Inheritable Long QT Syndrome

Authors: Michael Sanguinetti PhD, Mark Keating MD , and Katherine Timothy

In the 1990's, the Division of Cardiology at the University of Utah was an epicenter for research into the molecular basis of cardiac arrhythmia. The initial breakthrough was discovery of the genetic basis of inherited long QT syndrome: mutations in cardiac ion channel genes involved in repolarization of ventricular action potentials. Analysis of heterologously expressed wild-type and mutant channels revealed the underlying electrical dysfunction of inherited LQT and the structural basis of drug-induced QT prolongation. The following is a short history of these discoveries.

Inheritable long QT syndrome (LQT) is a cardiac disorder characterized by a prolonged QT interval measured on the body surface ECG and an increased risk of torsades de pointes, a ventricular arrhythmia that can degenerate into ventricular fibrillation and cause sudden cardiac death. A likely genetic basis for this disorder was first recognized by Jervell and Lange-Nielsen in 1957 (autosomal recessive) and Romano (autosomal dominant) in 1963, but its molecular basis was unknown. In 1985, Michael Vincent, a cardiologist at LDS hospital and founder of the Sudden Arrhythmia Death Syndromes (SADS) Foundation, was joined by Katherine Timothy and together they embarked on a quest to phenotypically describe a large Utah Mormon family, several members of whom had been diagnosed with LQT. After Timothy expanded the genealogy of the family to several thousand individuals and 7 generations, they met with Raymond White, then a Professor of Genetics at the University of Utah and a world-renown human molecular geneticist. They agreed to collaborate and soon thereafter Mark Keating moved to the University of Utah (in 1989) to start a faculty position in the Cardiology Division. Keating had just completed postdoctoral training in biochemistry at UC San Francisco and was drawn to Utah because of its growing reputation in cutting-edge approaches to study the genetic basis of human disease. During the late 1980's to 2000, the University of Utah was uniquely productive in the field of genetics due to the pioneering work of Mario Cappechi's lab in creating knockout mice and the availability of large, cooperative Mormon families that facilitated discovery of the genetic basis of several human diseases. After learning the ropes of molecular genetics from Mark Leppert (Human Genetics, Univ of Utah), Keating took over the LQT genetics project and together with Timothy and one technician (Don Atkinson) isolated DNA from transformed lymphocytes donated by family members of the large Utah family and initiated genomic screening using radioactive probes and Southern blots. In late Fall of 1991, after using 248 of the 250 probes available, linkage analysis succeeded in mapping the first known LQT locus to chromosome 11p15 with a LOD score ($\log_{10}$ of the odds ratio) of 16.4 (1). Since this was the first locus associated with the disorder, this type of LQT was later named "LQT1".

G. Michael Vincent, Mark T. Keating, Katherine Timothy

In 1993, Mike Sanguinetti was recruited to the University of Utah and the Cardiology Division by William Barry. Prior to this move, Sanguinetti was a research scientist at Merck Research Labs in Pennsylvania and was the cellular electrophysiologist tasked with discovery of novel antiarrhythmic agents. Soon after their initial meeting, Keating and Sanguinetti envisioned a soon to be fruitful collaboration on the molecular basis of inherited arrhythmias. As members of the HMBG (Human Molecular Biology and Genetics) program, then headed by Steve Prescott, Keating and Sanguinetti had laboratories located in the EIHG (Eccles Institute of Human Genetics) building. Most of their discussions took place while on almost daily runs or mountain biking adventures in the foothills that overlooked the EIHG.

Continued chromosomal mapping efforts soon made it clear that LQT was a heterogeneous disorder and the Keating lab continued investigations into the genetic basis of other cardiovascular diseases, including the discovery that chromosomal deletions that disrupt the elastin gene causes Williams syndrome and supravalvular aortic stenosis (2, 3). In 1995, in efforts led by Mark Curran, a graduate student and Qing Wang, a postdoctoral fellow in Keating's group discovered the genetic basis of LQT2 and LQT3, respectively. The causative gene for LQT2 was discovered by a candidate gene approach (4) soon after it was reported that a K^+ channel called *HERG* ("human *ether-a-go-go-related gene*", since renamed *KCNH2*) was on human chromosome 7q35-36, the location Keating et al had mapped for LQT2. In 1995 the Keating lab also reported that mutations in the cardiac sodium channel gene *SCN5A* was the cause of LQT3 (5). Although the locus for LQT1 was initially mapped in 1991, it took another 5 years before heroic positional cloning efforts led to the discovery of the genetic basis of LQT1 – mutations in a novel K^+ channel gene, *KVLQT1* (6), since renamed *KCNQ1*. Thus, in just two years, the genetic basis of the three most common forms of LQT was defined. With the cloned channels in hand, the next step was to heterologously express the wild-type and LQT-associated mutant forms of these human ion channels in *Xenopus* frog oocytes and characterize their normal and dysfunctional electrical properties using single cell voltage clamp techniques. These experiments were performed by the Sanguinetti lab and it was soon discovered that *HERG* encoded the I_{Kr} channel (7) and that *KVLQT1* encoded a novel K^+ channel that when coexpressed with a □-subunit called minK (*KCNE1* gene) produced heteromultimeric channels with properties that matched I_{Ks} (8) previously characterized in mammalian cardiomyocytes. As described below, the finding that the *HERG* gene encoded the channels that conduct I_{Kr} provided a mechanistic link between inherited and a common cause of acquired (drug-induced) LQT. A year later (1997) Splawski and Keating discovered that homozygous mutations in *KVLQT1* was the cause of autosomal recessive Jervell and Lange-Nielsen syndrome (9), characterized by congenital sensory deafness and an excessively prolonged QT interval, and that heterozygous mutations in KCNE1 caused a rare form of LQT (10), now referred to as LQT5. Functional analysis of LQT-associated mutations in *KCNE1* (10), *HERG* (11) and *KVLQT1* (12) by Martin Tristani-Firouzi (Pediatric Cardiology) and several postdocs including Jun Chen and Guiscard Seebohm in the Sanguinetti lab were found to cause loss of channel function by a variety of molecular mechanisms, including decreased current magnitude, altered properties of channel activation, deactivation and inactivation gating. Not unexpectedly, compound mutations in ion channel genes was found to cause more severe QT prolongation and increased risk of arrhythmia (13).

