Arrhythmia/Electrophysiology

Pathogenesis of the Novel Autoimmune-Associated Long-QT Syndrome

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Background—Emerging clinical evidence demonstrates high prevalence of QTc prolongation and complex ventricular arrhythmias in patients with anti-Ro antibody (anti-Ro Ab)—positive autoimmune diseases. We tested the hypothesis that anti-Ro Abs target the HERG (human ether-a-go-go–related gene) K+ channel, which conducts the rapidly activating delayed K+ current, I\(_{\text{Kr}}\), thereby causing delayed repolarization seen as QT interval prolongation on the ECG.

Methods and Results—Anti-Ro Ab–positive sera, purified IgG, and affinity-purified anti-52-kDa Ro Abs from patients with autoimmune diseases and QTc prolongation were tested on I\(_{\text{Kr}}\) using HEK293 cells expressing HERG channel and native cardiac myocytes. Electrophysiological and biochemical data demonstrate that anti-Ro Abs inhibit I\(_{\text{Kr}}\) to prolong action potential duration by directly binding to the HERG channel protein. The 52-kDa Ro antigen–immunized guinea pigs showed QTc prolongation on ECG after developing high titers of anti-Ro Abs, which inhibited native I\(_{\text{Kr}}\) and cross-reacted with guinea pig ERG channel.

Conclusions—The data establish that anti-Ro Abs from patients with autoimmune diseases inhibit I\(_{\text{Kr}}\) by cross-reacting with the HERG channel likely at the pore region where homology between anti–52-kDa Ro antigen and HERG channel is present. The animal model of autoimmune-associated QTc prolongation is the first to provide strong evidence for a pathogenic role of anti-Ro Abs in the development of QTc prolongation. It is proposed that adult patients with anti-Ro Abs may benefit from routine ECG screening and that those with QTc prolongation should receive counseling about drugs that may increase the risk for life-threatening arrhythmias. (Circulation. 2015;132:230-240. DOI: 10.1161/CIRCULATIONAHA.115.009800.)

Key Words: antibodies ■ arrhythmias, cardiac ■ immune system ■ long QT syndrome

The long-QT syndrome (LQTS) is one of the most studied channelopathies in which abnormal prolongation of ventricular repolarization predisposes to the life-threatening ventricular arrhythmia Torsades de Pointes.1-4 LQTS can be congenital or acquired. Although congenital LQTS is caused by mutations in ion channel protein-coding genes, acquired LQTS is often drug induced.3,5,6 In most cases of drug-induced QT prolongation, the target ion channel is the HERG (Human Ether-à-go-go-Related Gene) encoding the pore-forming subunits (Kv11.1) of the rapidly activating delayed K+ channel conducting I\(_{\text{Kr}}\).1,3-5 I\(_{\text{Kr}}\) plays a major role during repolarization of the cardiac action potential (AP).4,6 Its reduction by drug block or genetic defects causes delayed repolarization of the AP, which manifests as prolongation of the QT interval on the ECG.1,3,6

Clinical Perspective on p 240

A novel acquired autoimmune-associated LQTS has been recently reported in adult patients carrying anti-Ro antibodies (anti-Ro Abs),7-10 which result from an autoimmune response against the intracellular ribonucleoprotein, SSA/Ro antigen (Ro). The detection of circulating anti-Ro Abs is relatively

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frequent in the course of autoimmune diseases, particularly Sjögren syndrome, and systemic lupus erythematosus but also in other connective tissue diseases (CTDs) including mixed CTDs, undifferentiated CTDs, polymyositis /dermatomyositis, systemic sclerosis, rheumatoid arthritis, and even primary biliary cirrhosis.\(^{11,12}\) Interestingly, in the adult patients, anti-Ro Ab positivity is associated with repolarization abnormalities (QTc prolongation)\(^{7-10}\) but not with conduction abnormalities (complete atrioventricular block), which is well described in children born to mothers with anti-Ro Abs.\(^{13,14}\) Accordingly, (complete atrioventricular block), which is well described in children born to mothers with anti-Ro Abs.\(^{13,14}\) Accordingly, it is assumed that the adult heart does not represent an immunological target for anti-Ro Abs. However, emerging clinical observations suggest that anti-Ro Abs may be arrhythmogenic for the adult heart by causing QT interval prolongation.\(^{7-10}\) Specifically, in a cohort of CTD patients, more than half (58%) of patients with anti-Ro Abs displayed a prolonged QTc, with a mean QTc duration significantly longer in anti-Ro Ab–positive versus anti-Ro Ab–negative patients.\(^{7}\) In a subsequent study, patients with CTD and anti-Ro Ab showed 5-fold higher incidence of complex ventricular arrhythmias compared with anti-Ro Ab–negative patients.\(^{8}\) Despite this high incidence of QTc prolongation and ventricular arrhythmias in patients with CTD, the pathogenesis of this autoimmune-associated QTc prolongation in the adult remains poorly understood.

