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研究課題名（和文）Functional and pharmacological investigation on novel KCND3 variants identified in patients with early repolarization syndrome and refractory epilepsy

研究課題名（英文）Functional and pharmacological investigation on novel KCND3 variants identified in patients with early repolarization syndrome and refractory epilepsy

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交付決定額（研究期間全体）：（直接経費）　　2,200,000円

研究成果の概要（和文）：KCND3の変異体であるp.V401LとP.V401Mが同定され、KCND3によるこれらのItoチャネルの機能獲得が確認されました。これらのKCND3変異体によるItoチャネルは、キニジンやSSRIによって回復しました。したがって、これらの候補は、てんかんにおける心臓突然死を予防する治療選択肢と考えされます。

研究成果の学術的意義や社会的意義
De novo and heterozygous KCND3 variants, p.V401L and p.V401M were identified and the gain-of-functional changes of these Ito channels by a KCND3 variants were reversed by quinidine and SSRIs. Therefore, these candidates can be therapeutic options to prevent sudden cardiac death in epilepsy.

研究成果の概要（英文）： De novo and heterozygous KCND3 variants, p.V401L and p.V401M were identified in two young patients with refractory epilepsy. In the electrophysiological analysis, Chinese Hamster Ovary (CHO) cells expressing variant channels showed significant increase of current densities compared to those with WT. The activation curves of Ito with variants significantly shifted to leftward. In addition, significant slow inactivation time constants were observed. Related to the slow inactivation of variants, the recovery from inactivation in variants channels was significantly slow. We next conducted pharmacological investigation and examined the inhibitory effect of quinidine and Selective Serotonin Reuptake Inhibitors (SSRIs). Micromolar concentration of quinidine and SSRIs normalized the slow inactivation of variant channels in a concentration-dependent manner.
The variant carrying patients might have risk of sudden cardiac death in epilepsy, and quinidine and SSRIs can be therapeutic options.

研究分野：Cardiology

キーワード：KCND3　potassium channel　gain of function change　quinidine　SSRIs

１．研究開始当初の背景 Background and beginning of the research

Recent evidence suggests that acute and adaptive changes in heart rhythm in epilepsy implicate cardiac dysfunction as a potential pathogenic mechanism in sudden unexpected death in epilepsy (SUDEP), however, the underlying mechanism has not been fully understood yet. Therefore, the elucidation of its pathogenesis and the development of safe and effective therapy have been required. We have reported a gain-of-function (GOF) *KCND3* variant, p.G306A, which was identified in a young patient with early repolarization syndrome (ERS) and refractory epilepsy (RE). Kv4.3 encoded by *KCND3* is an α-subunit of the I_{to} channel and expressed in both brain and heart. Electrophysiological analysis of the variant revealed increased I_{to} and slower inactivation (Figure 1a). In the simulation study, the increased I_{to} produced the early repolarization in the electrocardiogram (ECG). Therefore, *KCND3*-G306A was confirmed as the cause of ERS. In addition, we evaluated the effect of quinidine and as shown in Figure 1b, quinidine suppressed the increased I_{to} current. After administration of quinidine to the patient, not only the diminish of ER in the ECG but also improvement of epilepsy was observed (Takayama K, et al. Heart Rhythm 2019). Recently, we identified two novel *KCND3* variants, p.V401L and p.V401M in 2-year-old female and 3-year-old male with refractory epilepsy (RE) without ER. These patients might have a risk of developing ER syndromes leading to SUDEP. However, the electrophysiological characteristics of these variants have not been analyzed yet, and the pharmacological effect to the variant I_{to} channel is unknown.

