



Autoimmune and inflammatory K⁺ channelopathies in cardiac arrhythmias: Clinical evidence and molecular mechanisms

This is the peer reviewed version of the following article:

Original:

Capecchi, P.L., Laghi-Pasini, F., El-Sherif, N., Qu, Y., Boutjdir, M., Lazzerini, P.E. (2019). Autoimmune and inflammatory K⁺ channelopathies in cardiac arrhythmias: Clinical evidence and molecular mechanisms. HEART RHYTHM, 16(8), 1273-1280 [10.1016/j.hrthm.2019.02.017].

Availability:

This version is available <http://hdl.handle.net/11365/1087500> since 2019-12-18T09:25:37Z

Published:

DOI: <http://doi.org/10.1016/j.hrthm.2019.02.017>

Terms of use:

Open Access

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. Works made available under a Creative Commons license can be used according to the terms and conditions of said license.

For all terms of use and more information see the publisher's website.

(Article begins on next page)

Accepted Manuscript

Autoimmune and inflammatory K⁺-channelopathies in cardiac arrhythmias: clinical evidence and molecular mechanisms.

Pier Leopoldo Capecchi, PhD/MD, Franco Laghi-Pasini, MD, Nabil El-Sherif, MD, Yongixa QU, MD/PhD, Mohamed Boutjdir, PhD, Pietro Enea Lazzzerini, MD

PII: S1547-5271(19)30139-0

DOI: <https://doi.org/10.1016/j.hrthm.2019.02.017>

Reference: HRTM 7916

To appear in: *Heart Rhythm*

Received Date: 28 November 2018

Please cite this article as: Capecchi PL, Laghi-Pasini F, El-Sherif N, QU Y, Boutjdir M, Lazzzerini PE, Autoimmune and inflammatory K⁺-channelopathies in cardiac arrhythmias: clinical evidence and molecular mechanisms., *Heart Rhythm* (2019), doi: <https://doi.org/10.1016/j.hrthm.2019.02.017>.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



Autoimmune and inflammatory K⁺-channelopathies in cardiac arrhythmias: clinical evidence and molecular mechanisms.

Pier Leopoldo CAPECCHI^{1†}, PhD/MD; Franco LAGHI-PASINI^{1†}, MD; Nabil EL-SHERIF, MD^{2†}; Yongixa QU, MD/PhD³, Mohamed BOUTJDIR^{2,4†}, PhD; Pietro Enea LAZZERINI^{1†*}, MD.

¹*Department of Medical Sciences, Surgery and Neurosciences, University of Siena, Italy;* ²*VA New York Harbor Healthcare System, SUNY Downstate Medical Center, New York, NY, United States;* ³*New York Presbyterian Brooklyn Methodist Hospital;* ⁴*NYU School of Medicine, New York, NY, United States.*

[†]These authors contributed equally to this work.

Running title: Autoimmune and inflammatory K⁺-channelopathies

Word count: 5,939

*Corresponding author:

Pietro Enea LAZZERINI, MD

Department of Medical Sciences, Surgery and Neurosciences, University of Siena, Italy

Tel.+39-0577-5585743; Fax+39-0577-233318; e-mail:lazzzerini7@unisi.it

CONFLICT OF INTEREST STATEMENT

On behalf of all authors, the corresponding author states that there is no conflict of interest

ABSTRACT

Cardiac K⁺-channelopathies account for a significant proportion of arrhythmias and sudden cardiac death (SCD) in subjects without structural heart disease. It is well recognized that genetic defects are key factors in many cases and in practice, the term cardiac channelopathies currently coincides with that of inherited cardiac channelopathies. However, mounting evidence demonstrate that not only genetic alterations but also autoimmune and inflammatory factors can cause cardiac K⁺-channel dysfunction and arrhythmias in the setting of a structurally normal heart. In particular, it has been demonstrated that specific autoantibodies as well as inflammatory cytokines can modulate expression and/or function of different K⁺-channels in the heart, resulting in a disruption of the cardiac action potential and arrhythmias/SCD. Awareness to the existence of these newly recognized forms is essential to identify and adequately manage affected patients.

In the present review, we focus on autoimmune and inflammatory K⁺-channelopathies as a novel mechanism for cardiac arrhythmias, and analyze the recent advancements in this topic providing complementary basic, clinical and population health perspectives.

Key words: cardiac K⁺-channels; cardiac arrhythmias; autoimmunity; inflammation; autoantibodies; cytokines; sudden cardiac death.

1.Introduction

Malignant arrhythmias are a recognized leading cause of sudden cardiac death (SCD) in Western countries, with coronary artery disease and heart failure representing the prevalent underlying substrate.¹ Up to 15% of subjects without any structural abnormality at autopsy died from cardiac arrest. This percentage increases to 40% in populations <40 years.² In a proportion of these cases, mutations in the genes encoding for cardiac ion channels and/or associated regulatory proteins (inherited channelopathies) are documented.³ In particular, it is well recognized that a wide number of inherited mutations of cardiac K⁺-channels can alter cardiomyocyte action potential (AP) via multiple mechanisms, eventually causing specific cardiac arrhythmia syndromes and SCD.⁴ However, in the majority of cases of unexplained SCD (~70%) the molecular autopsy (i.e. genetic testing of DNA extracted from postmortem blood/tissue sample) is negative.²

In such a scenario, the identification of novel, non-genetically determined arrhythmogenic mechanisms operating in structurally normal hearts represents a field of increasing interest in the recent years. In this regard, mounting evidence demonstrated that autoimmunity and inflammation can promote arrhythmias by directly interfering with the function of cardiac ion channels, particularly K⁺-channels. These factors operate regardless of evident histologic changes in the heart, thereby the terms of *autoimmune* and *inflammatory cardiac channelopathies* have been proposed, respectively.⁵⁻⁷ Specifically, it has been demonstrated that autoantibodies as well as inflammatory cytokines can modulate expression and/or function of different K⁺-channels in the heart, resulting in the disruption of the cardiac AP and arrhythmias/SCD.^{5,8}

In the present review, we focus on autoimmune and inflammatory K⁺-channelopathies as a novel mechanism for cardiac arrhythmias, and analyze the recent advancements in this topic providing complementary basic, clinical and population health perspectives.