**Methods**

**Study Population**

Sera from 6 CTD patients were studied (see Table 1). Three CTD patients were anti-Ro Ab positive with QTc >460 milliseconds, and 3 CTD patients were anti-Ro Ab negative with QTc <460 milliseconds.\(^{15}\) Patients did not have echocardiographic abnormalities or evidence of organic heart diseases, diabetes mellitus, renal failure, thyroid disease, or electrolyte abnormalities,\(^{16,17}\) and none were taking drugs that can potentially affect the QT interval. Genetic testing for mutations in the KCNH2, KCNQ1, SCN5A, KCNE2, KCNE1, and KCNJ2 genes was negative in the 3 anti-Ro Ab–positive patients studied. The Institutional Review Board approved the consent process for study participants, and patients gave informed consent. ECG recordings are described in the Methods section in the online-only Data Supplement.

**Purification of IgG and Affinity Purification of Anti–52-kDa Ro Antibodies From Patients’ Sera**

IgG and affinity purification of anti–52-kDa Ro (52Ro) Abs was performed as previously described with modifications\(^{16,20}\) and as detailed in the Methods section in the online-only Data Supplement. Western blot experiments were performed as described in the Methods section in the online-only Data Supplement.

**Table 1. Clinical Characteristics of the Patients Studied**

<table>
<thead>
<tr>
<th>Serum/Patients</th>
<th>Sex</th>
<th>Age, y</th>
<th>CTD Diagnosis</th>
<th>QTc, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Ro antibody positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>F</td>
<td>35</td>
<td>SS</td>
<td>566</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>37</td>
<td>SS</td>
<td>514</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>42</td>
<td>SS</td>
<td>498</td>
</tr>
<tr>
<td>Anti-Ro antibody negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>65</td>
<td>SS</td>
<td>404</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>23</td>
<td>SLE</td>
<td>451</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>31</td>
<td>SLE</td>
<td>430</td>
</tr>
</tbody>
</table>

CTD indicates connective tissue disease; SLE, systemic lupus erythematosus; and SS, Sjögren syndrome.

**Electrophysiology With HEK293 Cells Stably Expressing HERG Channel and Guinea Pig Ventricular Myocytes**

Details of DNA constructs and the methods of stable transfection of HEK293 cells with HERG channel were reported elsewhere.\(^{18}\) The electrophysiological recording and single ventricular myocytes enzymatic dissociation technique are detailed in the Methods section in the online-only Data Supplement and elsewhere.\(^{19}\)

**Western Blot Analysis**

Western blot experiments were performed as described in the Methods section in the online-only Data Supplement.

**Guinea Pig Immunization**

Seven adult guinea pigs (5 females and 2 males) were immunized with recombinant 52-kDa Ro antigen (Sigma), initially at day 1 and followed by boosters at days 14, 28, and 42 as described in the Methods section in the online-only Data Supplement and as previously reported with modification.\(^{16,20-22}\)

**ELISA Test**

Serum from each immunized guinea pig was analyzed for anti-Ro Ab reactivity by ELISA as previously described\(^{16,20}\) with some modifications reported in the Methods section in the online-only Data Supplement.

**Statistical Analysis**

Statistical comparisons were evaluated with paired Student \(t\) tests and with nonparametric Wilcoxon paired tests as appropriate. Data are presented as mean±SEM. A value of \(P<0.05\) was considered significant.

**Results**

**Anti-Ro Ab–Positive Sera From Patients With QTc Prolongation Inhibit \(I_{Kr}\)**

We first tested the electrophysiological effect of whole serum containing anti-Ro Abs from a patient who has CTD and a long QTc of 566 milliseconds (Figure 1A). Figure 1B shows the voltage protocol used to record \(I_{Kr}\). Anti-Ro Ab–positive serum 1 (200 μL; 83 U/mL) inhibited both the peak and tail of \(I_{Kr}\) recorded from HEK293 cells stably expressing the HERG channel (Figure 1C–1F). Selected current traces are shown before (Figure 1C) and after (Figure 1D) the application of anti-Ro Ab–positive serum. The I-V relationships for \(I_{Kr}\) peak and tail before and after the application of anti-Ro Ab–positive serum are shown in Figure 1E (n=10) and Figure 1F (n=10), respectively. Anti-Ro Ab–positive serum significantly reduced \(I_{Kr}\) peak density by 40.4% from 53.9±5.1 to 32.1±5.2 pA/pF at \(-10\) mV (\(P<0.0001\); n=10) and reduced \(I_{Kr}\) tail density by 38.4% from 93.4±8.7 to 57.5±10.2 pA/pF at \(-10\) mV (\(P<0.0001\); n=10). To ensure reproducibility of \(I_{Kr}\) inhibition, sera from 2 additional anti-Ro Ab–positive CTD patients with QTc of 514 and 498 milliseconds were tested (Table 1). The results show that serum 2 (200 μL; 241 U/mL) and serum 3 (200 μL; 241 U/mL) both significantly (\(P<0.0001\); n=10 each) inhibited \(I_{Kr}\) peak and tail densities (Figure 1G).

