A Genotype Dependent Intermediate ECG Phenotype in Patients with Persistent Lone Atrial Fibrillation

Husser: Genotype ECG-phenotype correlation in AF

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Abstract

Background: Atrial fibrillation (AF) is heterogeneous at the clinical and molecular levels. Association studies have reported that common single nucleotide polymorphisms (SNPs) in KCNE1 and SCN5A may predispose to AF. In this study, we tested the hypothesis that specific AF-associated genotypes confer variation on the appearance of AF assessed by analysis of fibrillatory rate of the atria.

Methods and Results: Twenty-six non-related patients (21 male, mean age 55 ± 12 years) with persistent lone AF (median AF duration 5 weeks) not taking class I or III antiarrhythmic drugs were studied. Fibrillatory rate was obtained by spatiotemporal QRST cancellation and time-frequency analysis of the index surface ECG. Genotypes at the AF-associated loci in KCNE1 (S38G) and SCN5A (H558R) were determined by direct DNA sequencing. The atrial fibrillatory rate was 418±50 fibrillations per minute (fpm, range 336–521) in the study cohort. Carriers of the 38GG KCNE1 genotype (n=13) had significantly lower fibrillatory rates (392±36 vs 443±49 fpm, p=.006) compared to those with GS or SS genotype (n=13). Six patients (23 %) with fibrillatory rates > 450 fpm, all had either the GS or SS genotype (chi² p=.008). In contrast, both the heterozygous and homozygous SCN5A H558R polymorphism had no effect on fibrillatory rate.

There were no significant associations between fibrillatory rate and clinical (age, gender, AF duration, drug treatment) or echocardiographic (left atrial diameter, LVEF) variables. In multivariable regression analysis, the KCNE1 S38G genotype (SS/GS coded 0, GG coded 1) was the only independent predictor of fibrillatory rate (β=-.437, p=.006) with a standard error of the estimate of 44 fpm.

Conclusions: This study suggests that atrial fibrillatory rate obtained from the surface ECG is at least in part determined by KCNE1 (S38G) genotype, implying that this variant exerts
functional effects on atrial electrophysiology. This intermediate ECG phenotype may be useful for elaborating genetic influences on AF mechanisms and identifying subsets of patients for variability in AF susceptibility or response to therapies.

**Keywords:** Atrial fibrillation – gene variants – ECG
Introduction

Atrial fibrillation (AF) is a heterogeneous arrhythmia at both the clinical and molecular level. Association studies have reported that common single nucleotide polymorphisms (SNPs) in genes encoding cardiac ion channels may predispose to AF development. For instance, the 38G allele of KCNE1 encoding the beta-subunit of IKs potassium channels and the R558 allele of the cardiac sodium channel gene SCN5A have been found to constitute risk factors for lone AF.

Experimental studies have revealed modulation of atrial refractoriness or conduction velocity by the KCNE1 (S38G) and SCN5A (H558R) polymorphisms, respectively. However, their association with an intermediate ECG phenotype in humans is unknown. One ECG-phenotype is atrial fibrillatory rate that can reliably be assessed from the surface ECG using spatiotemporal QRST cancellation and time frequency analysis. Fibrillatory rate of ECG lead V1 corresponds closely with fibrillatory rates of the high right atrium, coronary sinus and pulmonary veins and is a reproducible marker of atrial refractoriness.

It is a common observation that fibrillatory waves have various appearances from fine to coarse and from disorganized to organized. This inter-individual variance is also reflected by fibrillatory rates ranging from 240 to 540 fibrillations per minute (fpm). However, clinical and echocardiographic patient characteristics explain fibrillatory rate variance only in part.

In this study, we tested the hypothesis that specific AF-associated genotypes confer variation on the ECG appearance of AF assessed by analysis of fibrillatory rate.

Methods

Study population

The study was performed in patients prospectively enrolled in our AF registry, which consists of a clinical, a genetic and a digital ECG registry. Inclusion criteria include age ≥18 years, and a documented history of AF or atrial flutter. At enrollment into the registry, patients give
informed consent, a detailed medical and drug history is obtained in all patients in addition to a standard 12-lead ECG and a transthoracic echocardiogram.

For this study, patients were selected if they had persistent lone AF > 7 days with or without mild hypertension, not taking class I or III antiarrhythmic drugs at the time of ECG acquisition.

Left atrial and left ventricular measurements from the M-mode echocardiograms were performed by an experienced physician blinded to the genotype status of the patient. The echocardiograms were evaluated according to the recommendations of the American Society of Echocardiography.