2. K⁺-channels and their role in the cardiac action potential

K⁺-channels constitute the widest family of ion channels, encoded by >80 genes.⁹ Two main classes are currently recognized, i.e. voltage-gated (K_v) and inward-rectifying channels (K_{ir}). More details regarding K⁺-channels structure, and their involvement in AP duration (APD) are provided as supplementary material.

3. Clinical phenotypes

To date, two clinical phenotypes have been associated with autoimmune or inflammatory cardiac K⁺-channelopathies, i.e. long-QT syndrome (LQTS), in most cases, or short-QT syndrome (SQTS) (Figure 1).^{5,6,8} More information about general characteristics of these clinical phenotypes are provided as supplementary material.

4. Autoimmune K⁺-channelopathies

Different types of anti-K⁺-channel autoantibodies, recognizing specific cardiac K_v α subunits (i.e. K_v11.1 [also named human ether-a-go-go-related gene K⁺-channel, hERG], K_v7.1, K_v1.4), have been identified. By interfering with the function of K⁺-channels (*autoimmune K⁺-channelopathies*), these autoantibodies significantly impact APD of ventricular cardiomyocytes (Table 1) emerging in the clinical setting as LQTS or SQTS and related arrhythmias.^{5,10}

4.1. Anti-hERG-K⁺ channel autoantibodies

Mounting clinical and experimental evidence accumulated in the last decade demonstrated that, besides genetic and iatrogenic factors, the hERG-K⁺-channel function may be also disrupted by specific inhibiting autoantibodies, i.e. anti-Ro/SSA antibodies (anti-Ro/SSA), responsible for a novel LQTS of autoimmune origin.¹¹⁻¹³

Anti-Ro/SSA, characteristically detected in a large fraction of patients with connective tissue diseases (CTDs) such as Sjögren's syndrome (30–95%) and systemic lupus erythematosus (30–50%),¹⁴ can be also found in up to ~3% of apparently healthy populations.^{11,15} They include two

main subtypes, i.e. anti-Ro/SSA-52kD and anti-Ro/SSA-60kD, depending on the molecular weight of the specific subunits recognized on the Ro/SSA antigen, an intracellular ribonucleoprotein representing the primary molecular target of these antibodies.¹⁴ It is well recognized that transplacental passage of maternal anti-Ro/SSA is associated with the development of the autoimmune-congenital heart block (ACHB),¹⁶ at least in part resulting from an inhibitory cross-reaction with L-type Ca⁺⁺-channels in the foetal cardiac conduction system.^{11,17}

More recently, several studies demonstrated that anti-Ro/SSA-positive subjects, both newborns and adults affected or not by CTD, in 10-60% of cases can also show heart rate-corrected QT-interval (QTc) prolongation,¹⁸⁻²⁷ which correlate with autoantibody levels (particularly anti-Ro/SSA-52kD)^{23,24,27} and occurrence of complex ventricular arrhythmias (VA),²² including Torsades de pointes (TdP).^{28,29} From a molecular point of view, there is strong evidence that anti-Ro/SSA-52kD can induce LQTS as a result of a cross-reactivity with the pore region of hERG leading to an inhibition of the channel function (Table 1). In particular, our group demonstrated that serum, purified IgG, or affinity-purified anti-Ro/SSA-52kD from CTD patients with LQTS significantly inhibited I_{Kr}, both in native guinea-pig ventricular myocytes and in human embryonic kidney(HEK)-293-cells stably expressing the hERG channel.³⁰ Such effects occurred rapidly, in few minutes, were concentration-dependent and reversible. Moreover, guinea-pig immunization with the Ro52 antigens lead to high circulating anti-Ro/SSA levels, resulted in significant QTc prolongation due to APD lengthening, as demonstrated by incubating guinea-pig ventricular myocytes with anti-Ro/SSA-positive IgGs.³⁰ Enzyme-linked immunosorbent assay (ELISA) experiments also proved that anti-Ro/SSA directly cross-react with the hERG-channel by recognizing an epitope localized in the pore-forming subunit, at the extracellular loop between segments S5-S6, where significant sequence homology to the Ro52 antigen is present.³⁰ In addition, immunization of guinea-pigs with a 31-amino acid peptide corresponding to a portion of this extracellular region induced I_{Kr}-inhibiting antibodies as well as APD and QTc prolongation, in the absence of any structural heart change.³¹ These mechanisms are also actively involved in patients developing anti-Ro/SSA-

associated life-threatening VA, particularly TdP. Nakamura et al.²⁸ demonstrated that serum and purified IgG from an anti-Ro/SSA-positive patient with recurrent TdP cross-reacted with the hERG-protein and decreased I_{Kr} in HEK-293 cells. Such findings were confirmed and further characterized in our laboratory by studying 25 TdP patients consecutively enrolled from the general population, where circulating anti-Ro/SSA was found in 60% of cases.²⁹ In particular, experiments on anti-Ro/SSA-positive sera/IgGs from these subjects confirmed the strong aptitude of these autoantibodies to interfere with I_{Kr} function by specifically interacting with the hERG-channel pore-forming extracellular region.²⁹

Although these data strongly support the conclusion that anti-Ro/SSA can cause LQTS, some clinical studies found conflicting results,³²⁻³⁴ and even in studies demonstrating association, there was variability in the degree of QTc prolongation among anti-Ro/SSA-positive patients (10-60%).¹³ Disease-specific differences in anti-Ro/SSA-52kD titres (by most authors not separately evaluated), and QTc prolongation cut-offs used, as well as electrophysiological considerations, may account for such discrepancies throughout the studies. For example, one study involved systemic sclerosis patients, commonly showing anti-Ro/SSA-52kD-positivity but at a low concentration;⁵¹ another study used a very high QTc-cut-off (>500 milliseconds) thus excluding a wide range of possible abnormal QTc (440-500 ms).⁵⁰ In addition, it is well demonstrated that anti-Ro/SSA can also target cardiac Ca^{++} -channels,^{17,35,36} thus possibly leading to an opposite effect to that on I_{Kr} hence the variability. This hypothesis is supported by simulation studies showing that a concomitant inhibitory effect of anti-Ro/SSA on I_{CaL} during the plateau phase L-type Ca^{++} -channels can partially counteract the I_{Kr} inhibition-dependent prolongation of APD, and then the net duration of QTc on the ECG.³¹ Nevertheless, since I_{Kr} is activated after the peak of T wave,³⁷ specific measurement of the Tpeak-Tend interval (Tp-Te)³⁸ should in any case unmask if anti-Ro/SSA are interfering with the ventricular repolarization. Accordingly, Tufan et al.²⁷ demonstrated that Tp-Te is significantly prolonged in anti-Ro/SSA-52kD-positive CTD subjects, also when the QTc was found normal, thus pointing to Tp-Te as the ECG parameter of choice to assess the extent of anti-Ro/SSA-mediated I_{Kr}