**Anti-Ro Ab–Negative Sera From Patients With Normal QTc Did Not Affect \(I_{Kr}\)**

Next, we demonstrated that anti-Ro Ab–negative serum 4 (200 μL) from a control patient with a normal QTc of 404
milliseconds (Figure 2A) had no effect on \( I_{Kr} \). Figure 2 shows selected \( I_{Kr} \) traces before (Figure 2B) and after (Figure 2D) application of anti-Ro Ab–negative serum. Figure 2D and 2E shows I-V relationships of \( I_{Kr} \) peak and tail densities before and after the application of anti-Ro Ab–negative serum (n=10), respectively. \( I_{Kr} \) peak densities before and after the application of anti-Ro Ab–negative serum at −10 mV were 56.9±6.4 and 53.1±6.8 pA/pF (\( P=0.08; n=10 \)), and \( I_{Kr} \) tail densities were 94.5±9.2 and 89.2±7.3 pA/pF (\( P=0.09; n=10 \)). Similarly, additional sera from 2 anti-Ro–negative CTD patients with QTc of 451 and 430 milliseconds (Table 1) had no significant effect on \( I_{Kr} \) peak or tail current densities (Figure 2F).

Anti-Ro Ab–Positive Purified IgG From Patients With Long QTc Inhibits \( I_{Kr} \)

The effect of purified IgG containing anti-Ro Abs from serum 1 was tested on \( I_{Kr} \). Figure 3 shows that purified IgG (75 μg/mL) significantly inhibited both \( I_{Kr} \) peak and tail densities at several tested voltages. \( I_{Kr} \) peak density at −10 mV decreased by 33.1% from 50.1±4.0 to 33.6±3.8 pA/pF (\( P<0.0001; n=10 \)), and \( I_{Kr} \) tail density decreased by 35.4% from 95.5±5.4 to 61.7±5.7 pA/pF (\( P<0.0001; n=10 \)) on IgG application. The dose-dependent effect of IgG on \( I_{Kr} \) is shown in Figure 4A. Anti-Ro Ab–positive IgG inhibited \( I_{Kr} \) peak density in dose-dependent manner (n=6) with an EC\(_{50}\)=87.3 μg/mL. Figure 4B shows a representative time course for \( I_{Kr} \) peak inhibition by anti-Ro Ab–positive IgG (75 μg/mL). IgG inhibited \( I_{Kr} \) peak in a time-dependent manner with a steady-state effect reached after 8 minutes after IgG application. The inhibition of \( I_{Kr} \) by IgG was only partially reversible (washout). Figure 4C shows that anti-Ro Ab–negative IgG at −10 mV did not significantly inhibit \( I_{Kr} \) peak densities (from 49.4±7.1 to 46.3±5.2 pA/pF; n=6; \( P=0.1 \)) or \( I_{Kr} \) tail densities (from 92.2±7.4 to 88.3±8.1 pA/pF; n=6; \( P=0.09 \)). Figure 4D illustrates the time course of \( I_{Kr} \) peak without any intervention and shows minimal run-down seen in 6 experiments. To investigate whether the above IgG containing anti-Ro Ab inhibition of \( I_{Kr} \) occurs by affecting the kinetics of the HERG channel, the activation and deactivation time constants (\( \tau \)) were fitted by the Boltzmann equation. Figure 4E and 4F demonstrates that both activation and
deactivation were best fit by a single exponential and that anti-Ro Ab–positive IgG did not affect the kinetics of the HERG channel at all voltages (n=10; P=0.1).

**Anti-Ro Ab–Positive IgG but Not Anti-Ro Ab–Negative IgG Cross-Reacts With HERG Channels**

To test whether the inhibition of $I_{Kr}$ by anti-Ro Ab–positive IgG is due to a direct interaction with the HERG channel protein, Western blot experiments were performed with proteins from HEK293 cells stably expressing HERG channels (n=6) and untransfected HEK293 cells (negative control, n=6). Figure 5A shows that anti-Ro Ab–positive IgG did not recognize any bands in untransfected HEK293 cells (lane 1) but recognized 2 bands at ≈155 and 135 kDa (lane 2), corresponding to the glycosylated and endoplasmic reticulum–retained HERG channel, consistent with previous reports.18,23 Figure 5B shows no bands when anti-Ro Ab–negative IgG was used in both untransfected HEK293 cells (lane 3) and transfected HEK293 cells expressing HERG channels (lane 4). Similar to anti-Ro Ab–positive IgG in Figure 5A (lane 1), a commercial anti-Kv11.1 Ab did not recognize any bands from untransfected HEK293 cells (Figure 5C, lane 5, negative control) but did recognize the bands corresponding to the HERG channel (lane 6).