Keating lab in EIHG building, 1997

While Keating was investigating the genetic basis of LQT1 in Salt Lake City, Mike Sanguinetti was a research scientist at Merck Research Labs in Pennsylvania investigating the cellular mechanisms of action of novel "class III agents" that suppressed arrhythmias in animal models by prolonging ventricular repolarization. In 1990, Sanguinetti reported that experimental class III methanesulfonanilides (e.g., E4031, MK-499) specifically blocked a component of repolarizing delayed rectifier K^+ current ("I_K") in isolated cardiomyocytes, leaving another component untouched. He named the drug sensitive current I_{Kr} ("r" for rapid activating) and the E4031-insensitive current I_{Ks} ("s" for slowly activating) (14). Soon thereafter, Sanguinetti and his colleagues at Merck discovered highly potent and specific I_{Ks} blockers (modified benzodiazepines). Ironically, the U.S. patent for these I_{Ks} inhibitors was issued in 1995, a year before it was proven that decreased I_{Ks} caused LQT1. Needless to say, the findings that loss of function mutations in *HERG* (I_{Kr}) and *KVLQT1/KCNE1* (I_{Ks}) were the cause of the most commonly inherited arrhythmia significantly dampened enthusiasm for further development of class III anti-arrhythmic agents. Noncardiac medications that block I_{Kr} and prolong the QT interval as an unintended side effect are structurally diverse and span a wide spectrum of therapeutic drug classes, including psychiatric, antimicrobial and antihistaminic compounds. Moreover, unintended inhibition of I_{Kr} is by far the most common mechanism underlying drug-induced arrhythmia. Consequently, the screening of compounds for undesired block of hERG channels heterologously expressed in mammalian cell lines became a routine practice. The experimental protocols we developed to describe the biophysical properties of hERG channel current were adopted by the pharmaceutical industry and form the basis of one of the most widely used safety screening assays in the drug development process. To facilitate rapid in silico screening of the propensity for compounds to inhibit I_{Kr}, new insights into the molecular basis of drug binding to hERG channels was needed. Towards this goal, John Mitcheson and David Fernandez, postdocs in the Sanguinetti lab used site-directed mutagenesis and functional analysis of mutant channels to identify the key amino acid residues and their specific physiochemical properties that constitute the common binding site for drugs that block hERG (15, 16). A series of subsequent publications described the intimate details of the structural basis of drug binding and some unique features of hERG channel gating.

Mark Keating, Igor Splawski, Mike Sanguinetti, 1997

The most severe form of LQT is a very rare multi-organ disorder that includes excessive QT prolongation, lethal arrhythmias, webbing of fingers and toes, immune deficiency, intermittent hypoglycemia and autism. Katherine Timothy had identified several children with this syndrome and Igor Splawski in the Keating lab worked tirelessly for several years before discovering, after the group had moved to Harvard, the disease was caused by a single point mutation in the cardiac calcium channel gene *CACNA1A* (17, 18). The disorder was named Timothy syndrome to honor the invaluable contribution of Katherine Timothy in identification and phenotypic characterization of affected patients. Functional analysis of mutant channels by Niels Decher, a postdoc in Sanguinetti's lab revealed that both of the identified mutations slowed the rate of Ca current inactivation, a gain of channel function that slows repolarization and prolongs action potential duration. Over the last 15 years of continued genetic testing, Katherine has personally communicated with the parents of most of these children, and continues to include them in her ongoing studies. Timothy co-founded the Timothy Syndrome Alliance and continues to work with investigators to better understand the disorder and to improve treatment and care of affected children.

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