inhibition *in-vivo*. Notably, amino acid sequence comparison between $K_v11.1$ and other K^+ -channels ($K_v7.1$, $K_v1.5$, $K_v4.3$, $K_v4.2$, $K_v1.4$, $K_{ir6.2}$, $K_{ir2.1}$, $K_{ir2.2}$, $K_{ir3.1}$ and $K_{ir3.4}$) show low homology (6.5-11.6%) but a high interspecies homology(>95%) between mouse, rat, guinea pigs $K_v11.1$ compared to human- $K_v11.1$ (hERG). This suggests that anti-Ro/SSA may also affect $K_v11.1$ channel in these species, while additional cross-reactivity with other K^+ -channel is less likely.

In summary, many basic and clinical data support the ability of anti-Ro/SSA-52kD in directly impairing hERG- K^+ -channel function, resulting in an increased risk for developing LQTS and TdP. Nevertheless, given some discrepancies among studies, more investigation is warranted to better characterize all the specific determinants of such an effect, as well as population data to determine the percentage of anti-Ro/SSA52-kD carriers who actually manifest the channelopathy and/or develop arrhythmias.

4.2. Anti- $K_v7.1$ - K^+ channel autoantibodies

By generating the repolarising current I_{Ks} , the cardiac $K_v7.1$ - K^+ -channel plays a critical role in determining QT-interval duration^{37,39} (Figure 1). Indeed, it is well known that loss-of-function mutations in the encoding gene *KCNQ1* cause the most common form of congenital LQTS (LQT1), while gain-of function-mutations induce SQTs type 2 (SQT2).⁴

Six years ago, Li et al.⁴⁰ demonstrated that the function of this channel can be also altered by immune-mediated mechanisms, thus describing for the first time the existence of a SQTs of autoimmune origin. By studying a cohort of 150 dilated cardiomyopathy (DCM) patients, these authors found that in a subgroup of subjects (6%) showing a shortened QTc, circulating autoantibodies recognizing the $K_v7.1$ - K^+ -channel were detected by ELISA.⁴⁰ Although no direct data with purified autoantibodies are currently available,⁴¹ it is plausible that anti- $K_v7.1$ -antibodies exert an agonist-like effect on the channel resulting in I_{Ks} enhancement, possibly as a result of alterations in single-channel conductance and/or activation of membrane dormant channels. In fact, incubation of *KCNQ1*-expressing HEK293-cells with patients' sera containing anti- $K_v7.1$ -antibodies significantly increased I_{Ks} density, in the absence of any change in channel cell surface expression

by Western-blotting analysis.⁴⁰ Moreover, rabbits immunized with a peptide corresponding to the pore region of the channel (extracellular loop between the 5th and 6th transmembrane segments) developed high levels of circulating anti-K_v7.1-antibodies and showed QTc shortening, along with reduced ventricular effective refractory periods and increased vulnerability to induced ventricular tachyarrhythmias.⁴² In addition, electrophysiological characterization of ventricular cardiomyocytes isolated from these animals demonstrated I_{Ks} densities enhancement and APD decrease⁴² (Table 1). Notably, despite the fact that the rabbit myocardium showed extensive antibody deposition, no echocardiography changes or signs of inflammatory infiltration/fibrosis were observed.⁴² Although specific studies are warranted to define the clinical relevance of anti-K_v7.1-antibodies, altogether these data support the hypothesis that these autoantibodies may contribute, at least in a fraction of patients, to the increased incidence of malignant VA/SCD associated to DCM.

4.3. Anti-K_v1.4-K⁺ channel autoantibodies

The K_v1.4-K⁺-channel, which generates I_{to,s}, plays important roles both in the heart, where represents one of the main determinants of the early repolarization phase of the ventricular AP (Figure 1), and in the nervous system, by modulating the acetylcholine presynaptic release.^{37,39,43} Recent evidence demonstrated that in myasthenia gravis (MG), an autoimmune disease primarily targeting the neuromuscular junction, circulating autoantibodies against the K_v1.4-K⁺-channel can be detected by immunoprecipitation assay in up to 10-20% of patients,⁴⁴⁻⁴⁶ also associating with LQTS^{45,46} and life-threatening arrhythmic events.⁴⁶ Indeed, Suzuki et al. demonstrated that up to ~30% of anti-K_v1.4-positive subjects presented with QTc prolongation,^{45,46} and among these TdP/SCD occurred in 20% of cases.

While it is speculated that anti-K_v1.4-K⁺-channel-antibodies can increase QTc duration as a result of a direct channel binding leading to I_{to} inhibition, to date electrophysiological and molecular properties of these autoantibodies have not been characterized. Specifically, studies evaluating the functional impact of anti-K_v1.4 antibodies on I_{to} are missing, thus precluding at the moment any definitive conclusion regarding the mechanism(s) of QTc prolongation. In fact, a contributing role

to the genesis of electric abnormalities could be also played by a concomitant autoimmune-mediated inflammatory heart damage, as suggested by the fact that some of anti-Kv1.4-positive MG patients with LQTS showed clinical evidence of myocarditis.^{45,46}

5. Inflammatory K⁺-channelopathies

Inflammatory cytokines, specifically TNF α , IL-1 and IL-6, can complexly modulate both the expression and the function of several cardiac K⁺-channels, via direct effects on the cardiomyocyte (*inflammatory K⁺-channelopathies*).⁶ Although TNF α is the best characterized cytokine, current evidence indicates that, regardless the specific molecule involved, the common final result is a K⁺-channels loss-of-function, leading to APD prolongation and LQTS.^{6,8,47}

5.1. TNF α -induced K⁺-channelopathies

A lot of experimental studies documented the ability of TNF α to prolong APD/QT-interval and promote VA/SCD, as a result of inhibiting effects on several cardiac K⁺-channels, i.e. hERG, K_v7.1, K_v4.2/4.3 and K_v1.5 (Table 1).