**Affinity Purified Anti-52Ro Abs From a Patient With Long QTc Inhibits $I_{Kr}$**

To demonstrate the specificity the effect of anti-Ro Abs on $I_{Kr}$, affinity-purified anti-52Ro Abs from sera 1 were tested on $I_{Kr}$. Similar to purified IgG, affinity-purified anti-52Ro Abs (52 μg/mL) significantly inhibited $I_{Kr}$ peak densities by 28.5% (from 35.4±2.6 to 25.3±2.4 pA/pF; n=10; P<0.0001) and $I_{Kr}$ tail densities by 30.5% (from 62.7±5.9 to 43.5±4.7; n=10; P<0.0001). Figure 6 shows current tracings (Figure 6A and 6B), I-V relationships (Figure 6C and 6D), and dot plot (Figure 6E) before and after the application of affinity-purified anti-52Ro Abs.

**Immunization of Adult Guinea Pigs With SSA/52kDa Ro Antigen Causes QTc Prolongation**

To further demonstrate the potential role of anti-Ro Abs in the pathogenesis of QTc prolongation, we sought to establish an animal model of anti-Ro Ab–induced QTc...
prolongation. The guinea pig is a suitable animal model for QT studies because of the similarities of ECG features with humans. The results in Table 2 show significant QTc prolongation and high titers of anti-Ro Abs after immunization in 7 guinea pigs. No significant differences in heart rate, PR intervals, and QRS durations were observed before and after immunization. Figure 7 illustrates ECGs from a guinea pig before (Figure 7A) and after (Figure 7B) immunization with a ΔQTc of 36 milliseconds. If the QTc prolongation seen in the immunized guinea pigs is due, at least in part, to the inhibition of \( I_{kr} \) by anti-Ro Ab, then anti-Ro Abs should be expected to also lengthen the ventricular AP duration (APD). Thus, APs were recorded from guinea pig single ventricular myocytes before and after the application of anti-Ro–positive IgG at 0.5 Hz in the current clamp mode configuration. Figure 7C shows APs before (left) and after (right) the application of anti-Ro Ab–positive IgG (75 μg/mL). Anti-Ro Ab–positive IgG resulted in a prolongation of the APD at 90% (APD\(_{90}\)) by 39.7% without any changes in the resting membrane potential or the AP amplitude. Averaged data from a total of 6 experiments are shown in Figure 7D (bottom). Anti-Ro Ab–negative IgG did not have any significant effects on APD\(_{90}\) (348.4±32.1 milliseconds before and 350.2±39.3 milliseconds after negative IgG; \( P=0.1; n=6 \)). Figure 7D shows \( I_{kr} \) recorded from guinea pig ventricular myocytes using a short-duration (200 milliseconds) pulse protocol from a holding potential of −40 mV in which the slow delayed rectifier, \( I_{f} \), is still inactive (and in the presence of 10 μmol/L chromanol). Consistent with the observed APD lengthening, anti-Ro Ab–positive IgG (75 μg/mL), but not control anti-Ro Ab–negative IgG (75 μg/mL), inhibited \( I_{kr} \) peak by 30.8% and \( I_{kr} \) tail by 28.9% (dot plot in Figure 7D, bottom; \( n=6; P<0.0001 \)). To test whether the observed functional inhibition of \( I_{kr} \) is through direct interaction with the guinea pig Kv11.1 (ERG) channel proteins, Western blots experiments were performed. Anti-Ro Ab–positive IgG cross-reacted with the endogenous Kv11.1 proteins from guinea pig ventricles (Figure 7E, lane 3) and HEK293 cells expressing HERG/Kv11.1 channel (lane 2) but did not show reactivity to untransformed HEK293 cells (lane 1, negative control). The commercial anti-Kv11.1 Ab also recognized the same protein bands in guinea pig ventricles (lane 6) and HEK293 cells expressing HERG channel (lane 5) but not untransformed HEK293 cells, as did anti-Ro Ab–positive IgG (lane 3).

