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Short Communication

# Mouse as an Animal Model of Long QT Syndrome: A Reliable and Plausible Model?

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## Abstract

Mouse is widely used as an animal model in experimental research. Pharmacological trials and induction of human diseases are usually carried out in this animal. This animal model is also commonly used in cardiovascular research. Cost of using this model is relatively low [1] and it is easy to be modified genetically. Mouse can be used in *in-vivo, ex-vivo* and *in-vitro* studies. Those studies have their own characteristics and benefits, for example genetic modification such as Recombinase-mediated Cassette Exchange (RMCE) can be done in purpose of acquiring desired mutation. This method can be done *in-vivo* by removing one of the specific genes in the DNA of the mouse and replaces it with other exogenous genes [2]. Nowadays, induced mouse Pluripotent Stem Cells (iPSCs) become favourable *in-vitro* method to create 'unlimited' sample tissues, including cardiomyocytes [3,4] Functional heart diseases such as Long QT Syndrome can be induced in animal models by modification in SCN5A gene using one of those techniques [3,5,6]. Although mouse is advantageous for investigating structural heart diseases, [7] its role for investigating functional heart diseases is questioned because it has much faster heartbeat and different heart conduction mechanisms [8].

**Keywords:** Long QT Syndrome; Animal model; Arrhythmia; Cardiovascular

## Introduction & History of the Model

Long QT syndrome (LQTS) is a rare cardiac disease, which affects 1 in 2500 people and causes thousands of sudden death annually [9,10]. It is characterized by QT interval prolongation, which is prone to manifestation of ventricular arrhythmias.9 It also affects the electrical activity in the heart and causes delayed cardiac repolarization. Therefore, there are prolongations of Action Potential Duration (APD) and early after depolarization (EAD) [3,11]. The commonest effect of this disease is occurrence of polymorphic VT or Torsade de Pointes (TdP) [3,9,11] LQTS can be divided into several types, based on ion channels that are involved [3,12,13] Ion channels that are involved in LQTS can be seen in Table 1. Being female is one of renowned risk factors in occurrence of TdP in both congenital and acquired types [14].

From last few decades, genomic investigation has been one of the keys in understanding LQTS. Numerous mouse models have been created in purpose of studying pathophysiology of this disease. There are several types of arrhythmias that can be induced in mice. Of those are 2:1 AV block, sinus bradycardia, and ventricular tachycardia (both monomorphic and polymorphic). This animal model has also been a beneficial tool in investigating the role of ion channels in cardiac repolarization [15,8].

'Knock-out' mouse, one of the best accomplishments in genetic research, could be generated as an animal model for LQTS few years ago [1,9,16]. This 'knock-out' method was done through deletion of KPQ site in SCN5A gene. In human, similar mutation at the similar gene causes LQTS type 3, which may cause arrhythmia with low heart rate pattern.8 nowadays, more than 20 different types of mice with altered expression of potassium channels have been generated. Over expression of dominant-negative mutation and deletion of KCNQ1 have been done in mouse to mimic human LQTS type 1. Recently, some KCNQ1 'knock-out' mice have been generated to induce QT-interval prolongation, which leads to occurrence of spontaneous arrhythmia. Human LQTS type 2 can be induced by KCNH2 gene disruption in 'knock-out' mouse and this KCNH2 mutation is embryonic lethal in H/H form. Mouse model of LQTS4 has also been generated by genetic modification, thus give a better insight of novel arrhythmogenesis mechanism [8].

Based on the roles of Ito and IKs in mouse cardiac repolarization, several transgenic mouse models have been generated (Table 2). The aims of producing these models are to disrupt ion channel currents and observe whether disruption of specific ion channels will cause certain types of LQTS [8]. One of the latest technique is named Recombinase-mediated Cassette Exchange (RMCE), whereby specific genes can be put on specific locations without destructing other genes (Figure1) [2,17,18]. This method is commonly applied in mouse because mouse as an animal model brings some benefits, such as relatively low cost1, high accessibility to the genomic information, easy to manipulate in the laboratory, short breeding period (approximately three months per cycle) [19] and flexible ethical clearance. Nowadays, transgenic mouse