Different transgenic mouse models demonstrated that overexpression of TNF α is associated with APD prolongation, QT/QTc interval lengthening, and increased incidence of life-threatening arrhythmias with premature mortality.⁴⁸⁻⁵² Left ventricular myocytes isolated from these animals showed a significant decrease of both I_{to} and I_{Kur} currents, with a concomitant reduction in the expression of related channels K_v4.2/4.3 and K_v1.5 as revealed by both Western-blotting and real-time PCR.^{49,53} Moreover, different authors reported that TNF α incubation of rat ventricular myocytes down-regulates both I_{to} and K_v4.2 *in-vitro*, along with an increased nitric oxide synthase expression and reactive oxygen species (ROS) production, as well as a decrease in the potassium-channel-interacting protein-2 (KChIP-2).^{53,54} Dissecting the specific downstream pathway involved, accumulating data point to ROS-induced nuclear factor kappaB (NF- κ B) activation as the key molecular step responsible for K⁺-channels gene expression decrease induced by TNF α in cardiomyocytes.^{55,56} Similar inhibitory effects on I_{to} and I_{Kur} currents were also found by Grandy et

al.⁵⁷ by studying cardiomyocytes from ventricles of mice exposed to high circulating TNF α levels due to chronic injection of TNF α . Notably, the evidence that in the last model K⁺-current reduction occurred also in the absence of any decrease in K_v4.2/4.3 and K_v1.5 mRNA or protein expression (possibly depending on the lower cardiac tissue TNF α concentrations reached respect to transgenic mice),⁵⁷ points to additional inhibitory effects exerted by TNF α on the function of these channels. Other authors demonstrated that TNF α also significantly inhibits I_{Kr} and I_{Ks}. The group of Nattel⁵⁸ provided evidence that the hERG-K⁺-channel activity was impaired (I_{Kr} decrease) by TNF α in both HEK293-cells and canine cardiomyocytes, also associating with myocyte APD prolongation.⁵⁸ Electrophysiological changes, which occurred without altering hERG-protein expression (Western-blot), were prevented by cell pre-incubation with either an inhibiting anti-TNF-receptor-1 (TNFR1) antibody or anti-superoxide anion molecules, thus suggesting a interference with the channel at the functional level mediated by TNFR1 stimulation and ROS production.⁵⁸ In addition, by studying guinea-pig ventriculocytes *in-vitro* incubated with TNF α , Hatada et al.⁵⁹ found that isoprotenerol-activated I_{Ks} was markedly reduced, in the presence of significant sphingosine-1-phosphate generation and cyclic AMP decrease. Whether such decreasing effect results from TNF α -mediated changes in K_v7.1-K⁺-channel expression is not currently known, as specific expression studies are currently missing.

Overall, available studies consistently substantiate a significant inhibitory effect of TNF α on different K⁺-currents critically involved in ventricular repolarization. This activity, resulting from direct effects on the expression and/or function of specific K⁺-channels, can promote APD prolongation by inducing an inward shift in the balance of currents.

5.2. IL-1- and IL-6-induced K⁺-channelopathies

Although less information is currently available, recent basic studies provided evidence that both IL-6 and IL-1 can induce cardiac K⁺-channels dysfunction, associated with APD/QT-interval prolongation (Table 1). A recent *in-vitro* study provided evidence that IL-1 β incubation reduces I_{to} (no expression experiments on related K⁺-channels were performed), also augmenting APD in

ventricular rat myocytes.⁶⁰ Similar changes in the field potential duration (FPD) were demonstrated to occur in human-induced pluripotent stem-cell derived cardiomyocytes (hiPS-CM).⁶⁰ In addition, confocal imaging showed that IL-1 β increases diastolic sarcoplasmic reticulum Ca⁺⁺-leak, providing a cellular substrate for triggered arrhythmogenic activity.⁶⁰ Accordingly, in a diabetic murine model characterized by QTc prolongation and increased vulnerability to arrhythmias after caffeine/dobutamine challenge, IL-1 blockade via IL-1-receptor genetic deletion or IL-1-receptor antagonist (anakinra) treatment, abrogated the development of both electric alterations in these animals.⁶⁰

Regarding IL-6, our group demonstrated that in HEK293-cells stably expressing the hERG-K⁺-channel, IL-6 produced a significant dose-dependent depression of I_{Kr} peak and tail current densities. Western blot and patch-clamp data showed that such an effect was associated with an inhibition of both hERG-protein expression and activation kinetics, thus pointing to combined activities on channel trafficking and gating.⁶¹ In addition, electrophysiological experiments performed in guinea-pig ventricular myocytes pre-exposed to anti-IL-6R monoclonal antibody or Janus-kinase-I-inhibitor showed that IL-6 also prolonged APD through a pathway involving activation of the IL-6 receptor and JAK-pathway, thus pointing to the involvement of downstream complex effectors such as STAT3 (signal transducer and activator of transcription protein-3), MAPK (mitogen-activated protein-kinase), and Pi3K/Akt (phosphoinositol-3 kinase/protein kinase-B) as modulators of hERG-K⁺-channel gene expression and function.⁶¹

Altogether, these data provide mechanistic support to the hypothesis that not only TNF α , but also IL-1 and IL-6 represent key mediators of inflammation-induced QTc prolongation by inducing cardiac K⁺-channelopathies.

5.3. Inflammatory cytokines, LQTS and TdP/SCD risk: Clinical Evidence

Mounting evidence strongly suggests that the above described cytokine-induced K⁺-channelopathies may have an important impact in the clinical setting, by promoting LQTS

development and increasing TdP/SCD risk both in patients with inflammatory diseases and in apparently healthy subjects with low-grade systemic inflammation.

In rheumatoid arthritis (RA), a systemic inflammatory disease burdened by an high incidence of LQTS and a 2-times higher risk of risk of SCD than the general population,^{8,62,63} QTc interval duration positively correlated with circulating levels of TNF α , IL-6, and IL-1 β .⁶⁴ Similar aspects in terms of inflammatory activation and arrhythmic risk are also characteristic of patients with CTD,^{8,21,22,24,25,32} where the presence of QTc prolongation was independently predicted by IL-1 β serum levels.²⁵ More generally, we demonstrated that regardless of the specific disease involved,QTc prolongation was commonly observed in subjects with elevated C-reactive protein (CRP) levels from different inflammatory conditions (infective, immuno-mediated, other), also robustly correlating with IL-6 serum concentrations.⁶⁵ In addition, in a large study performed on 1716 subjects of the general population, Medenwald et al.⁶⁶ found that after covariate adjustment soluble TNF-receptor-1 levels were associated with QTc duration in women, while Kertai et al.⁶⁷ reported that high IL-1 β expressing gene variants were independent predictors of postoperative QTc prolongation among 957 patients undergoing cardiac surgery.