Molecular analysis
Genomic DNA was extracted according to standard protocols, and the KCNE1 (S38G) and SCN5A (H558R) polymorphisms were detected by direct sequencing blinded to the ECG findings.

ECG analysis
Standard 10-s, 12-lead surface ECG recordings were acquired in all patients with the subject relaxed in a supine position. Digital ECG (500 Hz sampling rate) were retrieved from the hospital ECG database for further signal processing.

After high-pass filtering to remove baseline wander, atrial fibrillatory activity was extracted in lead V1 using spatiotemporal QRST cancellation. Since the dominant frequency component of interest is within the 4–9 Hz range, the resulting fibrillatory baseline signal was downsampled to 50 Hz and subjected to spectral analysis. The time–frequency distribution of the atrial signal (obtained by short-term Fourier transform) was decomposed such that each spectrum can be modelled as a frequency-shifted and amplitude-scaled version of the spectral profile. This procedure is based on a spectral profile, dynamically updated from previous
spectra, which was matched to each new spectrum using weighted least squares estimation. The frequency shift needed to achieve optimal matching then yields a measure of instantaneous fibrillatory rate of a 2.5-s ECG segment (overlapping with one segment each second) and was trended as a function of time. Frequencies were converted to fibrillatory rates with its unit fibrillations per minute (fpm) as advocated previously (rate = frequency * 60). Mean fibrillatory rate (in fpm) defined as average of instantaneous fibrillatory rates over the 10-second ECG segment was determined (Figure 1).

Statistical analysis

Data are presented as mean ± one standard deviation for normally distributed continuous variables.

The possible relation between continuous clinical variables and fibrillatory rate was analyzed using bivariate correlations (Pearson). Student’s t-test for unpaired data was used to compare fibrillatory rates between two groups (e.g. male vs female patients) and ANOVA with post-hoc analysis when comparing fibrillatory rates between three groups (e.g. SCN5A genotypes).

With a sample size of 26 patients and a predicted standard deviation of fibrillatory rate of 60 fpm in variant carriers, fibrillatory rate differences of ±60 fpm between patients with a wild type and variant carriers would have been detectable with a power of 80 %. This difference is above the spontaneous fibrillatory rate variability and would have been considered a clinically relevant genotype – phenotype correlation. Multivariable regression analysis that included variables with a p-value < .1 found in univariate analysis was used to identify independent predictors of fibrillatory rate.

A p value of < .05 was considered statistically significant.

Statement of Responsibility
The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Patient characteristics

Patient characteristics are summarized in Table 1. The atrial fibrillatory rate was 418±50 fibrillations per minute (fpm, range 336–521).

The frequencies of the KCNE1 genotypes were SS in 4 %, GS in 46 %, and GG in 50 % and of the SCN5A genotypes HH in 57 %, HR in 31 %, and RR in 12 %. Patient characteristics according to genotype are summarized in Table 2.

Genotype – ECG phenotype correlations

Carriers of the 38GG KCNE1 genotype had significantly lower fibrillatory rates (392±36 vs 443±49 fpm, p=.006) compared to those with GS or SS genotype (Table 2). Six patients (23 %) with fibrillatory rates >450 fpm, all had either the GS or SS genotype (chi² p=.008; Figure 2). Their clinical characteristics were similar to patients with lower fibrillatory rates. In particular, their AF duration ranged from 2 to 42 weeks (median 5 weeks), similar to the remaining patients (1 to 52 weeks, median 6 weeks). In contrast, the SCN5A H558R polymorphism was not associated with fibrillatory rate (Table 2).

There were no significant associations between fibrillatory rate and clinical (age, gender, AF duration, drug treatment) or echocardiographic (left atrial diameter, LVEF) variables. In particular, the use of digoxin (426±67 vs. 409±42 fpm, p=.454) or beta blockers (394±52 vs. 418±47 fpm, p=.319) did not affect fibrillatory rate.

In multivariable regression analysis, the KCNE1 S38G genotype (SS/GS coded 0, GG coded 1) was the only independent predictor of fibrillatory rate (β=−.437, p=.006) with a standard error of the estimate of 44 fpm.
Discussion

Main finding

To the best of our knowledge, this study is the first in patients with persistent lone AF to assess possible genotype–phenotype correlations using atrial fibrillatory rate of the surface ECG. In contrast to clinical and echocardiographic variables, KCNE1 (S38G) but not SCN5A (H558R) genotype was associated with fibrillatory rate. It was demonstrated that carriers of the 38GG KCNE1 genotype had significantly lower rates than those with the GS or SS genotype. Of interest, in none of the patients with the GG genotype did fibrillatory rate exceed 450 fpm.

