（報告様式４）

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I.　基本情報

事　業　名：（日本語）循環器疾患・糖尿病等生活習慣病対策実用化研究事業
（英　語）Practical Research Project for Life-Style related Diseases including Cardiovascular Diseases and Diabetes Mellitus

研究開発課題名：（日本語）特発性心室細動の発症予測精度向上のための遺伝子解析研究
（英　語）Genetic-based risk stratification in patients with idiopathic ventricular fibrillation

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実　施　期　間：　平成 28 年 10 月 3 日　～　平成 29 年　3 月 31 日

① 分担研究開発課題名：（日本語）ゲノムデータの遺伝統計解析による特発性心室細動の遺伝子解析
（英　語）Bioinformatics analysis of gene data in idiopathic VF patients

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② 分担研究開発課題名：（日本語）特発性心室細動患者の臨床データの取得
（英　語）Clinical data management of idiopathic VF patients

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③ 分担研究　（日本語）特発性心室細動患者の心電図解析
開発課題名：　（英　語）ECG analysis of idiopathic VF patients
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④ 分担研究　（日本語）外部医療機関からの試料・臨床情報収集システムの構築
開発課題名：　（英　語）Construct of clinical data and sample storage system
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⑤ 分担研究　（日本語）臨床・ゲノム情報のデータストレージ
開発課題名：　（英　語）Storage of clinical and genome information
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⑥ 分担研究　（日本語）特発性心室細動患者の遺伝子と臨床情報の収集
開発課題名：　（英　語）Clinical and genetic analysis for idiopathic VF patients.
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開発課題名：　（英　語）Clinical and genetic analysis for idiopathic VF patients.
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開発課題名：　（英　語）Clinical and genetic analysis for idiopathic VF patients.
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⑨ 分担研究　（日本語）Brugada 症候群の候補遺伝子との比較検証
開発課題名：　（英　語）Comparison and validation with candidate genes for Brugada syndrome
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⑩ 分担研究　（日本語）LQTS 関連の候補遺伝子との比較検証
開発課題名：　（英　語）Comparison and validation with candidate genes for Long-QT syndrome
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⑪ 分担研究　（日本語）特発性心室細動患者の遺伝子と臨床情報の収集
開発課題名：　（英　語）Clinical and genetic analysis for idiopathic VF patients.
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⑫ 分担研究　（日本語）特発性心室細動患者の遺伝子と臨床情報の収集
開発課題名：　（英　語）Clinical and genetic analysis for idiopathic VF patients.
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成果の概要（総括研究報告）

・ 研究開発代表者による報告の場合

和文

特発性心室細動の発症予測精度向上のための遺伝子解析研究

本研究は堀江稔教授（滋賀医科大学)、蒔田直昌教授（長崎大学)、清水渉教授（日本医科大学)、中野由紀子准教授（広島大学)、林研至助教（金沢大学)、八木原伸江助教（新潟大学）らのグループとともに、原因不明の心室細動（VF）蘇生後、いわゆる特発性心室細動患者の遺伝学的要因を網羅的遺伝子解析 Exome 解析を世界で初めて行った。

方法：国立循環器病研究センターならびに各共同研究施設から QT 延長症候群および Brugada 症候群以外の器質的心疾患のない原因不明の心室細動蘇生後患者のゲノム計 210 サンプルを用いて全エクソン解析を実施、得られたゲノムデータの中から対照群として東北メガバンク・ゲノムデータ、1000 ゲノム、dbSNP, ESP6500 および各分担研究者の in-house のデータから頻度 0.1%以上のものは SNP として除外した。さらに対照群として家族性高コレステロール（FH）患者の Exome 解析データを用いて VF 患者に共通する遺伝子多型の解析を行った。

結果：①：既存の不整脈関連遺伝子の検索を行ったが、210 名中 5 人以上が共通して有するものはなく、*RYR2* 異常（3 例)、*CASQ2*（１)、*KCNJ2*（１)、また *PKP2*（１）などカテコラミン誘発性多形性心室頻拍（CPVT）あるいは不整脈原性右室心筋症（ARVC）の初期と思われる例を認めた。②：既知の不整脈・心筋症遺伝子以外に VF 例で 5 人以上かつ FH は 3 人以下が共有する遺伝子多型は全部で 313 個あり、内訳は 5 人が共有（146 SNP)、6 人（86 SNP)、7 人（35 SNP)、8 人（13 SNP)、9 人（8 SNP)、10 人（4 SNP)、最高で 11 人（1 SNP）であった。この中には Ca チャネル、ハンドリングに関係するもの、電位依存性 K チャネルに関係するもの、デスモゾーム関連、Na チャネルや Purkinje 細胞などに関係するもの、心房細動の原因の候補遺伝子などが含まれていた。これらの遺伝子多型を発端者（VF 蘇生例）とその家族で保有する個数を比較すると、発端者では一人あたり平均 14 SNP を有しているのに対して、一方家族例では平均 4 SNP しか有していなかった。SNP の多くは日本人に特異なもので病的意義については今後検討が必要と思われるが、いくつかは疾患原因（pathogenicity）が疑われるものであった。

