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Electrophysiological study of mutant Kv7.1 ion channel, responsible for LQT syndrome

Li B.[1], *Zhang H.*[2]

1 - Университет МГУ-ППИ в Шэньчжэне, Шэньчжэнь, Китай, *E-mail: 2120200033@smbu.edu.cn*;

2 - Университет МГУ-ППИ в Шэньчжэне, Шэньчжэнь, Китай, *E-mail: zhanghan@smbu.edu.cn*

Congenital long QT syndrome is a type of heart disease caused by genetic mutation. LQTS is an extended QT interval in the electrocardiogram (ECG) that can trigger torsade-de-pointes(Tdp) and even sudden cardiac arrest. In the United States, LQTS cause about 4,000 deaths per year (Tester, 2014), But because the disease lacks clinical manifestations and many carriers of the related mutation are asymptomatic, LQTS are easily overlooked. The QT interval of the ECG corresponds to ventricular depolarization and repolarization, which is the ventricular action potential interval. The decrease of slow delay rectified potassium current (Iks) caused by mutation of Kv7.1 volt-gated potassium channel which is coded by KCNQ1 and Mink gene can lead to LQTS. The KCNQ gene family encodes the voltage-gate potassium channel Kv7 family, which includes five Kv7 channel sub-units (Kv7.1-5) (Schwartz, 2009). We utilized Kv7.1 to investigate for probable pathways and focused on the most prevalent mutation. KCNQ1 encoded a channel protein with six transmembrane domains, short N-teminal and a C-terminal region. KCNQ1 carboxyl terminal domain bind with Yotiao protein which encoded by AKAP9 gene to regulate and mediate the PKA-dependent phosphorylation of Kv7.1 channel (Marx, 2002). Therefore, the mutation of amino acids at C-terminal of Kv7.1 channel which is the binding site of Yotiao protein will not only affect the structure and function of Kv7.1 channel, but also affect the response of the volt-gated potassium channel to the PKA signaling pathway. Therefore, it is very important to study the point mutation at the junction of KCNQ1 and Yotiao.

The mutation site we studied was c.1748 G>A. This site is the C-terminal of alpha-subunit in the Kv7.1 channel encoded by KCNQ1 gene. A mutation of the nucleotide at this location leads to p.583 R>H. We first demonstrated the influence of this mutation site on the channel by comparing KCNQ1-p.R583H and KCNQ1-WT. We used pcDNA3.1A as the plasmid and introduced the mutant gene sequence into the plasmid using Site-directed mutation (SDM) method. Then, HEK293(Human embryonic kidney) was selected as eukaryotic cells expressing mutated and normal ion channels. Calcium phosphate co-precipitation was used to transfect the plasmid containing point mutations and those with normal expression channels, while GFP was used as fluorescent label. Finally, the patch-clamp in whole-cell recording mode was used to detect the properties of the channels.

Our results show that the point mutation of c.1748 G>A does lead to decreased channel conductance and thus decreased IKs current, but the effect of this mutation is very small when only Kv7.1 channel is expressed. We hypothesized that the mutation site of p.R583H was located at the interaction site between C-terminal of kv7.1 channel and Yotiao protein. Therefore, the main effect of the mutation was not the function of KCNQ1 channel itself, but the interaction between Yotiao protein and KCNQ1 channel. Furthermore, the regulation of Kv7.1 channel by PKA signaling pathway is affected, and this mutation is also one of the factors that prevent LQT1 patients from vigorous exercise.

In order to test our hypothesis, our current and future studies are to co-transfect AKAP9(Yotiao) gene with KCNQ1 gene and measure and compare their electrophysiological properties. If the hypothesis is true, we not only explore the influence of a new mutation site, but also provide a new idea to explore the joint design of ion channel mutation and signaling pathway.

Keywords: long QT syndrome; IKs; KCNQ1; Kv channel; Yotiao.

References

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Illustrations

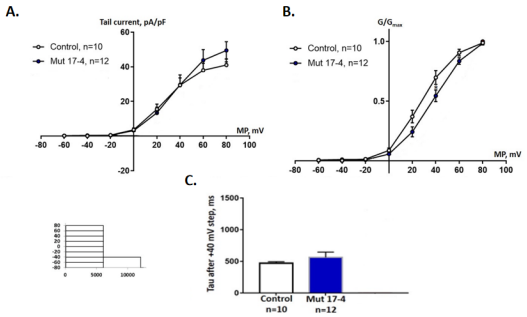

Рис. : Patch clamp result of 1748 G-A point mutation KCNQ1 channel.