Figure 8A shows that anti-Ro Ab–positive serum from immunized guinea pigs inhibited \( I_{kr} \) expressed in HEK293 cells. Anti-Ro Ab–positive serum (100 μL) from immunized guinea pigs inhibited \( I_{kr} \) peak densities by 29% (from 44.6±6.1 to 32.5±5.2 pA/pF; \( P<0.0001; n=6 \)) and \( I_{kr} \) tail densities by 28% (from 20.1±3.1 to 14.3±2.4 pA/pF; \( P<0.0001; n=6 \)). These effects on \( I_{kr} \) were not seen with anti-Ro Ab–negative serum from preimmunized guinea pigs (\( I_{kr} \) peak by 30.8% and \( I_{kr} \) tail by 28.9%) or with anti-Ro Ab–negative serum from patients (\( I_{kr} \) peak by 30.8% and \( I_{kr} \) tail by 28.9%). With the use of guinea pig ventricles, anti-Ro Ab–positive (lane 1) but not anti-Ro Ab–negative (lane 2) serum recognized Kv11.1 proteins.

**Homology Analysis and Reactivity of Sera to the Peptide Corresponding to HERG Extracellular Loop at the Pore Region**

Linear homology analysis showed that there is 44% homology between 52Ro protein (aa302-aa321) and HERG \( \alpha_1 \) subunit (aa574-aa598) at the pore region (Figure 8C-a–8C-c), of which 25% are identical. The presence of this homology at the pore region may account for anti-Ro Ab off-target binding to HERG channel at this epitope mimic, especially in the tetrameric conformation model of the channel where the 4 pore extracellular loops come together and are accessible to the antibodies (Figure 8C-a). This was tested by performing ELISA with patients’ sera and a synthesized 31–amino acid peptide corresponding to the pore-forming region of the HERG channel (Figure 8C-d). Figure 8C-d shows that anti-Ro Ab–positive sera (n=3) but not anti-Ro Ab–negative sera (n=3) exhibited high reactivity with the peptide. The average...
reactivity reading was 2.19±0.04 optical density in anti-Ro Ab–positive sera versus 0.36±0.03 optical density in anti-Ro Ab–negative sera. The difference was statistically significant (n=6; P<0.0001).

**Discussion**

Here, we describe the pathogenesis underlying a novel form of acquired QT prolongation of autoimmune origin associated with anti-Ro Abs in adults. The in vitro data show that serum and purified IgG containing anti-Ro Abs and affinity-purified anti-52Ro Abs from CTD patients with QTc prolongation functionally inhibit \( I_{Kr} \) in a time- and dose-dependent manner by direct interaction with the HERG/Kv11.1 channel protein. The in vivo data from guinea pigs are the first to establish an animal model whereby induction of anti-Ro Abs by immunization results in QTc prolongation on the ECG. Furthermore, with the use of native cardiomyocytes, anti-Ro Ab–positive IgG prolonged APD and inhibited \( I_{Kr} \) by directly interacting with the \( I_{Kr} \) channel, likely at the pore region where homology between the Ro antigen and HERG channel was identified and reactivity to anti-Ro Ab–positive sera was observed.

**Autoimmune-Associated Electric Abnormalities in Clinical Settings**

The most well-characterized autoimmune disease in which anti-Ro Abs are associated with electrical abnormalities is congenital heart block detected at or before birth in a structurally normal heart.\(^{14,27}\) The hallmark of congenital heart block is the irreversible complete atrioventricular block that affects exclusively the fetus or the newborn heart but curiously not the adult maternal heart despite exposure to the identical circulating anti-Ro Abs.\(^{14,27}\) QTc prolongation has also been reported in newborns\(^{28,29}\) of mothers with anti-Ro Abs but resolves over time with the disappearance of Abs,\(^{30}\) thus supporting the involvement of anti-Ro Abs in the pathogenesis of QTc prolongation. These findings affected the 2002 guidelines of the European Society of Cardiology in which...
Close ECG monitoring of anti-Ro Ab–positive neonates was proposed.31 Recently, clinical evidence has demonstrated a correlation between the presence of anti-Ro Abs and QTc prolongation in adult patients with autoimmune diseases.7,9,10,32 Our experimental findings support and are consistent with these clinical observations from different groups that the presence of anti-Ro Abs correlates with QTc prolongation.7–10,32 A study by Bourré-Tessier et al10 analyzing a large cohort of 278 patients affected with systemic lupus erythematosus found that the occurrence of QTc prolongation was ≈8-fold more likely in anti-Ro Ab–positive than–negative patients. Similarly, Lazzerini et al7,9 found that about half of anti-Ro/SSA–positive patients displayed a prolonged QTc and increased propensity for ventricular arrhythmias.9 However, other studies did not show any significant differences in mean QTc, likely because of the type of autoimmune disease studied, the age of the patients, the titers of Abs, and the methods used for analysis of Abs.33–35

