Reply to Christ et al.: LQT1 and JLNS phenotypes in hiPSC-derived cardiomyocytes are due to KCNQ1 mutations

In their letter on our recent publication on modeling Jervell and Lange-Nielsen syndrome (JLNS) using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) (1), Christ et al. question: (*i*) the role of the delayed rectifier potassium current (I_{Ks}) under baseline conditions in the absence of sympathetic stimulation, (*ii*) the rapid activation kinetics of I_{Ks}, and (*iii*) the causality of mutant *KCNQ1* in the disease phenotypes we observed in hiPSC-CMs (2).

Concerning the role of I_{Ks} under baseline conditions, we would like to point out that this is more controversial than Christ et al. (2) suggest. The conclusion that I_{Ks} plays no role in repolarization under resting conditions has been challenged using an I_{Ks} inhibitor, JNJ303, with improved potency. Towart et al. found that JNJ303 consistently evoked Torsade de Pointes arrhythmia in (anesthetized) dogs (3). Similarly, JLNS patients, including the individual in our study, show markedly prolonged QT intervals under resting conditions, and this is indeed a defining diagnostic feature of the disease (1). This is clear evidence that I_{Ks} is important even in the absence of sympathetic stimulation, and the observed action potential prolongation in our JLNS hiPSC-CMs, as well as in independent studies, confirm this (1, 4). Given the known immaturity of hiPSC-CMs, it is conceivable that this phenotype was more pronounced than in primary cardiomyocytes in vivo.

The activation kinetics of JNJ303-sensitive currents in our hiPSC-CMs were relatively fast. However, KVLQT1 kinetics may be greatly influenced by temperature, the cellular context/species under investigation, as well as the KVLQT1/KCNE1 subunit stochiometry. For example, I_{Ks} is activated rapidly and tends to show low temperature-dependence when expressed heterologously in CHO cells but is significantly more temperature-dependent in other systems, including cardiomyocytes. Furthermore, we cannot rule out that KCNE1 levels may be rate limiting in hiPSC-CMs, although we did demonstrate KCNE1 expression in our cell lines (1, 5). We do not therefore agree that I_{Ks} activation kinetics are slow in general. Rather, our data illustrate that the situation may be more complex.

Finally, concerning the question of whether *KCNQ1* mutations actually cause the observed phenotypes, we wish to point out that in both of our JLNS models, we used genetic engineering to create isogenic pairs of hiPSC lines that only differed in the mutation. This process allowed us to attribute any phenotypic differences to the underlying gene defects in *KCNQ1* (1). We believe that this demonstrates unequivocally the causative role of mutant KVLQT1 in the electrical changes measured.

We hope that the discussion above resolves any apparent discrepancies it was felt that our data raised. Christ et al. do, however, challenge the validity of hiPSC-CMs as models of human cardiac health and disease in general (2), and we certainly agree that further investigation is necessary to establish conditions under which hiPSC-CMs have added value.

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1 Zhang M, et al. (2014) Recessive cardiac phenotypes in induced pluripotent stem cell models of Jervell and Lange-Nielsen syndrome: Disease mechanisms and pharmacological rescue. *Proc Natl Acad Sci USA* 111(50):E5383–E5392.

2 Christ T, Horvath A, Eschenhagen T (2015) LQT1-phenotypes in hiPSC: Are we measuring the right thing? *Proc Natl Acad Sci USA* 112:E1968.

3 Towart R, et al. (2009) Blockade of the I(Ks) potassium channel: An overlooked cardiovascular liability in drug safety screening? *J Pharmacol Toxicol Methods* 60(1):1–10.

4 Moretti A, et al. (2010) Patient-specific induced pluripotent stem-cell models for long-QT syndrome. *N Engl J Med* 363(15):1397–1409.

5 Wang K, et al. (2011) Biophysical properties of slow potassium channels in human embryonic stem cell derived cardiomyocytes implicate subunit stoichiometry. *J Physiol* 589(Pt 24):6093–6104.

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The authors declare no conflict of interest.

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