NOS1AP polymorphisms reduce NOS1 activity and interact with prolonged repolarization in arrhythmogenesis

Ronchi C.1*, Bernardi J.1, Mura M.2, Stefanello M.2, Badone B.1, Rocchetti M.1, Crotti L.3,4,5, Brink P.6, Schwartz PJ.3, Gnegchi M.2,7,8, Zaza A.1,9

*carlotta.ronchi@unimib.it
1Department of Biotechnology and Bioscience, University of Milano-Bicocca, Milano (IT);
2Coronary Care Unit and Laboratory of Experimental Cardiology for Cell and Molecular Therapy, Fondazione IRCCS Policlinico San Matteo, Pavia, (IT);
3Istituto Auxologico Italiano, IRCCS, Center for Cardiac Arrhythmias of Genetic Origin, Milan, Italy
4Department of Medicine and Surgery, University of Milano-Bicocca, Milano, (IT);
5Department of Cardiovascular, Neural and Metabolic Sciences, San Luca Hospital, IRCCS Istituto Auxologico Italiano, Milan, (IT);
6Department of Medicine, University of Stellenbosch, Tygerberg (SA);
7Department of Molecular Medicine, Unit of Cardiology, University of Pavia, Pavia, (IT)
8Department of Medicine, University of Cape Town, South Africa.
9Cardiovascular Research Institute (CARIM), Maastricht University (NL).

Keywords (maximum 8): arrhythmogenesis; NOS1 inhibition; LQT1; Sarcoplasmic Reticulum instability

NOS1AP SNPs correlate with QT prolongation and cardiac sudden death in patients affected by long QT syndrome type 1 (LQT1). NOS1AP targets NOS1 to intracellular effectors. We hypothesize that NOS1AP SNPs cause NOS1 dysfunction and this may converge with prolonged action potential duration (APD) to facilitate arrhythmias.

Aims: To test 1) the effects of NOS1 inhibition and their interaction with prolonged APD in a guinea pig cardiomyocyte (GP-CMs) LQT1 model; 2) whether pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) from LQT1 patients differing for NOS1AP variants and mutation penetrance display a phenotype compatible with NOS1 deficiency.

Methods and Results: In GP-CMs NOS1 was inhibited by SMTC (or L-VNIO); LQT1 was mimicked by I\textsubscript{ks} blockade (JNJ203) and \beta-adrenergic stimulation (isoproterenol). hiPSC-CMs were obtained from symptomatic (S) and asymptomatic (AS) KCNQ1-A341V carriers, harboring the minor and major alleles of NOS1AP SNPs (rs16847548 and rs4657139) respectively. In GP-CMs: NOS1 inhibition prolonged APD, enhanced I\textsubscript{CaL} and I\textsubscript{NaL}, slowed Ca\textsuperscript{2+} decay and induced delayed afterdepolarizations. Under action-potential clamp, switching to shorter APD suppressed “transient inward current” events induced by NOS1 inhibition and reduced cytosolic Ca\textsuperscript{2+}. In S (vs AS) hiPSC-CMs: APD was longer and I\textsubscript{CaL} larger; NOS1AP and NOS1 expression and colocalization were decreased.

Conclusions: the minor NOS1AP alleles are associated with NOS1 loss of function. The latter likely contributes to APD prolongation in LQT1 and converges with it to perturb Ca\textsuperscript{2+} handling. This establishes a mechanistic link between NOS1AP SNPs and aggravation of the arrhythmia phenotype in prolonged repolarization syndromes.