Animal Model of Autoimmune-Associated QTc Prolongation
To correlate the arrhythmogenic effects of anti-Ro Abs with the genesis of QT interval prolongation, guinea pigs were immunized with 52Ro antigen to generate anti-52Ro Abs. The data from this in vivo study are the first to establish an animal model of autoimmune-associated QTc prolongation and to provide evidence for a pathogenic role of anti-52Ro Abs in the development of QTc prolongation. Moreover, anti-52Ro Ab–positive but not –negative sera from immunized guinea pigs also inhibited $I_{Kr}$ and cross-reacted with Kv11.1 channel protein. Together with the anti-Ro Ab–positive IgG lengthening of APD in guinea pig ventricular myocytes,

Table 2. ECG Parameters and Antibody Levels Before and After Immunization

<table>
<thead>
<tr>
<th></th>
<th>QTc, ms</th>
<th>Heart Rate, bpm</th>
<th>PR Interval, ms</th>
<th>QRS, ms</th>
<th>Anti-Ro Antibody Range, OD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>248.3±30</td>
<td>294.4±12</td>
<td>50.5±2.9</td>
<td>19.1±1.0</td>
<td>0.02–0.04</td>
</tr>
<tr>
<td>(n=7)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Immunized</td>
<td>272.5±23*</td>
<td>280.2±42</td>
<td>56.5±1.4</td>
<td>19.2±2.0</td>
<td>1.14–2.16*</td>
</tr>
<tr>
<td>(n=7)</td>
<td></td>
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OD indicates optical density.
*P<0.05.
these findings are consistent with the clinical observation that the sole presence of anti-Ro Abs correlates with QTc prolongation.7,8,10,32

**Pathogenesis of Autoimmune-Associated QTc Prolongation**

The proposed mechanism for the autoimmune-associated QTc prolongation is that anti-Ro Abs cause APD and QTc prolongations by direct block of the HERG/Kv11.1 channels. Several lines of evidence support this proposed mechanism: (1) Only sera, IgG, and affinity-purified anti-Ro Abs from anti-Ro Ab-positive patients, not from anti-Ro–negative patients, inhibited $I_{Kr}$ and directly interacted with HERG/Kv11.1 channel proteins both in expression systems and in native ventricular myocytes; (2) anti-Ro Abs did not affect $I_{Kr}$ kinetics, and their effect was observed within minutes, suggesting a direct block of the HERG channel; (3) the homology between the 52Ro antigen and HERG channel at the pore-forming region may account for anti-Ro Abs targeting and blocking the pore of the HERG channel (This is supported by the tetrameric 3-dimensional model of the HERG pore region showing the accessibility of the extracellular loop between S5 and S6 to antibodies; Figure 8C-c); (4) the reactivity of anti-Ro Ab–positive but not anti-Ro Ab–negative sera to the peptide corresponds to the pore-forming region of HERG; (5) the sole presence of anti-Ro Abs induced by immunization of guinea pigs resulted in QTc prolongation on the surface ECG; and (6) anti-Ro Abs do not affect other currents such as the delayed rectifier K current, $I_{Kr}$; the transient outward current, $I_{to}$; the inward rectifier K current, $I_{K1}$; and the Na current, $I_{Na}$.36,37

Because it is generally accepted that the QT interval is a function of ventricular APD and that the 2 variables are very closely correlated in humans38,39 and animals,40 the combined in vitro and in vivo data from this study provided important clues to the relationship among ion channel block, APD lengthening, and QT interval prolongation. Abnormal QTc interval prolongation is known to predispose to ventricular arrhythmias known as Torsades de Pointes via early afterdepolarization–induced triggered

![Figure 7](https://example.com/figure7.png)
activity. Thus, anti-Ro Ab–induced QTc prolongation confers an increased risk of ventricular arrhythmias not only for patients with anti-Ro Abs but also for patients with genetic or drug-induced QTc prolongation. In fact, anti-Ro Ab is the most frequent autoantibody found in the general population (up to \( \approx 3\% \)), but anti-Ro Ab–positive patients are in most cases asymptomatic for autoimmune diseases.\(^{41}\) The involvement of anti-Ro Abs and the HERG channel in the pathogenesis of QTc prolongation is further supported by a case report in which \( I_{Kr} \) has been shown to be inhibited by anti-Ro Abs from a female patient (asymptomatic for autoimmune diseases) with acquired LQTS with no known cause of QTc prolongation except positive anti-Ro Abs.\(^{42}\) Both clinical and experimental evidence supports the association of anti-Ro Abs with QTc prolongation in adult patients and pinpoints the inhibition of the HERG/Kv11.1 channel by anti-Ro Abs as a plausible underlying mechanism.

**Significance**

Patients with QTc prolongation are prone to complex ventricular arrhythmias, including Torsades de Pointes, syncope, and sudden death.\(^{3,4}\) QTc prolongation associated with anti-Ro Abs per se may confer an increased risk for developing ventricular arrhythmias and represents an additional risk factor for patients with drug-induced or congenital QTc prolongation. The finding from the present study supports the recommendations that adult patients with anti-Ro Abs may benefit from routine ECG screening for QTc prolongation and that those already identified with anti-Ro Ab–associated QTc prolongation should receive counseling, including education about avoiding drugs and other conditions known to prolong the QT interval.