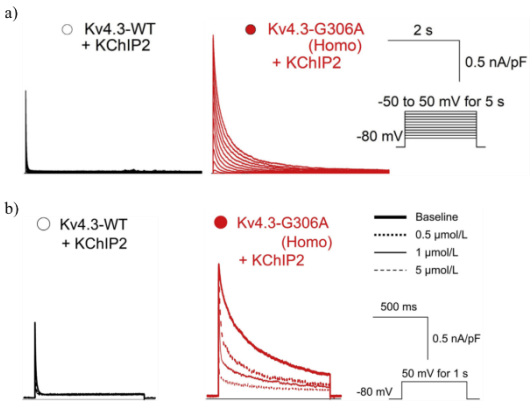

Figure 1 (a) Functinoal analysis of Kv4.3-G306A (b) effect of quinidine to the variant channel (Takayama et al. 2019)

２．研究の目的 Purpose of the study

The main purpose of this study is to investigate the electrophysiological characteristics of novel *KCND3* variants and evaluate the pharmacological effect of quinidine and other candidate therapeutics, Selective Serotonin Reuptake Inhibitors (SSRIs) to the variants.

３．研究の方法 Methods of research

Using cultured cells transfected with *KCND3* variants, we conducted the electrophysiological and pharmacological investigation.

- Mutagenesis: Full-length cDNA encoding the short isoform of human *KCND3* was subcloned into pIRES-GFP expression vector and full-length cDNA encoding WT *KChIP2* encoding K^+ channel interacting protein (β-subunit of the channel) was subcloned into the PCMV-IRS expression vector. The *KCND3* missense variants were introduced to the plasmid using a KOD FX Neo (TOYOBO) DNA polymerase.
- Transfection: WT or mutation introduced plasmids (*KCND3*-V401M or *KCND3*-V401L) were transiently transfected with *KChIP2* into Chinese hamster ovary (CHO) cells using Lipofectamine LXT (Invitrogen).
- Electrophysiological analysis: Using whole-cell patch-clamp method, I_{to} were recorded at $+37^0$C with an Axon instruments patch-clamp amplifier (Axon™ Digidata® 1550B). Obtained data were analyzed using IgorPro 8 (WaveMetrics) and SAS9.4 software (SAS). $p<0.05$ was determined by 1-way ANOVA followed by Dunnett post hoc analysis comparing with Kv4.3-WT
- Pharmacological evaluation of quinidine and SSRIs to the *KCND3* variants: After confirming the effect of quinidine to the variant I_{to} channels, which is expected to normalize the gain-of-function disorder, we applied several SSRIs and examined the effect whether these candidates suppress the increased I_{to}. Effects of SSRIs are analyzed using paired t-test comparing values after drug application to baseline values and $p<0.05$ considered significant.

４．研究成果 Research results

A novel *KCND3* variant, c.1201 G>C, p.V401L, was identified a 2-year-old girl with epileptic encephalopathy, global developmental delays, and chorea. The second novel variant, c.1201 G>A, p.V401M, was identified in a 3-year-old boy with a prominent and progressive ataxia, as well as chorea and dystonia and due to medically refractory epilepsy, he required multiple anti-seizure medications. Codon 401 is located in the intracellular side of segment 6 and the valine at 401 is highly conserved among species. In silico analysis, both variants were predicted as deleterious and pathogenic.

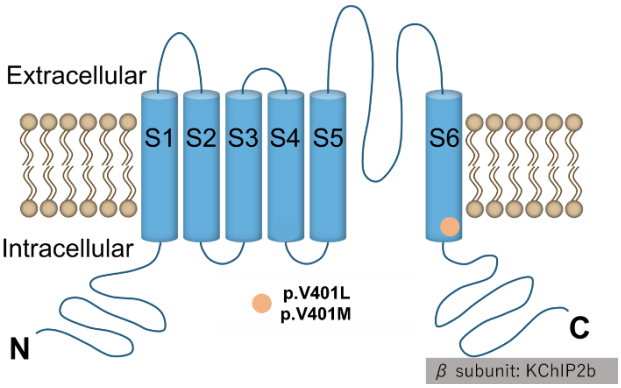

Figure 2 Topology of Kv4.3 encoded by *KCND3*

Electrophysiological characteristics

We investigated the functional effect of the novel *KCND3* variants on I_{to}. Figure 1 shows representative current traces, and CHO cells expressing variant channels showed significant increase in current densities. Activation and inactivation kinetics were assessed by double-step voltage protocol and activation curves of I_{to} with variants significantly shifted to leftward. The half-maximal activation and inactivation voltages obtained from Boltzmann equation were significantly different from that of WT. The half-maximal inactivation voltages of mutant channels were significantly different from

that of WT. Recovery from inactivation in variant channels both homozygous V401L and V401M was slower comparing to that of WT (Figure 3).