Modulation of atrial electrophysiology by gene variants

Few previous studies have assessed in vitro modulatory effects of gene variants on atrial electrophysiological properties such atrial refractoriness or conduction velocity. Of special importance for our study are findings on KCNE1 (S38G) and SCN5A (H558R) polymorphisms. Ehrlich and co-workers found that the KCNE1 38G isoform is associated with reduced IKs likely due to decreased KvLQT1 membrane expression. This in turn leads to longer atrial action potential duration and refractoriness. Since longer refractory periods are associated with lower fibrillatory rates, our observation that lower rates not exceeding 450 fpm are present in patients with the GG genotype is in agreement with these findings. It needs to be pointed out that the increased risk for AF initiation with this genotype due to early afterdepolarizations is not in conflict with our observations that refer to AF persistence.

Although the SCN5A H558R polymorphism has been suggested to be associated with non-functioning or poorly functioning sodium channels and a subsequently reduced conduction velocity, we have found no association between this genotype and slower fibrillatory rates. On the one hand, it needs to be emphasized, however, that atrial refractoriness is the main determinant of fibrillatory rate, while conduction velocity seems to be similar in a narrow
range among patients with persistent AF. On the other hand, the sodium channel function is also affected by the amino acid splice variant that is unknown in the clinical setting.

Very limited in vivo data is available showing that certain gene variants may indeed exert functional effects on atrial electrophysiology. For instance, the promoter polymorphism -44G>A of the connexin 40 (Cx40) gene is associated with reduced transcriptional activity thereby influencing atrial Cx40 expression. Conceptually similar to our study, Firouzi and colleagues have shown that this Cx40 polymorphism was associated with atrial refractoriness dispersion. In their study, the prevalence of the minor Cx40 allele (-44A) and -44AA genotype was significantly higher in subjects with increased dispersion. Interestingly, as with our study, this difference was revealed already in a small population (n=30).

Previous studies have shown that a lower fibrillatory rate is associated with a better response to antiarrhythmic drugs and less AF recurrence following cardioversion. Similarly, certain genotypes such as the ACE I/D polymorphism may modulate the response to antiarrhythmic therapy via increased ACE and consequently angiotensin II levels. The latter is known to promote atrial fibrosis and electrophysiological remodeling. In consequence, the presence of the ACE ID or DD polymorphisms may indicate a more severe remodeling process in AF that is refractory to class I and III antiarrhythmics.

In summary, only limited data is available on few polymorphisms that point to a variety of mechanisms and pathways involved in the electrical and structural remodeling during AF. The elaboration of their individual contribution that may, moreover, vary over time is in its infancy. Nevertheless, the findings of our study are hypothesis generating and suggest that fibrillatory rate is an intermediate genotype dependent ECG phenotype which - together with its underlying genotype - may be useful for predicting response to therapy.
Limitations

This study is limited by a small but homogenous patient population. However, in agreement with a previous study, our current approach assessing genotype – ECG phenotype correlations among patients with AF requires a much smaller sample size than classic association studies. Nevertheless, certain genotypes (e.g. \textit{KCNE1} 38SS, \textit{SCN5A} 588RR) were underrepresented in this population and require further evaluation.

Using the candidate gene approach, this study was limited to the investigation of two SNPs of two cardiac ion channel genes. However, as discussed above, other genes may also influence atrial electrophysiology and consequently the ECG-phenotype.

This study was limited to the analysis of ECG lead V1. It can be argued that this is a closer reflection of right atrial but not left atrial activity. However, fibrillatory rates are similar among different sites in patients with persistent AF as in our population. Consequently, it is not surprising that ECG lead V1 closely reflects right atrial, but is also related to pulmonary venous/left atrial rates, especially when AF is persistent and the left to right atrial frequency gradient diminishes.

Aside from P wave parameters and ventricular rate, fibrillatory rate during AF can be considered as one specific ECG phenotype. Although there are several theories about AF sustenance including multiple wavelets, focal activation, spiral wave; in the individual patient, the underlying mechanism is currently difficult (if not impossible) to assess. Consequently, the association between fibrillatory rate and the dominant underlying AF mechanisms has not been explored. But even with this limited knowledge, fibrillatory rate of ECG lead V1 can be considered as a general marker of atrial remodeling as it is also associated with spontaneous and drug-induced AF termination and recidivism after cardioversion.