The clinical relevance of the link between inflammatory cytokines and LQTS is also supported by studies demonstrating that anti-cytokine targeted therapy can reverse QTc prolongation. In ankylosing spondylitis patients, a 6-months treatment with the anti-TNF α monoclonal antibody infliximab significantly reduced both inflammatory markers and QTc duration.⁶⁸ Moreover, two recent studies consistently reported that anti-IL6 therapy with tocilizumab led to a rapid (3-6 months) QTc shortening in RA, also correlating with TNF α levels decrease.^{69,70}

Finally, we demonstrated that in a cohort of 40 unselected TdP patients, elevated IL-6 serum concentrations were present, comparable to those observed in severe active RA, and 15-20 times higher than in controls.⁶⁵ These data, together with the evidence emerging from large prospective population studies that IL-6 is a strong independent predictor of SCD in apparently healthy

persons,^{71,72} further points to a key role for inflammatory cytokines in promoting LQTS and associated malignant arrhythmias, specifically TdP degenerating to VF.

6. Clinical perspectives

Until recently and largely overlooked, the potential role of autoimmune and inflammatory K^+ -channelopathies as a novel mechanism of cardiac arrhythmias/SCD is now increasingly recognized, not only in patients with manifest immune-inflammatory diseases, but also in the general population. Thus, unrecognized autoimmune/inflammatory K^+ -channelopathies may underlie a percentage of unexplained arrhythmias/SCDs, whose origin could be revealed only if specific testing is performed. Moreover, these factors may also importantly contribute to increase cardiac electric instability in patients already predisposed to arrhythmias. Indeed, accumulating evidence indicates that immune-inflammatory activation, also subclinical, can precipitate life-threatening arrhythmias/electrical storms in congenital LQTS subjects,⁷³⁻⁷⁵ and cardiomyopathies.⁷⁶⁻⁷⁸ Accordingly, suitable patients for testing may be those developing rhythm disorders/aborted cardiac arrest in the absence of any genetic or acquired recognized factor despite extensive examination, as well as subjects with structural heart disease or inherited channelopathies refractory to conventional treatments. However, while CRP and anti-Ro/SSA-antibodies testing are routinely accessible, other specific anti- K^+ -channel autoantibodies and circulating inflammatory cytokines are currently restricted to research settings only.

Overcoming such practical limitations could have very important clinical implications. In fact, demonstrating a causative role for autoimmune or inflammatory K^+ -channelopathies in these patients could open to innovative anti-arrhythmic therapies targeting the immune-inflammatory system (i.e. immunomodulating drugs, anti-cytokine monoclonal antibodies, plasmapheresis, immunoabsorption), also including the potential use of short decoy peptides diverting pathogenic autoantibodies from K^+ -channel binding-sites.

In this novel perspective, an increased awareness to the existence of autoimmune and inflammatory K⁺-channelopathies is a crucial step forward for a more comprehensive approach to the patient with cardiac arrhythmias.

ACCEPTED MANUSCRIPT

This work has received funding from “Fondo Aree Sottoutilizzate-Salute ToRSADE project” (FAS-Salute 2014, Regione Toscana), and from Biomedical Laboratory Research & Development Service of Veterans Affairs Office of Research and Development as a Merit Review grant I01BX002137 to M.B.

ACCEPTED MANUSCRIPT

1. Krokhalava Y, Vaseghi M. Update on prevention and treatment of sudden cardiac arrest. *Trends Cardiovasc Med*. 2018.
2. Semsarian C, Ingles J. Molecular autopsy in victims of inherited arrhythmias. *J Arrhythm*. 2016;32(5):359-365.
3. Cerrone M, Priori SG. Genetics of sudden death: focus on inherited channelopathies. *Eur Heart J*. 2011;32(17):2109-2118.
4. Giudicessi JR, Ackerman MJ. Potassium-channel mutations and cardiac arrhythmias--diagnosis and therapy. *Nat Rev Cardiol*. 2012;9(6):319-332.
5. Lazzerini PE, Capecchi PL, Laghi-Pasini F, Boutjdir M. Autoimmune channelopathies as a novel mechanism in cardiac arrhythmias. *Nat Rev Cardiol*. 2017;14(9):521-535.
6. Lazzerini PE, Capecchi PL, El-Sherif N, Laghi-Pasini F, Boutjdir M. Emerging Arrhythmic Risk of Autoimmune and Inflammatory Cardiac Channelopathies. *J Am Heart Assoc*. 2018;7(22):e010595.
7. Lazzerini PE, Laghi-Pasini F, Boutjdir M, Capecchi PL. Cardioimmunology of arrhythmias: the role of autoimmune and inflammatory cardiac channelopathies. *Nat Rev Immunol*. 2019;19(1):63-64.
8. Lazzerini PE, Capecchi PL, Laghi-Pasini F. Systemic inflammation and arrhythmic risk: lessons from rheumatoid arthritis. *Eur Heart J*. 2017;38(22):1717-1727.
9. Amin AS, Wilde AA. Genetic Control of Potassium Channels. *Card Electrophysiol Clin*. 2016;8(2):285-306.
10. Lazzerini PE, Capecchi PL, Guideri F, et al. Autoantibody-mediated cardiac arrhythmias: mechanisms and clinical implications. *Basic Res Cardiol*. 2008;103(1):1-11.
11. Lazzerini PE, Capecchi PL, Laghi-Pasini F. Anti-Ro/SSA antibodies and cardiac arrhythmias in the adult: facts and hypotheses. *Scand J Immunol*. 2010;72(3):213-222.
12. Lazzerini PE, Capecchi PL, Acampa M, et al. Arrhythmogenic effects of anti-Ro/SSA antibodies on the adult heart: more than expected? *Autoimmun Rev*. 2009;9(1):40-44.
13. Boutjdir M, Lazzerini PE, Capecchi PL, Laghi-Pasini F, El-Sherif N. Potassium Channel Block and Novel Autoimmune-Associated Long QT Syndrome. *Card Electrophysiol Clin*. 2016;8(2):373-384.
14. Franceschini F, Cavazzana I. Anti-Ro/SSA and La/SSB antibodies. *Autoimmunity*. 2005;38(1):55-63.
15. Hayashi N, Koshiba M, Nishimura K, et al. Prevalence of disease-specific antinuclear antibodies in general population: estimates from annual physical examinations of residents of a small town over a 5-year period. *Mod Rheumatol*. 2008;18(2):153-160.
16. Brito-Zerón P, Izmirly PM, Ramos-Casals M, Buyon JP, Khamashta MA. The clinical spectrum of autoimmune congenital heart block. *Nat Rev Rheumatol*. 2015;11(5):301-312.
17. Karnabi E, Boutjdir M. Role of calcium channels in congenital heart block. *Scand J Immunol*. 2010;72(3):226-234.
18. Cimaz R, Stramba-Badiale M, Brucato A, Catelli L, Panzeri P, Meroni PL. QT interval prolongation in asymptomatic anti-SSA/Ro-positive infants without congenital heart block. *Arthritis Rheum*. 2000;43(5):1049-1053.
19. Gordon PA, Khamashta MA, Hughes GR, Rosenthal E. Increase in the heart rate-corrected QT interval in children of anti-Ro-positive mothers, with a further increase in those with siblings with congenital heart block: comment on the article by Cimaz et al. *Arthritis Rheum*. 2001;44(1):242-243.
20. Jaeggi E, Laskin C, Hamilton R, Kingdom J, Silverman E. The importance of the level of maternal anti-Ro/SSA antibodies as a prognostic marker of the development of cardiac neonatal lupus erythematosus a prospective study of 186 antibody-exposed fetuses and infants. *J Am Coll Cardiol*. 2010;55(24):2778-2784.
21. Lazzerini PE, Acampa M, Guideri F, et al. Prolongation of the corrected QT interval in adult patients with anti-Ro/SSA-positive connective tissue diseases. *Arthritis Rheum*. 2004;50(4):1248-1252.
22. Lazzerini PE, Capecchi PL, Guideri F, et al. Comparison of frequency of complex ventricular arrhythmias in patients with positive versus negative anti-Ro/SSA and connective tissue disease. *Am J Cardiol*. 2007;100(6):1029-1034.