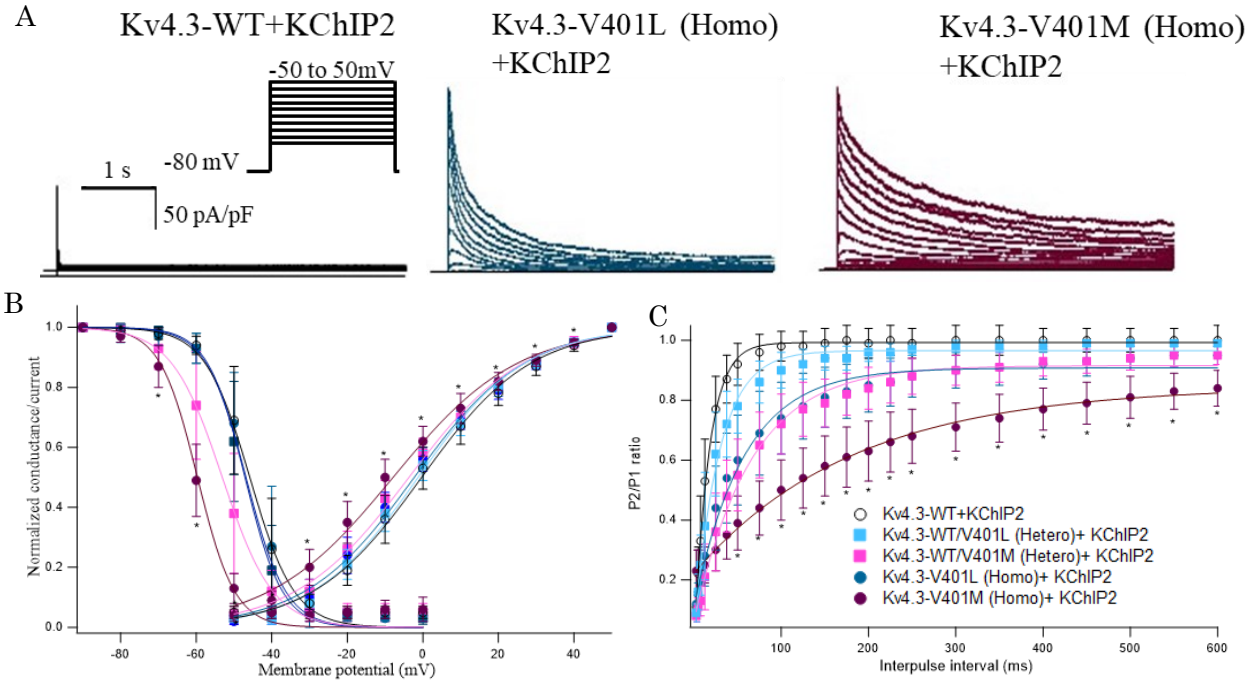

Figure 3 Functional analysis of Kv4.3 with KChIP2. A. Representative Kv4.3 currents with KChIP2. Inset shows the protocol. B. Normalized activation conductance and inactivation current, fitted to the Boltzmann equation. C. Recovery from inactivation.

Effect of quinidine on the Function of I_{to}

The inhibitory effect of quinidine on WT and variant channels at concentrations of 1 and 5 μmol/L was investigated. Figure 4 shows the representative current traces elicited by depolarizing pulses to +50 mV from a holding potential of -80 mV at baseline and after quinidine administration. Extracellular application of quinidine resulted in decrease of the current densities in concentration dependently. Furthermore, slower inactivation time constants and leftward shift of activation in variant channels were corrected by quinidine.

