantages
over fluctuating and non-standardized laboratory measurements. Although research into the fundamentals of structural remodelling 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. Since 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 male and female AF patients. Further and larger studies are required to replicate this finding and to explore mechanisms and consequences.

The use of RAAS 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 RAAS modulation offers benefits is unknown and should be explored in the future.

While 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 AF patients.28

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 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.

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Conflict of Interest Disclosures: None.

References:

Hindricks G, Iesaka Y, Jackman W, Jalife J, Jais P, Kalman J, Keane D, Kim YH, Kirchhof P, Klein G, Kottkamp H, Kumagai K, Lindsay BD, Mansour M, Marchlinski FE, McCarthy PM, Mont JL, Morady F, Nademane K, Nakagawa H, Natale A, Nattel S, Packer DL, Pappone C, Prystowsky E, Raviele A, Reddy V, Ruskin JN, Shemin RJ, Tsao HM, Wilber D, Heart Rhythm Society Task Force on C, Surgical Ablation of Atrial F. 2012 HRS/EHRA/ECAS expert consensus statement on catheter and surgical ablation of atrial fibrillation: Recommendations for patient selection, procedural techniques, patient management and follow-up, definitions, endpoints, and research trial design: A report of the heart rhythm society (HRS) task force on catheter and surgical ablation of atrial fibrillation. Developed in partnership with the european heart rhythm association (EHRA), a registered branch of the european society of cardiology (ESC) and the european cardiac arrhythmia society (ECAS); and in collaboration with the american college of cardiology (ACC), american heart association (AHA), the asia pacific heart rhythm society (APHRS), and the society of thoracic surgeons (STS). Endorsed by the governing bodies of the american college of cardiology foundation, the american heart association, the european cardiac arrhythmia society, the european heart rhythm association, the society of thoracic surgeons, the asia pacific heart rhythm society, and the heart rhythm society. Heart Rhythm. 2012;9:632-696 e621.


Table 1. Characteristics of the study population and stratified by ACE I/D genotype.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>II/ID genotype</th>
<th>DD genotype</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=238</td>
<td>n=160</td>
<td>n=78</td>
<td></td>
</tr>
<tr>
<td>Age, yrs</td>
<td>58 ± 11</td>
<td>58 ± 12</td>
<td>59 ±10</td>
<td>0.823</td>
</tr>
<tr>
<td>Male (%)</td>
<td>69</td>
<td>64</td>
<td>78</td>
<td>0.030</td>
</tr>
<tr>
<td>Persistent AF (%)</td>
<td>41</td>
<td>42</td>
<td>40</td>
<td>0.696</td>
</tr>
<tr>
<td>Lone AF (%)</td>
<td>58</td>
<td>59</td>
<td>56</td>
<td>0.710</td>
</tr>
<tr>
<td>AF history, month</td>
<td>74 ± 76</td>
<td>72 ± 73</td>
<td>79 ± 81</td>
<td>0.498</td>
</tr>
<tr>
<td>BB/ CCB* use (%)</td>
<td>86</td>
<td>84</td>
<td>90</td>
<td>0.261</td>
</tr>
<tr>
<td>Digitalis use (%)</td>
<td>22</td>
<td>22</td>
<td>22</td>
<td>0.989</td>
</tr>
<tr>
<td>AAD† use (%)</td>
<td>42</td>
<td>40</td>
<td>45</td>
<td>0.498</td>
</tr>
<tr>
<td>Diuretic use (%)</td>
<td>33</td>
<td>33</td>
<td>33</td>
<td>0.898</td>
</tr>
<tr>
<td>ACEi/ARB/Ri‡ use (%)</td>
<td>65</td>
<td>59</td>
<td>76</td>
<td>0.014</td>
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<tr>
<td>LAD§, mm</td>
<td>43 ± 6</td>
<td>43 ± 6</td>
<td>43 ± 5</td>
<td>0.548</td>
</tr>
<tr>
<td>LVEF‡ (%)</td>
<td>60 ± 9</td>
<td>59 ± 8</td>
<td>61 ± 9</td>
<td>0.120</td>
</tr>
<tr>
<td>IVSd¶, mm</td>
<td>12 ± 2</td>
<td>12 ± 2</td>
<td>13 ± 3</td>
<td>0.170</td>
</tr>
<tr>
<td>LVED*, mm</td>
<td>49 ± 6</td>
<td>49 ± 7</td>
<td>50 ± 5</td>
<td>0.312</td>
</tr>
<tr>
<td>Ablation time, sec</td>
<td>3118 ± 1387</td>
<td>3141 ± 1379</td>
<td>3073 ± 1415</td>
<td>0.771</td>
</tr>
<tr>
<td>Additional linear lesions (%)</td>
<td>41</td>
<td>40</td>
<td>43</td>
<td>0.677</td>
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</tbody>
</table>

*angiotensin-converting-enzyme inhibitor/ angiotensin II blocker/ renin inhibitor
†antiarrhythmic drug
*beta blocker/ calcium channel blocker
‡interventricular septal enddiastolic dimension
§LAD = left atrial diameter
¶interventricular septal enddiastolic diameter
#left ventricular end diastolic diameter
Table 2. Comparison of patient characteristics with and without AF recurrence. Please note that one patient was lost to follow-up.