23. Bourré-Tessier J, Clarke AE, Huynh T, et al. Prolonged corrected QT interval in anti-Ro/SSA-positive adults with systemic lupus erythematosus. *Arthritis Care Res (Hoboken)*. 2011;63(7):1031-1037.
24. Lazzerini PE, Capecchi PL, Acampa M, et al. Anti-Ro/SSA-associated corrected QT interval prolongation in adults: the role of antibody level and specificity. *Arthritis Care Res (Hoboken)*. 2011;63(10):1463-1470.
25. Pisoni CN, Reina S, Arakaki D, Eimon A, Carrizo C, Borda E. Elevated IL-1 β levels in anti-Ro/SSA connective tissue diseases patients with prolonged corrected QTc interval. *Clin Exp Rheumatol*. 2015;33(5):715-720.
26. Sham S, Madheshwaran M, Tamilselvam T, Rajeswari S. Correlation of QT interval with disease activity in newly detected SLE patients at baseline and during flare. *Indian J Rheumatol*. 2015;10(3):121-124.
27. Tufan AN, Sag S, Oksuz MF, et al. Prolonged Tpeak-Tend interval in anti-Ro52 antibody-positive connective tissue diseases. *Rheumatol Int*. 2017;37(1):67-73.
28. Nakamura K, Katayama Y, Kusano KF, et al. Anti-KCNH2 antibody-induced long QT syndrome: novel acquired form of long QT syndrome. *J Am Coll Cardiol*. 2007;50(18):1808-1809.
29. Lazzerini PE, Yue Y, Srivastava U, et al. Arrhythmogenicity of Anti-Ro/SSA Antibodies in Patients With Torsades de Pointes. *Circ Arrhythm Electrophysiol*. 2016;9(4):e003419.
30. Yue Y, Castrichini M, Srivastava U, et al. Pathogenesis of the Novel Autoimmune-Associated Long-QT Syndrome. *Circulation*. 2015;132(4):230-240.
31. Fabris F, Yue Y, Qu Y, et al. Induction of autoimmune response to the extracellular loop of the HERG channel pore induces QTc prolongation in guinea-pigs. *J Physiol*. 2016;594(21):6175-6187.
32. Costedoat-Chalumeau N, Amoura Z, Hulot JS, et al. Corrected QT interval in anti-SSA-positive adults with connective tissue disease: comment on the article by Lazzerini et al. *Arthritis Rheum*. 2005;52(2):676-677; author reply 677-678.
33. Teixeira RA, Borba EF, Pedrosa A, et al. Evidence for cardiac safety and antiarrhythmic potential of chloroquine in systemic lupus erythematosus. *Europace*. 2014;16(6):887-892.
34. Massie C, Hudson M, Tatibouet S, et al. Absence of an association between anti-Ro antibodies and prolonged QTc interval in systemic sclerosis: a multicenter study of 689 patients. *Semin Arthritis Rheum*. 2014;44(3):338-344.
35. Xiao GQ, Hu K, Boutjdir M. Direct inhibition of expressed cardiac I- and t-type calcium channels by igg from mothers whose children have congenital heart block. *Circulation*. 2001;103(11):1599-1604.
36. Karnabi E, Qu Y, Wadgaonkar R, et al. Congenital heart block: identification of autoantibody binding site on the extracellular loop (domain I, S5-S6) of alpha(1D) L-type Ca channel. *J Autoimmun*. 2010;34(2):80-86.
37. Grant AO. Cardiac ion channels. *Circ Arrhythm Electrophysiol*. 2009;2(2):185-194.
38. Drew BJ, Ackerman MJ, Funk M, et al. Prevention of torsade de pointes in hospital settings: a scientific statement from the American Heart Association and the American College of Cardiology Foundation. *J Am Coll Cardiol*. 2010;55(9):934-947.
39. Bartos DC, Grandi E, Ripplinger CM. Ion Channels in the Heart. *Compr Physiol*. 2015;5(3):1423-1464.
40. Li J, Seyler C, Wiedmann F, et al. Anti-KCNQ1 K⁺ channel autoantibodies increase IKs current and are associated with QT interval shortening in dilated cardiomyopathy. *Cardiovasc Res*. 2013;98(3):496-503.
41. Lazzerini PE, Capecchi PL, Laghi-Pasini F, Boutjdir M. Autoimmune cardiac channelopathies: the heart of the matter. *Nat Rev Cardiol*. 2017;14(9):566.
42. Li J, Maguy A, Duverger JE, et al. Induced KCNQ1 autoimmunity accelerates cardiac repolarization in rabbits: potential significance in arrhythmogenesis and antiarrhythmic therapy. *Heart Rhythm*. 2014;11(11):2092-2100.
43. Nerbonne JM. Molecular Basis of Functional Myocardial Potassium Channel Diversity. *Card Electrophysiol Clin*. 2016;8(2):257-273.
44. Romi F, Suzuki S, Suzuki N, Petzold A, Plant GT, Gilhus NE. Anti-voltage-gated potassium channel Kv1.4 antibodies in myasthenia gravis. *J Neurol*. 2012;259(7):1312-1316.
45. Suzuki S, Satoh T, Yasuoka H, et al. Novel autoantibodies to a voltage-gated potassium channel Kv1.4 in a severe form of myasthenia gravis. *J Neuroimmunol*. 2005;170(1-2):141-149.