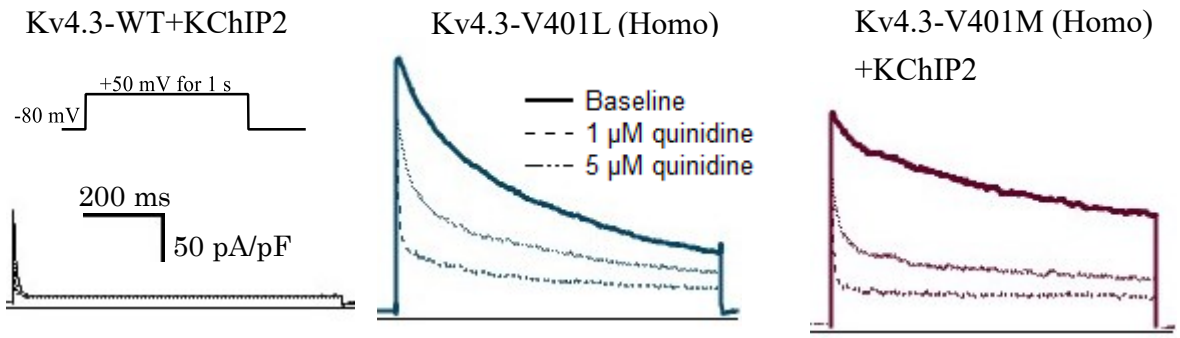

Figure 4 Effect of quinidine (1 and 5 μmol/l) on WT and variant Kv4.3 currents.

Effect of SSRIs on the Function of I_{to}

Besides quinidine, we searched for new candidate therapeutics and found that a Selective Serotonin Reuptake Inhibitor (SSRI) - fluoxetine had the blocking effect on wild type (WT) Kv4.3 (Jeong I *et al.* Brain Res. 2013). We applied fluoxetine to WT and mutant Kv4.3, and it suppressed the increased I_{to} with concentration dependently (Figure 5).

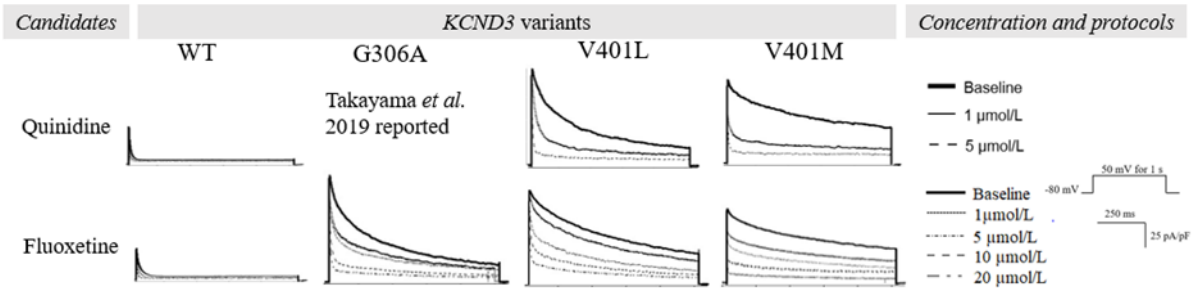

Figure 5. Effect of quinidine and fluoxetine to the variant channels (unpublished data)

Conclusion: The GOF effect of I_{to} channel by a *KCND3* variants (V401L and V401M) was reversed by quinidine and SSRI, fluoxetine. The variant carrying patients might have a risk of SUDEP, and our investigated therapeutics, quinidine and fluoxetine, can be a therapeutic option to prevent sudden cardiac death in epilepsy.

５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計2件（うち招待講演　0件／うち国際学会　1件）

１．発表者名 Byambajav Tserenlkham
２．発表標題 KCND3 Variants Identified in Young Refractory Epilepsy Patients Showed Similar Gain-of-Functional Change with those in patients with Early Repolarization Syndrome
３．学会等名 第87回日本循環器学会学術集会
４．発表年 2023年

１．発表者名 Byambajav Tserenlkham
２．発表標題 Gain-of-Function KCND3 Variants Identified in Young Patients with Refractory Epilepsy might be a Cause of Sudden Unexpected Death in Epilepsy
３．学会等名 Heart Rhythm 2023（国際学会）
４．発表年 2023年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関