ECG recordings in this study were limited to 10 sec and variability over time was not assessed. However, previous studies have shown that fibrillatory rate obtained from the surface ECG is reproducible over 24 hours in clinically stable patients with persistent AF.
Finally, although we did not invasively assess ERP in our population, previous studies have shown the association between the KCNE1 (S38G) genotype and atrial refractoriness \(^4\) as well as between atrial refractoriness and fibrillatory rate.\(^{11}\)

**Conclusions**

This study suggests that atrial fibrillatory rate obtained from the surface ECG is at least in part determined by KCNE1 (S38G) genotype, implying that this variant exerts functional effects on atrial electrophysiology. This intermediate ECG phenotype may be useful for elaborating genetic influences on AF mechanisms and identifying subsets of patients for variability in AF susceptibility or response to therapies.

**Acknowledgments**

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**Conflict of Interest Disclosures**
References


**Figure 1.** Time-frequency analysis of AF. ECG recording of lead V1 from a patient with AF (upper panel), and the same interval after spatiotemporal QRST cancellation (middle panel). Please note the overlapping 2.5-second ECG windows, which are then subjected to short-term Fourier transform resulting in a power frequency spectrum (bottom left), and a frequency trend (bottom right).

**Figure 2.** *KCNE1* S38G and fibrillatory rate. The full circles represent the individual fibrillatory rates while the open circles represent the mean +/- one standard deviation (line).
Figure 1.
Table 1. Patient characteristics (n=26).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>55±12 (22 – 82)</td>
</tr>
<tr>
<td>Male/Female</td>
<td>21/5</td>
</tr>
<tr>
<td>AF duration, weeks</td>
<td>10±11 (1 – 52)</td>
</tr>
<tr>
<td>Mild hypertension</td>
<td>15</td>
</tr>
<tr>
<td>LAD, mm</td>
<td>43±8 (31 – 49)</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>54±8 (45 – 65)</td>
</tr>
<tr>
<td>Digitalis</td>
<td>6</td>
</tr>
<tr>
<td>Beta and/or calcium channel blocker</td>
<td>8</td>
</tr>
<tr>
<td>Atrial fibrillatory rate, fpm</td>
<td>418±50 (336 – 521)</td>
</tr>
<tr>
<td>Ventricular rate, bpm</td>
<td>88±21 (61 – 123)</td>
</tr>
</tbody>
</table>

LAD = left atrial diameter, LVEF = left ventricular ejection fraction

Mean ± one standard deviation (range)
Table 2. Patient characteristics according to genotype.

<table>
<thead>
<tr>
<th></th>
<th>KCNE1</th>
<th></th>
<th></th>
<th>SCN5A</th>
<th></th>
<th></th>
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<tr>
<td></td>
<td>SS</td>
<td>GS</td>
<td>GG</td>
<td>HH</td>
<td>HR</td>
<td>RR</td>
</tr>
<tr>
<td>N=1</td>
<td></td>
<td>N=12</td>
<td>N=13</td>
<td>N=15</td>
<td>N=8</td>
<td>N=3</td>
</tr>
<tr>
<td>Age, years</td>
<td>50</td>
<td>58±14</td>
<td>54±11</td>
<td>56±13</td>
<td>55±14</td>
<td>60±5</td>
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<tr>
<td>Male/Female</td>
<td>1/0</td>
<td>8/4</td>
<td>12/1</td>
<td>12/3</td>
<td>7/1</td>
<td>2/1</td>
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<tr>
<td>AF duration, weeks (median)</td>
<td>5</td>
<td>9±9 (4)</td>
<td>11±14 (8)</td>
<td>8±8 (4)</td>
<td>12±17 (5)</td>
<td>12±12 (7)</td>
</tr>
<tr>
<td>Mild hypertension</td>
<td>0</td>
<td>6</td>
<td>9</td>
<td>5</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>LAD, mm</td>
<td>43</td>
<td>43±9</td>
<td>44±7</td>
<td>42±7</td>
<td>46±10</td>
<td>42±3</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>45</td>
<td>54±7</td>
<td>53±9</td>
<td>54±9</td>
<td>54±7</td>
<td>50±2</td>
</tr>
<tr>
<td>Digitalis</td>
<td>0</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Beta and/or calcium channel blocker</td>
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<td>4</td>
<td>4</td>
<td>5</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Atrial fibrillatory rate, fpm</td>
<td>470</td>
<td>431±52*</td>
<td>392±36*</td>
<td>403±50</td>
<td>434±50</td>
<td>408±28</td>
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<tr>
<td>Ventricular rate, bpm</td>
<td>115</td>
<td>82±12</td>
<td>91±27</td>
<td>91±18</td>
<td>79±13</td>
<td>96±40</td>
</tr>
</tbody>
</table>

LAD = left atrial diameter, LVEF = left ventricular ejection fraction, * p<.05
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