46. Suzuki S, Baba A, Kaida K, et al. Cardiac involvements in myasthenia gravis associated with anti-Kv1.4 antibodies. *Eur J Neurol*. 2014;21(2):223-230.
47. Lazzerini PE, Capecchi PL, Laghi-Pasini F. Long QT Syndrome: An Emerging Role for Inflammation and Immunity. *Front Cardiovasc Med*. 2015;2:26.
48. Saba S, Janczewski AM, Baker LC, et al. Atrial contractile dysfunction, fibrosis, and arrhythmias in a mouse model of cardiomyopathy secondary to cardiac-specific overexpression of tumor necrosis factor- α . *Am J Physiol Heart Circ Physiol*. 2005;289(4):H1456-1467.
49. Petkova-Kirova PS, Gursoy E, Mehdi H, McTiernan CF, London B, Salama G. Electrical remodeling of cardiac myocytes from mice with heart failure due to the overexpression of tumor necrosis factor- α . *Am J Physiol Heart Circ Physiol*. 2006;290(5):H2098-2107.
50. Sakkou M, Chouvardas P, Ntari L, et al. Mesenchymal TNFR2 promotes the development of polyarthritis and comorbid heart valve stenosis. *JCI Insight*. 2018;3(7).
51. Ntari L, Sakkou M, Chouvardas P, et al. Comorbid TNF-mediated heart valve disease and chronic polyarthritis share common mesenchymal cell-mediated aetiopathogenesis. *Ann Rheum Dis*. 2018;77(6):926-934.
52. London B, Baker LC, Lee JS, et al. Calcium-dependent arrhythmias in transgenic mice with heart failure. *Am J Physiol Heart Circ Physiol*. 2003;284(2):H431-441.
53. Kawada H, Niwano S, Niwano H, et al. Tumor necrosis factor- α downregulates the voltage gated outward K⁺ current in cultured neonatal rat cardiomyocytes: a possible cause of electrical remodeling in diseased hearts. *Circ J*. 2006;70(5):605-609.
54. Fernández-Velasco M, Ruiz-Hurtado G, Hurtado O, Moro MA, Delgado C. TNF- α downregulates transient outward potassium current in rat ventricular myocytes through iNOS overexpression and oxidant species generation. *Am J Physiol Heart Circ Physiol*. 2007;293(1):H238-245.
55. Panama BK, Latour-Villamil D, Farman GP, et al. Nuclear factor kappaB downregulates the transient outward potassium current I_(to,f) through control of KChIP2 expression. *Circ Res*. 2011;108(5):537-543.
56. Moe KT, Khairunnisa K, Yin NO, Chin-Dusting J, Wong P, Wong MC. Tumor necrosis factor- α -induced nuclear factor- κ B activation in human cardiomyocytes is mediated by NADPH oxidase. *J Physiol Biochem*. 2014;70(3):769-779.
57. Grandy SA, Fiset C. Ventricular K⁺ currents are reduced in mice with elevated levels of serum TNF α . *J Mol Cell Cardiol*. 2009;47(2):238-246.
58. Wang J, Wang H, Zhang Y, Gao H, Nattel S, Wang Z. Impairment of HERG K(+) channel function by tumor necrosis factor- α : role of reactive oxygen species as a mediator. *J Biol Chem*. 2004;279(14):13289-13292.
59. Hatada K, Washizuka T, Horie M, et al. Tumor necrosis factor- α inhibits the cardiac delayed rectifier K current via the sphingomyelin pathway. *Biochem Biophys Res Commun*. 2006;344(1):189-193.
60. Monnerat G, Alarcón ML, Vasconcellos LR, et al. Macrophage-dependent IL-1 β production induces cardiac arrhythmias in diabetic mice. *Nat Commun*. 2016;7:13344.
61. Aromolaran AS, Srivastava U, Alí A, et al. Interleukin-6 inhibition of hERG underlies risk for acquired long QT in cardiac and systemic inflammation. *PLoS One*. 2018;13(12):e0208321.
62. Lazzerini PE, Acampa M, Capecchi PL, et al. Association between high sensitivity C-reactive protein, heart rate variability and corrected QT interval in patients with chronic inflammatory arthritis. *Eur J Intern Med*. 2013;24(4):368-374.
63. Chauhan K, Ackerman MJ, Crowson CS, Matteson EL, Gabriel SE. Population-based study of QT interval prolongation in patients with rheumatoid arthritis. *Clin Exp Rheumatol*. 2015;33(1):84-89.
64. Adlan AM, Panoulas VF, Smith JP, Fisher JP, Kitas GD. Association between corrected QT interval and inflammatory cytokines in rheumatoid arthritis. *J Rheumatol*. 2015;42(3):421-428.
65. Lazzerini PE, Laghi-Pasini F, Bertolozzi I, et al. Systemic inflammation as a novel QT-prolonging risk factor in patients with torsades de pointes. *Heart*. 2017;103(22):1821-1829.
66. Medenwald D, Kors JA, Loppnow H, et al. Inflammation and prolonged QT time: results from the Cardiovascular Disease, Living and Ageing in Halle (CARLA) study. *PLoS One*. 2014;9(4):e95994.

