A Knockout May Not Always Be a Knockout

To the Editor:

In a recent issue of Circulation, Hoornjte et al.1 presented data on a family with a duplication in exon 4 of HERG. A consanguineous marriage resulted in 2 homozygous offspring: one died before birth and the other had a severe cardiac phenotype characterized by arrhythmias and conduction defects. The importance of these findings regarding the role of HERG in the heart is clear.

The authors conclude that these homozygous individuals will lack functional Ik, and that HERG likely plays no critical roles in other organs. Various laboratories have reported the existence of alternate HERG isoforms.2,3 One isoform, HERGb, begins at an alternate transcription start site between exons 5 and 6, is expressed in the heart, and encodes channels with deactivation kinetics more rapid than those of the longer HERGa isoform. A duplication in exon 4 of HERG would probably not affect HERGb, and homozygotes would probably have an isoform-specific “knockout” of HERGa. In addition, HERG expression could be upregulated in response to the absence of HERGa, and the mutation could lead to altered splicing and some functional HERGa protein.

When a gene is knocked out in an animal model, investigators design the targeting construct to abolish protein expression and/or function. In channels, a transmembrane domain is usually disrupted. The absence of RNA, protein, and protein activity is then confirmed. This is often not possible in humans homozygous for a cardiac mutation because cardiac tissue and cells are rarely available. Given these difficulties, the conclusions from patients with homozygous HERG mutations are necessarily limited, especially regarding the potential role of HERG in other organs. In addition, the term “knockout” should be restricted to animals models in which the absence of channel function is directly proven.

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Response

We appreciate Dr. London’s comments and, although we carefully formulated our phrases (“putatively leading to ‘a human HERG knockout’” and “suggesting a limited role of HERG in other human organs”),1 we agree that defining our patients as human-isoform–specific HERGa-knockouts would have been more accurate. HERG-associated long-QT syndrome is caused by different pathophysiological mechanisms,2 including mutation-based accelerated deactivation kinetics.3 Acceleration of the deactivation time course, which is also observed for HERGb currents,4 can reduce outward current flow on repolarization and potentially prolong the action potential.3 However, HERGb also displays enhanced activation kinetics,4 which should increase outward currents and abbreviate the action potential. It is hard to predict what the result of these counterbalancing forces will be, but from the severe cardiac phenotype of our patients, it can be concluded that the eventual presence of HERGb or some functional HERGa protein produced by altered splicing in no way replaces absent HERGa. Indeed, we could not tell whether the phenotype we observed was the result of an exclusive expression of HERGb or a total absence of HERG.

HERGb is a relatively heart-specific isoform.3 Therefore, it seems unlikely that HERGb can replace HERGa in other organs. On the basis of the unremarkable development of the currently 2-year-old patient, our statement that HERG might have a limited role in other human organs still seems valid.

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