| Variant | Gene | Chromosome | Function |
|---|---|---|---|
| LQT1 | *KCNQ1* | 11p15.5 | $I_{Ks}$ alpha subunit |
| LQT2 | *KCNH2* | 7q35-35 | $I_{Kr}$ alpha subunit |
| LQT3 | *SCN5A* | 3p21-23 | $I_{Na}$ alpha subunit |
| LQT4 | *ANK2* | 4q25-2 | Targeting protein |
| LQT5 | *KCNE1* | 21p22.1-22-2 | $I_{Ks}$ beta subunit |
| LQT6 | *KCNE2* | 21p22.1-22-2 | $I_{Kr}$ beta subunit |
| LQT7 | *KCNJ2* | 17p23.1-24.2 | $I_{K1}$ |
| LQT8 | *CACNA1C* | 12p13.3 | $I_{Ca}$ alpha subunit |
| JLN1 | *KCNQ1* | 11p15.5 | $I_{Ks}$ alpha subunit |
| JLN2 | *KCNE1* | 21p22.1-22-2 | $I_{Kr}$ beta subunit |

**Table 1:** Several types of Long QT Syndrome in humans. Long QT Syndromes are mostly caused by mutation in potassium ion channel regulating genes. The exception is LQTS type-3, which is caused by mutation of cardiac sodium channel in SCN5A gene 5, 15 and LQTS type-8 which is caused by mutation in calcium channel regulating genes. (adapted from: http://cardiocompass.cardiosource.org/)

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| Locus | Gene | Chromosome | Protein | Mouse models | Current | ↑APD? | ↑QTc? | Arrhythmias? | Reference |
|---|---|---|---|---|---|---|---|---|---|
| LQT1 | *KCNQ1* | 11p15.5 | KvLQT1 | KO: KvLQT1$^{-/-}$ | $\downarrow I_{Ks}$ | No | No | n.d. | Lee *et al.* (2000) |
| | | | | KO: KvLQT1$^{-/-}$ | $\downarrow I_{Ks}$ | Yes | Yes | n.d. | Casimiro *et al.* (2001) |
| | | | | TG: KvLQT1DN | $\downarrow I_{Ks}$ | Yes | Yes | Brady: Moblitz I | Demolombe *et al.* (2001) |
| LQT2 | *KCNH2* | 7q35–36 | HERG1 | KO: Merg1$^{+/-}$ | $\downarrow I_{Kr}$ | Yes | Yes | Tachy: I | London *et al.* (1998*b*) |
| | | | | KO: Merg1b$^{-/-}$ | $\Delta I_{Kr}$ | n.d. | No | Brady: Sinus | Lees-Miller *et al.* (2003) |
| | | | | TG: HERGDN | $\downarrow I_{Kr}$ | Yes | No | n.d. | Babij *et al.* (1998) |
| LQT3 | *SCN5A* | 3p21 | $Na_v1.5$ | KI: Scn5A$\Delta$KPQ$^{+/-}$ | $\uparrow I_{Na}$ | Yes | Yes | Tachy: S, I | Nuyens *et al.* (2001) |
| LQT4 | *ANK-B* | 4q25–27 | Ankyrin-B | KO: AnkB$^{-/-}$ | $\uparrow I_{Na}$ | Yes | Rate-related | Brady | Chauhan *et al.* (2000) |
| LQT5 | *KCNE1* | 21q21–22 | MinK/IsK | KO: minK$^{-/-}$ | $\downarrow I_{Ks}$ | n.d. | Rate related | n.d. | Drici *et al.* (1998) |
| | | | | KI: LacZ$^{+/+}$ | $\downarrow I_{Ks}$ | No | No | n.d. | Kupershmidt *et al.* (1999) |
| | | | | TG: KvLQT1-minK | $\uparrow I_{Ks}$ | No | No | n.d. | Marx *et al.* (2002) |
| LQT6 | *KCNE2* | 21q21–22 | MiRP1 | n.d. | | | | | |
| LQT7 | *KCNJ2* | 17q23 | $K_{ir}2.1$ | KO: $K_{ir}2.1^{-/-}$ | $\downarrow I_{K1}$ | Yes | Yes | Brady: Sinus | Zaritsky *et al.* (2001) |
| LQT8 | *CACNA1* | 12p13.3 | $Ca_v1.2$ | n.d. | | | | | |

**Table 2:** Mouse Model of Human LQTS. Until now, there are 8 types of human Long QT Syndromes (LQTS) that have been discovered. However, there are only 6 of them can be genetically modified using mouse model [9].