67. Kertai MD, Ji Y, Li YJ, et al. Interleukin-1 β gene variants are associated with QTc interval prolongation following cardiac surgery: a prospective observational study. *Can J Anaesth*. 2016;63(4):397-410.
68. Senel S, Cobankara V, Taskoylu O, Guclu A, Evrengul H, Kaya MG. Effect of infliximab treatment on QT intervals in patients with ankylosing spondylitis. *J Investig Med*. 2011;59(8):1273-1275.
69. Lazzerini PE, Acampa M, Capecchi PL, et al. Antiarrhythmic potential of anticytokine therapy in rheumatoid arthritis: tocilizumab reduces corrected QT interval by controlling systemic inflammation. *Arthritis Care Res (Hoboken)*. 2015;67(3):332-339.
70. Kobayashi H, Kobayashi Y, Yokoe I, et al. Heart Rate-corrected QT Interval Duration in Rheumatoid Arthritis and Its Reduction with Treatment with the Interleukin 6 Inhibitor Tocilizumab. *J Rheumatol*. 2018.
71. Empana JP, Jouven X, Canoui-Poitrine F, et al. C-reactive protein, interleukin 6, fibrinogen and risk of sudden death in European middle-aged men: the PRIME study. *Arterioscler Thromb Vasc Biol*. 2010;30(10):2047-2052.
72. Hussein AA, Gottdiener JS, Bartz TM, et al. Inflammation and sudden cardiac death in a community-based population of older adults: the Cardiovascular Health Study. *Heart Rhythm*. 2013;10(10):1425-1432.
73. Silva Marques J, Veiga A, Nóbrega J, Correia MJ, de Sousa J. Electrical storm induced by H1N1 A influenza infection. *Europace*. 2010;12(2):294-295.
74. Amin AS, Herfst LJ, Delisle BP, et al. Fever-induced QTc prolongation and ventricular arrhythmias in individuals with type 2 congenital long QT syndrome. *J Clin Invest*. 2008;118(7):2552-2561.
75. Rizzo S, Basso C, Troost D, et al. T-cell-mediated inflammatory activity in the stellate ganglia of patients with ion-channel disease and severe ventricular arrhythmias. *Circ Arrhythm Electrophysiol*. 2014;7(2):224-229.
76. Streitner F, Kuschyk J, Veltmann C, et al. Prospective study of interleukin-6 and the risk of malignant ventricular tachyarrhythmia in ICD-recipients--a pilot study. *Cytokine*. 2007;40(1):30-34.
77. Wu KC, Gerstenblith G, Guallar E, et al. Combined cardiac magnetic resonance imaging and C-reactive protein levels identify a cohort at low risk for defibrillator firings and death. *Circ Cardiovasc Imaging*. 2012;5(2):178-186.
78. Ajjola OA, Hoover DB, Simerly TM, et al. Inflammation, oxidative stress, and glial cell activation characterize stellate ganglia from humans with electrical storm. *JCI Insight*. 2017;2(18).

Figure 1. Arrhythmogenicity of autoimmune and inflammatory K⁺-channelopathies: from the molecules to the clinics.

Anti-K⁺-channel autoantibodies (i.e. anti-Ro/SSA, anti-Kv7.1, anti-Kv1.4) and inflammatory cytokines (i.e. TNF α , IL-6, IL-1) directly affect cardiac K⁺-channel function, resulting in a modulation of K⁺-currents (I_{to} , I_{Kur} , I_{Kr} , I_{Ks}) and action potential duration (APD) changes. Such electrophysiological effects can be reflected on the surface electrocardiogram (ECG) by the development of long-QT-syndrome (LQTS) or short-QT-syndrome (SQTS) and related life-threatening ventricular arrhythmias/sudden cardiac death (SCD).

Table 1. Autoimmune and inflammatory potassium channelopathies: molecular and electrophysiological basis

<i>Potassium current</i>	<i>Autoantibody/cytokine</i>	<i>Ion channel</i>	<i>Functional effect</i>	<i>Molecular mechanisms</i>	<i>Effect on APD/QT interval</i>
I_{Kr}	anti-hERG- K^+ -channel antibodies (anti-Ro/SSA)	$K_v11.1/hERG$	inhibition (I_{Kr} decrease)	direct autoantibody binding to hERG pore region (extracellular loop between S5-S6)	prolongation
	$TNF\alpha$	$K_v11.1/hERG$	inhibition (I_{Kr} decrease)	functional impairment of hERG, mediated by TNF-receptor I engagement and ROS production	prolongation
	IL-6	$K_v11.1/hERG$	inhibition (I_{Kr} decrease)	reduced expression and function of hERG, mediated by IL-6-receptor engagement and Janus-kinase pathway activation	prolongation
I_{Ks}	anti- $K_v7.1-K^+$ -channel antibodies	$K_v7.1$	agonist-like (I_{Ks} increase)	direct autoantibody binding to $K_v7.1$ pore region (extracellular loop between S5-S6)	shortening
	$TNF\alpha$	$K_v7.1$	inhibition (I_{Ks} decrease)	inhibiting effect mediated by sphingosine-1-phosphate generation and cyclic-AMP decrease	NA
I_{to}	$TNF\alpha$	$K_v4.2/ K_v4.3$	inhibition ($I_{to,f}$ decrease)	reduced $K_v4.2/K_v4.3$ expression and function mediated by iNOS, with ROS generation and KChIP-2 inhibition	prolongation
	IL-1	NA	inhibition (I_{to} decrease)	NA	prolongation
	anti- $K_v1.4-K^+$ -channel antibodies	$K_v1.4$	Inhibition ($I_{to,s}$ decrease)*	NA	prolongation
I_{Kur}	$TNF\alpha$	$K_v1.5$	inhibition (I_{Kur} decrease)	NA	prolongation

$TNF\alpha$: tumor necrosis factor- α ; IL-1:interleukin-1; IL-6: interleukin-6; ROS: reactive oxygen species; hERG: human ether-a-go-go-related gene K^+ -channel; I_{to} : transient K^+ -outward current; I_{Kur} : ultra-rapidly activating component of the delayed-outward-rectifying current; I_{Kr} : rapidly activating component of the delayed-outward-rectifying current; I_{Ks} : slowly activating component of the delayed-outward-rectifying current; iNOS: inducible nitric oxide synthase; KChIP-2: K^+ -Channel-Interacting Protein; SHP/ERK: Src homology-2 domain-containing phosphatase/extracellular signal-regulated kinase; APD: action potential duration; NA: data not available.

*Proposed, as no direct evidence is currently available.

ACCEPTED MANUSCRIPT