<table>
<thead>
<tr>
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<th>AF recurrence -</th>
<th>AF recurrence +</th>
<th>p-value</th>
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<td></td>
<td>n=145</td>
<td>n=92</td>
<td></td>
</tr>
<tr>
<td>Age, yrs</td>
<td>58 ± 11</td>
<td>59 ± 11</td>
<td>0.280</td>
</tr>
<tr>
<td>Male (%)</td>
<td>70</td>
<td>69</td>
<td>0.848</td>
</tr>
<tr>
<td>Persistent AF (%)</td>
<td>37</td>
<td>50</td>
<td>0.043</td>
</tr>
<tr>
<td>Lone AF (%)</td>
<td>60</td>
<td>53</td>
<td>0.393</td>
</tr>
<tr>
<td>AF history, months</td>
<td>68 ± 78</td>
<td>84 ± 72</td>
<td>0.134</td>
</tr>
<tr>
<td>BB/ CCB use (%)</td>
<td>86</td>
<td>87</td>
<td>0.755</td>
</tr>
<tr>
<td>Digitalis use (%)</td>
<td>24</td>
<td>19</td>
<td>0.305</td>
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<tr>
<td>AAD use (%)</td>
<td>36</td>
<td>51</td>
<td>0.023</td>
</tr>
<tr>
<td>Diuretic use (%)</td>
<td>28</td>
<td>41</td>
<td>0.029</td>
</tr>
<tr>
<td>ACEi/ARB/Ri use (%)</td>
<td>65</td>
<td>64</td>
<td>0.913</td>
</tr>
<tr>
<td>LAD, mm</td>
<td>42 ± 6</td>
<td>45 ± 6</td>
<td>&lt;0.001</td>
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<tr>
<td>LVEF (%)</td>
<td>60 ± 9</td>
<td>60 ± 8</td>
<td>0.828</td>
</tr>
<tr>
<td>IVSd, mm</td>
<td>12 ± 2</td>
<td>12 ± 2</td>
<td>0.262</td>
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<tr>
<td>LVEDd, mm</td>
<td>48 ± 6</td>
<td>50 ± 7</td>
<td>0.046</td>
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<tr>
<td>Additional linear lesions (%)</td>
<td>33</td>
<td>55</td>
<td>0.001</td>
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<tr>
<td>ACE DD genotype (%)</td>
<td>28</td>
<td>40</td>
<td>0.043</td>
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</table>

Abbreviations as in Table 1.
Table 3. Univariate and multivariable* analysis of predictors for AF recurrence.

<table>
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<th></th>
<th>Univariate analysis</th>
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<th>Unadjusted multivariable analysis</th>
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<th>Adjusted multivariable analysis</th>
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<tr>
<td></td>
<td>Odds ratio</td>
<td>95 % CI</td>
<td>p value</td>
<td>Odds ratio</td>
<td>95 % CI</td>
<td>p value</td>
<td>Odds ratio</td>
<td>95 % CI</td>
<td>p value</td>
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<tr>
<td>Persistent AF</td>
<td>1.731</td>
<td>1.015 - 2.950</td>
<td>0.043</td>
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<td></td>
<td>0.765</td>
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<td>0.465</td>
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<td>AF history</td>
<td>1.003</td>
<td>0.999 - 1.006</td>
<td>0.134</td>
<td></td>
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<td></td>
<td>0.141</td>
<td></td>
<td>0.114</td>
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<tr>
<td>AAD use</td>
<td>1.848</td>
<td>1.086 - 3.145</td>
<td>0.023</td>
<td></td>
<td></td>
<td></td>
<td>0.447</td>
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<td>0.288</td>
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<tr>
<td>Diuretic use</td>
<td>1.847</td>
<td>1.063 - 3.209</td>
<td>0.029</td>
<td></td>
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<td>0.147</td>
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<td>0.111</td>
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<td>LAD</td>
<td>1.108</td>
<td>1.044 - 1.177</td>
<td>&lt;0.001</td>
<td></td>
<td>1.111</td>
<td>1.040 - 1.187</td>
<td>0.002</td>
<td>1.117</td>
<td>1.032 – 1.209</td>
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<td>LVED</td>
<td>1.055</td>
<td>1.000 - 1.144</td>
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<td></td>
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<td>Additional linear lesions</td>
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<td>1.420 - 4.230</td>
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<td></td>
<td></td>
<td>0.060</td>
<td></td>
<td>0.068</td>
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<tr>
<td>ACE DD genotype</td>
<td>1.766</td>
<td>1.015 - 3.071</td>
<td>0.043</td>
<td></td>
<td>2.251</td>
<td>1.056 - 4.798</td>
<td>0.036</td>
<td>2.724</td>
<td>1.161 – 6.395</td>
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</tbody>
</table>

Abbreviations as in Table 1.
*All variables with a p<0.15 in univariate analysis were included. Adjusted multivariable analysis has been adjusted for gender and ACEi/ARBi/Ri use.
Figure Legends:

Figure 1. Determination of the ACE I/D polymorphism using 2% agarose gel electrophoresis. - A representative gel demonstrates genotype of 7 patients. Fragment sizes are 190 bp for D allele and 490 bp for I allele (M=marker). Please note the following genotypes below: 1) DD, 2) II, 3) ID, 4) ID, 5) II, 6) DD.

Figure 2. AF recurrence in relation to ACE genotype and left atrial diameter. – ACE genotype (left panel, 34.4 % vs. 48.1 %, OR=1.766, 95 % CI 1.015 - 3.071, p=.043) and left atrial diameter (right panel, 42±5mm vs. 45±6mm, OR=1.108 per mm increase, 95 % CI 1.044 – 1.177, p<.001) are predictors for AF recurrence.

Figure 3. AF recurrence in relation to the presence of risk factors i.e. left atrial enlargement ≥ 45 mm and ACE DD genotype. – Note the increased risk for AF recurrence if none, one or two risk factors are present (21.6 % vs. 39.7 % vs. 70.0 %, OR 2.754 per risk factor, 95 % CI 1.657 – 4.577, p<.001).
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.