京都大学	博士（　医　学）	氏　名	高　景山
論文題目	Novel calmodulin variant p.E46K associated with severe CPVT produces robust arrhythmogenicity in human iPSC-derived cardiomyocytes （重症 CPVT を引き起こす新規カルモジュリン変異 p.E46K は、ヒト iPS 細胞由来心筋細胞において重度な催不整脈性を示す）		

（論文内容の要旨）

[Background] Calmodulin (CaM) is a multifunctional intermediate calcium-binding messenger protein expressed in all eukaryotic cells and involved in multiple cell process. In humans, CaM is encoded by three different genes (*CALM1-3*) which produce identical amino acid sequences. Recently missense variants occurred in any of *CALM* genes were identified to cause malignant inherited arrhythmias such as long QT syndrome (LQTS) and catecholamine polymorphic ventricular tachycardia (CPVT). CPVT is a rare genetic condition, which causes an irregular heart rhythm and can be life-threatening. However, it remains unclear how such severe disease is caused by only one mutated allele of six CaM alleles. This study sought to investigate the arrhythmogenic mechanism of CaM-related CPVT using human induced pluripotent stem cell derived cardiomyocyte (iPSC-CM) models and biochemical assays.

[Methods and Results] A novel *de novel* heterozygous variant, *CALM2* p.E46K was identified in two unrelated CPVT patients. The two patients showed unequivocal CPVT features as exercise-induced bidirectional or polymorphic ventricular tachycardia, and they showed other similar clinical phenotypes, such as sinus bradycardia, a similar T-U wave morphology, patent ductus arteriosus, and neurodevelopmental disorders. To evaluate the arrhythmogenic features of *CALM2* p.E46K, a patient-derived E46K iPSC line was established. In addition, a control line, 201B7, established from a healthy individual and an isogenic control line generated by CRISPR/Cas9-based gene correction were utilized. Furthermore, to compare CaM-E46K with other CaM variants, a previously established iPSC line from an LQTS patient bearing *CALM2* p.N98S (also reported in CPVT) was utilized. The CMs differentiated from E46K and control iPSC lines showed similar morphology and sarcomere organization, and with a high proportion (> 90%) of ventricular-type CMs. In addition, the E46K and control iPSC-CMs showed equivalent mRNA expression levels in the CPVT-related genes, including *RYR2* and *CALM1-3*. In electrophysiological analyses, the E46K-CMs exhibited significantly more frequent abnormal depolarizations in membrane potential recordings and more abnormal Ca^{2+} waves in Ca^{2+} imaging than controls and N98S lines. And these abnormal events in E46K-CMs occurred more frequently after adrenergic stimulation by isoproterenol. In Ca^{2+} homeostasis analysis, compared to controls, the E46K and N98S CMs exhibited larger Ca^{2+} leak via cardia ryanodine receptors (RyR2s) and smaller Ca^{2+} storage in the sarcoplasmic reticulum; and specifically, E46K exhibited significantly larger alterations than N98S. Subsequently, the severe RyR2 dysregulation caused by *CALM2* p.E46K was confirmed in a RyR2 expressing HEK293 cells system. The RyR2-HEK293 cells overexpressed with E46K-CaM showed higher frequency of Ca^{2+} oscillation, and lower endoplasmic reticulum luminal [Ca^{2+}] threshold levels (indicating larger Ca^{2+} leak) than wild-type-CaM and N98S-CaM. To further investigate the pathogenic mechanism of CPVT caused by *CALM2* p.E46K, several biochemical analyses were performed. The [^{3}H]ryanodine binding assay revealed that recombinant E46K-CaM significantly facilitated RyR2 function, especially by activating at low [Ca^{2+}] levels. The real-time dynamic CaM-RyR2 binding analysis demonstrated that E46K-CaM had a tenfold increased binding affinity to RyR2 compared to the wild-type, which may account for the dominant effect of the mutant CaM. However, E46K-CaM did not affect CaM-Ca^{2+} binding or L-type calcium channel function. Finally, the antiarrhythmic agents, nadolol and flecainide are confirmed to efficiently alleviate abnormal Ca^{2+} waves in E46K iPSC-CMs, which is consistent with the clinical response in the index patients.

[Conclusion] The study, for the first time, analyze the CaM-related CPVT with iPSC-CM models. The E46K-CMs successfully recapitulated severe arrhythmogenic features resulting from E46K-CaM dominantly binding and facilitating RyR2. In addition, the findings in iPSC-based drug testing will contribute to precision medicine.

（論文審査の結果の要旨）

カルモジュリンは、Ca^{2+}センサータンパク質として広く存在し、様々なタンパク質の機能を調節しています。近年、カルモジュリン遺伝子異常により、重症型の遺伝性不整脈疾患（先天性 QT 延長症候群やカテコラミン誘発性多形性心室頻拍（CPVT））が引き起こされることが報告されたが、その疾患発症機序はまだ十分に解明されていない。

本研究では、カルモジュリン遺伝子異常による CPVT 発症機序を解明することを目的とし、患者由来 iPS 細胞、生化学的解析手法を用いた検討を行った。*CALM2* ヘテロミスセンス変異（p.E46K）を有する運動誘発性心室頻拍を呈した症例より iPS 細胞を樹立し、分化心筋細胞における電気生理学的解析を行い、筋小胞体から細胞質への Ca^{2+}リークによる異常な電気的興奮や Ca^{2+}上昇を認め、CPVT の特徴を再現していた。また、組換えタンパク質を用いた生化学的解析により、変異型カルモジュリンは、野生型と比べて心臓リアノジン受容体に優先的に結合し筋小胞体からの Ca^{2+}リークを促進していることが分かり、催不整脈性に関するメカニズムを明らかにした。さらに、臨床で使用される抗不整脈薬（ナドロール、フレカイニド）が、患者 iPS 細胞由来心筋細胞において抗不整脈効果を有することを示した。

以上の研究はカルモジュリン遺伝子異常による CPVT の疾患発症機序の解明に貢献し、新規治療法の開発に寄与するところが多い。

したがって、本論文は博士（　医学　）の学位論文として価値あるものと認める。

なお、本学位授与申請者は、令和 5 年 7 月 11 日実施の論文内容とそれに関連した試問を受け、合格と認められたものである。

要旨公開可能日：　　年　　月　　日　以降