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**Disclosures**

None.

**References**


CLINICAL PERSPECTIVE

QT prolongation has been attributed to either a congenital origin resulting from ion channel mutations or an acquired origin generally resulting from QT-prolonging drugs. Patients with QTc prolongation are prone to complex ventricular arrhythmias, including Torsades de Pointes, syncope, and sudden death. Here, we propose a novel form of acquired QT prolongation of autoimmune origin induced by anti-Ro antibodies. QTc prolongation associated with anti-Ro antibodies per se may confer an increased risk for developing ventricular arrhythmias and represents an additional risk factor for patients with drug-induced or congenital QTc prolongation. The finding from the present study supports the recommendations that adult patients with anti-Ro antibodies may benefit from routine ECG screening for QTc prolongation and that those already identified with anti-Ro antibodies associated QTc prolongation should receive counseling, including education about avoiding drugs and other conditions known to prolong the QT interval.
Pathogenesis of the Novel Autoimmune-Associated Long-QT Syndrome
Yuankun Yue, Monica Castrichini, Ujala Srivastava, Frank Fabris, Krupa Shah, Zhiqiang Li, Yongxia Qu, Nabil El-Sherif, Zhengfeng Zhou, Craig January, M. Mahmood Hussain, Xian-Cheng Jiang, Eric A. Sobie, Marie Wahren-Herlenius, Mohamed Chahine, Pier-Leopoldo Capecchi, Franco Laghi-Pasini, Pietro-Enea Lazzerini and Mohamed Boutjdir

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Supplemental Methods

ECG recordings in patients: In this study, QTc was considered prolonged if ≥460 ms in accordance with the AHA/ACC/HRS recommendations\(^1\). QT interval was manually measured on a 12-lead ECG from the onset of the Q wave or the onset of the QRS complex to the end of the T wave, defined as the return to the T-P baseline. When U waves are present, the QT interval was measured to the nadir of the curve between the T and U waves. QT interval, determined as the longest measured QT interval in any lead, was corrected for heart rate by Bazett’s formula (dividing the QT interval by the square root of the R-R interval) to yield the QTc value. QTc is measured by a single investigator who was blinded to the patient’s Abs status.

Purification of IgG and affinity purification of anti-52kDa/Ro antibodies from patients’ sera: IgG purification was performed using Melon Gel IgG spin purification Kit (Thermo scientific). Briefly, 10-100 μL of serum per 100 μL of settled gel was used and the Melon Gel IgG purification support and purification buffer was equilibrated to room temperature. To obtain an even suspension, bottle containing the purification support was swirled and a 500 μL of slurry and dispensed into a spin column placed in a micro centrifuge tube. The uncapped column/tube assembly was centrifuged at 2,000-6,000 ×g for 1 minute. Purification buffer (300 μL) was added to the column, pulse centrifuged for 10 seconds and flow through discarded. 100-500 μL of diluted or 10-100 μL of buffer-exchanged serum was added to the column and incubated for 5 minutes at room temperature with end-over-end mixing. Centrifugation for 1 minute was performed to collect the purified Ab. For affinity purification of anti-52kDa Ro (52Ro) Abs, purified 52Ro protein was separated by 4-15% SDS-PAGE. The protein was transferred to nitrocellulose membrane and membrane areas with bound 52Ro protein were identified by immunoblotting. The membrane was blocked in 5% fat free milk in PBS-Tween (0.05%) for 30
minutes to prevent non-specific binding. Membrane areas with bound 52Ro were then excised and incubated overnight at 4°C in patient serum at a 1:10 dilution in PBS-Tween. The membrane was then washed thrice in PBS-Tween for 5 minutes each and the bound anti-52Ro Abs were eluted by incubating membrane in 0.1M Glycine (pH 2.8). Tris (1M) pH 9 was added in a 10:1 ratio to neutralize the pH of the final solution with anti-52Ro Abs.

**Electrophysiology**

**HEK293 cells stably expressing HERG channel:** The stably transfected cells were cultured in minimum essential medium (MEM) supplemented with 10% fetal bovine serum and 400 µg/ml geneticin (G418). For electrophysiological studies, cells were washed twice with standard MEM medium, and stored in this medium at room temperature for later use. Cells were superfused with HEPES-buffered Tyrode's solution containing (in mM): 137 NaCl, 4 KCl, 1.8 CaCl₂, 1 MgCl₂, 10 glucose, and 10 HEPES (pH 7.4 with NaOH). The internal pipette solution contained (in mM): 130 KCl, 1 MgCl₂, 5 EGTA, 5 MgATP, 10 HEPES (pH 7.2 with KOH). IKr was recorded using a standard protocol shown in Figure 1B. IKr kinetics were studied by fitting the activation and deactivation of IKr using Boltzmann equation as previously reported. Experiments were performed at room temperature.