③病態との関係では VF 発症の年齢層、発症誘因（運動・非運動性)、発症時間などと各患者の心電図情報を集積し解析を行った。VF 心停止の発生状況から大まかに交感神経優位型（20 才）と副交感神経型（45 才：中央値）に分けられ、それぞれに関連すると思われる Variant が同定された。

結語：今回の結果から VF 発症の誘因は単一の遺伝子異常によるものの可能性は必ずしも高いとはいえず多因子遺伝であると考えられる。全ての Variant の病的意義を正確に評価することは難しいが、今回見つかった VF に関係する遺伝子多型のをスコア化し、その組み合わせと臨床情報とを将来的には人口知能（AI）などを用いることにより、リスクの層別化を測ることが可能となる。また得られた結果は今後別な母集団において再検討(replication study)を行う必要があると考えられる。すなわち本研究成果は特発性 VF のゲノムデータベース化の入り口であり、今後の生物統計や臨床情報との組み合わせによって心室細動の発症予測精度が向上すると期待される。

English

Genetic-based risk stratification in patients with idiopathic ventricular fibrillation

Genetic background in idiopathic ventricular fibrillation (VF) cannot fully be understood.
Whole exome analysis was performed for 210 patients with resuscitated after idiopathic VF in the National Cerebral and Cardiovascular Center collaborated with: Shiga Medical University (Prof. Minoru Horie), Nagasaki University (Professor Naomasa Makita), Nippon Medical School (Professor Wataru Shimizu), Hiroshima University (Yukiko Nakano, Associate Professor), Kanazawa University (Kenshi Hayashi, Assistant Professor), and Niigata University (Nobue Yagihara Assistant Professor).
Data obtained from TOHOKU Mega-bank, 1000 Genomes, dbSNP, ESP6500 and each investigator's in-house data were used as control, in which an allele frequency of ≥0.1% were excluded as the SNP. Moreover, exome data from 200 patients with familial hypercholesterolemia (FH) were used as control polymorphisms.
Results: In the p arrhythmia-related genes previously reported, no mutation was found in at least five of the 210 patients had overlapped. However, mutations or rare variants in RYR2 (n=3), CASQ2 (n=1), KCNJ2 (n=1), PKP2 (n=1) such as CPVT or ARVC associated genes, could be obtained in a small number of patients.
On the other hand, in more common variants, total 313 variants or SNPs which were overlapped in at least five VF patients as well as at most three control (FH) subjects, including 146 variants overlapped in 5 cases, 86 (6 cases), 35 (7 cases), 13 (8 cases), 8 (9 cases), 4 (10 cases) and 1 (11 cases) could be obtained in VF patients. In these variants, although most of them had not functionally been analyzed, some were suggested as functionally associated with Ca channels, Ca handling, voltage-dependent K channels, desmosome, Na channels, Purkinje cells, or atrial fibrillation. In VF cases 14 of these variants could be found on average, whereas only 4 in their families.
These findings suggest that idiopathic VF occurred mainly due to multiple genetic disorders or polymorphisms but not monogenic mutations. In this study, we could found some candidate genetic polymorphism associated with the VF-risk. Further replication study to investigate how many or much of these common polymorphism in Japanese can increase the risk of VF should be evaluated.

II. 成果の外部への発表

（1） 学会誌・雑誌等における論文一覧（国内誌　6 件、国際誌　89 件）

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（３）「国民との科学・技術対話社会」に対する取り組み

1. 致死性遺伝性不整脈の遺伝子診断と治療. 教育講演, 口頭, 清水　渉, 第52回日本小児循環器学会総会・学術集会 (東京), 2016/7/6. 国内
2. 「きょうの健康」『忍び寄る！心臓突然死を防ぐ』口頭, 清水　渉, NHK Eテレ出演
   2017年 4/3(月)～4/6(木)　8:30～8:45PM　4夜連続放送
   ① 突然死はなぜ起こる？ (VF/VT 虚血性心疾患)
   ② 若年・中年を襲う突然死　(LQTS、Brugada、CPVT)
   ③ 心筋症による突然死　(HCM、DCM、ARVC)
   ④ 突然死を防げ　＝AED などの実演あり＝

（４）特許出願

なし