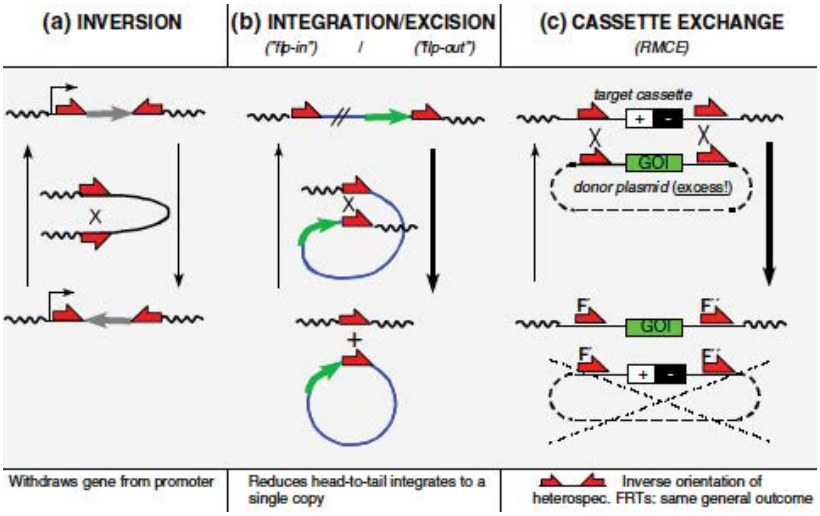


**Figure 1:** Basic Technique of 'Recombinase-mediated Cassette Exchange'. There are four main steps in recombination such as inversion, integration and excision, also cassette exchange. Then two-targeted sites are combined using recombinase [17].

has also been used in another approach. It is used to produce induced Pluripotent Stem Cells (iPSCs) with specific mutation then develop those cells *in-vitro* to get desired type of cells or tissues. Identical with embryonic stem cells, mouse iPSCs have a capability to differentiate into all types of cell [4]. This method is chosen to be used in *in-vitro* study because of some reasons, such as high safety, easy availability and 'unlimited' cell sources. Now, this method is used in many different studies for cell therapy, drug discovery and disease modeling [20].

## Characteristic of Mouse as an Animal Model of LQTS

Mouse (Mus musculus) has been widely used for more than 95% of animal experiments, the results of which are to be extrapolated in human. Mouse has a short reproductive/gestation cycle [21], large litters and its breeding cost is relatively cheap [22]. Mouse genome is widely discovered; therefore it is commonly used in genome manipulation experiments such as transgenesis and gene targeting [23]. Mouse embryonic stem cells have also been manipulated to produce large amount of cardiomyocytes with high success rates. Mouse heart morphology has some similarities with human heart. They have four chambers [7]. Both of them are equal with 0.5% body weight, have similar atrial and Mean Arterial Pressure (MAP), and similar size of capillaries. It also has similar cardiac ion channels and resting membrane potential with human [22].

Although ion channels in mouse and human are quite similar, the action potential and ion channels regulation mechanism are different [22]. Mouse has shorter action potential8 and faster heart beat ~ 10 times higher than human (approximately 350-600x/min) [22]. Unlike human, repolarization in mouse is dominated by transient outward current (Ito) [8]. ECG intervals such as PR, QRS, QT and RR in mouse are also shorter than human. There is no clear ST segment that can be distinguished as well because the T-wave merges with final part of the QRS-complex [22]. As the implication of having smaller heart size, ventricular arrhythmia pattern in mouse is transient. Overall, this model has been very useful in exploring ionic mechanisms that affect repolarization but it has not enough applicability in human disease state [8]. A head-to-head comparison between mouse and human cardiac morphology discovers several findings. They have different heart sizes, dimensions, cellular contents and cardiomyocyte metabolisms [22]. Moreover, they have different myosin heavy chain isoforms (human has V3 myosin heavy chain that causes slower resting heart and mouse has V1 myosin heavy chain that is associated with high cross-bridge turnover, ATPase and high resting heart rate) [24]. This myosin heavy chain participates in myoenergetic changes. Some differences should also be noticed in *ex-vivo* studies. In classical preparations, such as isolated ejecting hearts and cardiac papillary muscles, hearts from larger mammals show positive force-frequency. Inversely, mouse heart shows

negative force-frequency. It means that increasing stimulation frequency causes reduction of forces generated by cardiac muscle [22,25].

## Conclusion

Mouse is not a perfect model of LQTS. However, its contribution to the cardiovascular research should still be taken into account. The decision of choosing the right animal model in cardiovascular research, particularly LQTS, should be based on the aim of the research, with regards to the advantages and disadvantages of the animal model.

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