**Guinea-pig ventricular myocytes:** All experiments were performed in accordance with the IACUC at the VA New York Harbor Healthcare System and conform to the NIH guidelines. Guinea pigs were anesthetized with isoflurane and the heart was rapidly excised and Langendorff perfused with the following Tyrode’s solution (in mM): 140 NaCl, 4.5 KCl, 2 CaCl₂, 10 dextrose, 1 MgCl₂, and 10 HEPES (pH = 7.4) for 2 min. The heart was then perfused with a Ca-free Tyrode’s solution followed by collagenase B (final concentration, 1mg/ml; Boehringer Mannheim, Indianapolis, IN). The dispersed cells were resuspended in KB solution and
maintained at room temperature before use. The external solution used for IKr recordings contained (in mM): 145 NaCl, 4.5 KCl, 1 MgCl₂, 1.8 CaCl₂, 10 HEPES, and 10 glucose (pH 7.35). Ca currents were blocked by the addition of 10 µM nifedipine in the bath solution and the slow delayed rectifier K current (I_{Ks}) was blocked with 10 µM chromanol. The pipette solutions contained (in mM): 140 KCl, 10 HEPES, 11 EGTA, 1 MgCl₂, 1 CaCl₂, 5 MgATP, and 5 K₂ATP; the pH adjusted to 7.2 with KOH. Currents were recorded in the whole-cell, voltage clamp configuration of the patch-clamp technique using an Axopatch-200B amplifier (Axon Instruments, Inc., Burlingame, CA). IKr was recorded using a short 200 ms depolarizing pulse from a holding potential of -40 mV and test pulses were applied at various voltages from -40 to +40 mV in a 10 mV increment prior to returning to -40 mV for tail current recording. Measurements were repeated every two minutes to allow for complete tail current deactivation. Action potentials were recorded from single ventricular myocytes in current-clamp mode by passing depolarizing currents at subthreshold (1.4 X) intensity.

**Western blots analysis:** Un-transfected and transfected HEK293 cells stably expressing HERG/Kv11.1 channels were harvested and lysed in RIPA buffer (25mM Tris-HCl (pH 7.6), 150mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) for 30 minutes at 4°C and centrifuged at 14,000 rpm for 15 minutes. Likewise, guinea-pig ventricles were minced in RIPA buffer and homogenized with a Polytron Homogenizer. After a 30 minutes incubation at 4°C, the homogenate was centrifuged at 14,000 rpm for 15 minutes. The supernatant after centrifugation was resolved by SDS-PAGE on a 4-15% Tris-HCl gel (Bio-Rad) and transferred on PVDF membrane (Bio-Rad). Blots were blocked with 5% milk for an hour and probed with HERG/Kv11.1 antibody (Sigma; 1:200), IgG (1:100) and GAPDH antibody (Sigma; 1:5000) overnight at 4°C. It was further probed with anti-rabbit IgG HRP (Santa Cruz) and anti-Human
IgG antibody (Jackson Immunolab) at a 1:5000 and 1:10,000 dilution. The signal was detected with Clarity ECL substrate (Bio-Rad) and blots were scanned in a C-Digit blot scanner (LI-COR) at high sensitivity to obtain the image. Because HERG channel delineates Human K channel, we used Kv11.1 to indicate the ERG K channel from guinea-pigs.

**Guinea-pigs immunization:** Antibody levels were checked at days 14, 28 and 42 by ELISA as described below. The ECG was recorded from slightly anesthetized (2-4% isoflurane) guinea-pigs in lead II and analyzed using a digital acquisition and analysis system (Power Lab/4SP). QTc was calculated using Bazett’s formula as it is more suitable for anesthetized guinea-pigs³.

**ELISA:** Briefly, wells were coated overnight with 0.2 µg of 52Ro protein in PBS, washed with PBS containing 0.05% Tween 20 (PBS Tween), blocked with 3% BSA/PBS Tween, washed with PBS Tween, and incubated with serial dilutions of antibody in PBS Tween (1/100, 1/500, and 1/1000) for 1 h at 22 °C. Goat anti-guinea-pig IgG conjugated to alkaline phosphatase was used at the second stage for 1 hour at 22 °C. OD was read at 405 nm. Results were considered positive if they are >2 SD greater than the mean obtained with sera prior to immunization. The peptide (GNMEQPHMDSRIGWLHNLGDQIGKPYNSSGL) corresponding to the pore forming region of HERG channel α₁ subunit was synthesized by GenScript (Piscataway, NJ) at >90% purity. ELISA using patients’ sera and the peptide was performed as above except for the use of goat anti-Human IgG.

References:

