

Gain-of-Function KCNH2 Mutations in Patients with Brugada Syndrome.

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発行年	2015-03-10
その他の言語のタイトル	Brugada症候群患者における機能獲得型KCNH2変異 Brugada ショウコウグン カンジャ ニ オケル キノウ カクトクガタ KCNH2 ヘンイ
URL	http://hdl.handle.net/10422/10238

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学 位 の 種 類	博 士 (医 学)
学 位 記 番 号	博 士 甲 第 7 3 2 号
学 位 授 与 の 要 件	学 位 規 則 第 4 条 第 1 項 該 当
学 位 授 与 年 月 日	平 成 2 7 年 3 月 1 0 日
学 位 論 文 題 目	Gain-of-Function KCNH2 Mutations in Patients with Brugada Syndrome. (Brugada 症 候 群 患 者 に お け る 機 能 獲 得 型 KCNH2 変 異)
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論文内容要旨

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学位論文題目	Gain-of-Function KCNH2 Mutations in Patients with Brugada Syndrome (Brugada 症候群患者における機能獲得型 KCNH2 変異)		
<p>[Background] Brugada syndrome (BrS) is a cardiac electrical disease characterized by right precordial ST-segment elevation on electrocardiograms (ECG) that predisposes patients to sudden cardiac death as a result of ventricular fibrillation (VF). In BrS patients, except for <i>SCN5A</i>, mutations in other responsible genes are poorly elucidated.</p> <p><i>KCNH2</i> gene encodes an α-subunit of the rapid component of the delayed rectifier K^+ channel (Kv11.1). The Kv11.1 channel carries I_{Kr}, which plays an important role in regulating the repolarization of the cardiac action potential. In 2005, two novel <i>KCNH2</i> mutations (G873S and N985S) were identified in patients with BrS who had no <i>SCN5A</i> mutation. Functional analyses of the two mutant channels and the computer simulation revealed that they caused gain-of-function of the Kv11.1 channel. In 2009, we reported a novel <i>KCNH2</i> mutation, R1135H, in a patient showing a short QT interval and Brugada ECG. However, it remains unknown how these <i>KCNH2</i> mutations are associated with the clinical features.</p> <p>[Objective] Our objectives were to identify novel <i>KCNH2</i> mutations in BrS patients and to investigate the relationship between the channel function and clinical phenotype. Clinical characteristics were compared between the patients with BrS or Brugada-like ECG patterns of different genotypes on <i>KCNH2</i>, <i>SCN5A</i> or <i>CACNA1C</i> mutations.</p> <p>[Methods] The study population consisted of 236 consecutive probands, who were diagnosed as BrS or with Brugada-like ECG. We screened genetic variants of BrS candidate genes, using Denature High Performance Liquid Chromatograph (dHPLC), high-resolution melting method and direct sequencing. The human wild-type <i>KCNH2</i> cDNA was subcloned into a pRC-CMV vector, and <i>KCNH2</i>-mutant plasmids were constructed using a site-directed mutagenesis. Chinese Hamster Ovary cells were co-transfected with 0.5 μg of GFP and 1 μg WT or respective mutant <i>KCNH2</i> plasmid using lipofectamine and cultured at 37°C. The whole-cell configuration of patch-clamp recordings was conducted at 37\pm1°C using an EPC-8 patch-clamp amplifier 48-72h after transfection.</p>			

- (備考) 1. 論文内容要旨は、研究の目的・方法・結果・考察・結論の順に記載し、2千字程度でタイプ等で印字すること。
2. ※印の欄には記入しないこと。

[Results] We identified four *KCNH2* mutations, T152I, R164C, W927G, and R1135H, in 236 consecutive probands with BrS or Brugada-like ECG. Three of these mutation carriers showed QTc intervals shorter than 360 ms and one experienced VF. Biophysical analyses showed that three mutations, R164C, W927G, and R1135H, increased I_{Kr} densities. Three mutations, T152I, R164C, and W927G, caused a negative shift in voltage-dependent activation curves. Only the R1135H mutant channel prolonged the deactivation time constants. Clinical characteristics comparison of different genotypes showed that QT intervals were significantly shorter in *KCNH2* mutation group, and QRS durations were significantly longer in *SCN5A* mutation group.

[Discussion] N588K was the first *KCNH2* mutation that showed gain-of-function effects and responsible for short QT syndrome but not BrS. Brugada and colleagues identified this mutation in the S5-P loop region in two unrelated families with hereditary short QT syndrome (QTc < 300 ms). Functional analyses of N588K channels revealed that it caused a complete loss of rectifying properties of $K_{v11.1}$ channels and did not inactivate over the physiological range of potentials.

In 2005, two first reported *KCNH2* mutations (G873S and N985S) in cases with Brugada-like ECG pattern that modulated I_{Kr} properties: these two mutant channels showed increased I_{Kr} current densities. Then we found a mutation *KCNH2*-R1135H in a patient with Brugada ECG and short QT interval. In a simulation study, Wilders and colleagues then demonstrated that the *KCNH2*-R1135H mutation not only shortened the action potential but also increased the susceptibility to all-or-none repolarization (loss-of-dome of action potentials in the right ventricle epicardium). Mutation-induced increase in I_{Kr} during initial activation may contribute to the premature repolarization, particularly in epicardium, a mechanism proposed for the BrS. This result suggests that, although not causative, these mutations may contribute to the Brugada phenotype in these families.

Similar to previous reports, in the present study, we showed that three novel *KCNH2* mutations (T152I, R164C, and W927G) exerted gain-of-function effects on I_{Kr} channels without changing the inactivation kinetics. Clinical data confirmed that these four mutation carriers displayed BrS with or without relatively short QT intervals. However, the mechanism underlying the gain-of-function of these novel mutations (T152I, R164C, and W927G) appeared quite different from that of N588K, in which the voltage dependence for inactivation was significantly shifted to more depolarized direction.

[Conclusions] All *KCNH2* mutations that we identified in probands with BrS exerted gain-of-function effects on I_{Kr} channels, which may partially explain the ECG findings in our patients.

学位論文審査の結果の要旨

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<p>(学位論文審査の結果の要旨) (明朝体 11ポイント、600字以内で作成のこと。)</p> <p>ブルガダ症候群は、心電図で特徴的な所見を呈し、心室細動によって突然死を引き起こすこともある疾患である。約 20%の患者にナトリウムチャネル遺伝子 (<i>SCN5A</i>) の異常が認められるが、その他の遺伝子異常については情報が乏しかった。最近、申請者のグループを始めとして、カリウムチャネル遺伝子 (<i>KCNH2</i>) の異常が同定されている。そこで、<i>KCNH2</i> 遺伝子を含むブルガダ症候群に関連した 9 つの遺伝子について、236 名の患者を対象に遺伝子変異をスクリーニングした。さらに、同定した変異体の機能について、パッチクランプ法で解析するとともに、機能変化と臨床的特徴の関連について、他の遺伝子変異も併せて精査し、以下の点を明らかにした。</p> <p>1) <i>KCNH2</i> 遺伝子について、3 つの新規変異を含む 4 つの変異を同定した (T152I, R164C, W927G, R1135H)。</p> <p>2) 3 名の <i>KCNH2</i> 遺伝子変異患者では、QT 間隔が短縮し、うち 1 名は心室細動が起こっていた。</p> <p>3) いずれの変異体も K チャネル活性が亢進する機能変化をもたらした。</p> <p>4) <i>KCNH2</i> 遺伝子変異患者では QT 間隔が短縮するのに対して、<i>SCN5A</i> 遺伝子変異患者では、QRS 時間の延長が認められる。</p> <p>本論文は、ブルガダ症候群の発症機構に新しい知見を与えたものであり、最終試験として論文内容に関連した試問を受け合格したので、博士 (医学) の学位論文に値するものと認められた。</p> <p style="text-align: right;">(総字数 599 字)</p> <p style="text-align: right;">(平成 27 年 1 月 28 日)</p>			