（報告様式４）

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I. 基本情報

事　業　名：（日本語）ゲノム医療実現推進プラットフォーム事業
（英　語）Platform Program for Promotion of Genome Medicine

研究開発課題名：（日本語）心臓突然死の発症リスク遺伝子の解明と層別化システムの構築
（英　語）Elucidation and stratification of genetic risks for sudden cardiac death

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実　施　期　間：　平成 28 年 4 月 1 日～平成 29 年 3 月 31 日

分担研究開発課題名：
(日本語）ブルガダ症候群以外の致死性不整脈のゲノム解析およびブルガダ症候群のゲノムワイド関連解析
(英　語）Genomic analysis of lethal arrhythmias other than Brugada syndrome and genome-wide association of study of Brugada syndrome
研究開発分担者 所属 役職 氏名：
(日本語）東京医科歯科大学疾患バイオリソースセンター 教授　田中 敏博
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分担研究開発課題名：
(日本語）国立循環器病研究センター以外で収集されたブルガダ症候群のゲノム解析
(英　語）Genomic analysis of Brugada syndrome enrolled from institutions other than NCVC
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分担研究開発課題名：
(日本語）致死性不整脈患者 iPS 心筋細胞を用いた機能評価
(英　語）Functional evaluation of iPS-derived cardiomyocytes from patients with lethal arrhythmias
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分担研究開発課題名：
(日本語）関西における致死性不整脈の臨床情報データベース構築
(英　語）Implementation of clinical database of patients with lethal arrhythmias in Kansai district
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分担研究開発課題名：
(日本語）東京における致死性不整脈の臨床情報データベース構築
(英　語）Implementation of clinical database of patients with lethal arrhythmias in Tokyo
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分担研究開発課題名：
(日本語）関東(東京周辺)における致死性不整脈の臨床情報データベース構築
(英　語）Implementation of clinical database of patients with lethal arrhythmias in Kanto district
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分担研究開発課題名：
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(英　語）Implementation of clinical database of patients with lethal arrhythmias in Chugoku and Shikoku districts
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分担研究開発課題名：
(日本語）東北・北海道における致死性不整脈の臨床情報データベース構築
(英　語）Implementation of clinical database of patients with lethal arrhythmias in Tohoku & Hokkaido districts
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（日本語）心臓突然死の発症リスク遺伝子の解明と層別化システムの確立
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(日本語）北陸における致死性不整脈の臨床情報データベース構築
(英　語）　Implementation of clinical database of patients with lethal arrhythmias in Hokuriku district

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分担研究開発課題名：
(日本語）北関東における致死性不整脈の臨床情報データベース構築
(英　語） Implementation of clinical database of patients with lethal arrhythmias in Kita-Kanto district
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(日本語）東京における致死性不整脈の臨床情報データベース構築
(英　語）Implementation of clinical database of patients with lethal arrhythmias in Tokyo
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II. 成果の概要（総括研究報告）

心臓突然死のあらたな病因を解明しリスクに応じた予防医療を実現するために、遺伝性致死性不整脈の QT 延長症候群(LQTS)とブルガダ症候群(BrS)とに焦点をあて、次世代シークエンサーを用いた網羅的ゲノム解析とゲノムワイド関連解析(GWAS)によって、突然死に関連する遺伝的要因の同定を目指した。

① LQTS の deep sequence ：東京医科歯科大学の田中教授のグループは、臨床解析チームから現在までに LQTS を 624 例収集し、そのうち 583 例の deep sequencing を完了した。*in silico* 解析で、既知遺伝子のプロモータ領域に変異が集積するという予備知見を得た。変異により転写因子の結合部位が消失する可能性が示唆され有望である。その他の 4 つの候補遺伝子において、複数の *in silico* 解析ツールでタンパク機能の障害が予測される変異の集積も見出した。

② LQTS 新規遺伝子の機能解析：京都大学の牧山助教のグループは、LQTS の新規遺伝子 *CALM2* の変異による QT 延長の機序を解明するために、患者 iPS 由来心筋細胞を作成し、電気生理学的を含めた機能解析を行った。さらにゲノム編集の技術 CRISPR/Cas9 で患者 iPS 心筋細胞の異常な活動電位を正常化することに成功した(Yamamoto, *et al*. Hum Mol Genet, in press)。

③ BrS のエクソーム：長崎大学の蒔田教授、吉浦教授、石川助教のグループは、臨床解析チームから現在までに 363 例の有症候性 BrS 発端者のゲノムと臨床情報を集積し、そのうち 296 例のエクソームを終了した。現在新たな疾患遺伝子の同定を目指して、*in silico* 解析を行っている。一方、BrS に多因子疾患要因が関与するという我々の知見をもとに(Bezzina, Makita et al. Nat Genet 2013)、今回得

られたエクソームと、コントロール日本人 372 例のエクソームデータを用いて、レアバリアント関連解析(Burden test)を開始している。

④ BrS の GWAS: 長崎大学の蒔田教授、石川助教、東京医科歯科大学の田中教授のグループは臨床解析チームとの共同で、BrS における突然死のリスクの遺伝的要因を解明するための GWAS をおこなった。有症候性 BrS 436 人、無症候性 BrS 321 人、非心疾患 1,154 人で解析したところ、症状に関連する新規 SNP を染色体 19 上に同定した。現在、アジア人・白人のサンプルでレプリケーションを行う準備をしている。この研究は心臓突然死というまれな遺伝性疾患の病態には多因子疾患的な要素が関与していることを示すとともに、GWAS が稀な遺伝性不整脈の病態解明に重要な役割を果たすことを示している。

⑤ 心臓突然死の層別化：BrS 患者の突然死や重症不整脈などの予後予測にたいする *SCN5A* 変異の有用性には否定的な見解が多かった。これを日本人 BrS で独自に検証するために、10 年ほど前、日本医科大学の清水教授が中心となって本邦の多施設 BrS 登録研究が始まった。本研究班班員の多くが参画している。日本人 BrS 発端者 415 人長期予後を見たこの研究から、*SCN5A* 変異キャリアは非キャリアに比べて最初の心事故発生が有意に早期であることが判明した。この結果は、*SCN5A* 変異の臨床的な意義だけでなく、BrS 症例における遺伝子解析の有用性を改めて確認したと言える(Yamagata *el al*. Circulation, in press)。

II. Summary of research findings

In order to elucidate novel pathophysiology underling sudden cardiac death (SCD) and to implement personalized preventive medicine based on the individuals genetic risk for SCD, we performed high throughput sequencing using next-generation sequencers and genome-wide association study (GWAS) in patients with long QT syndrome (LQTS) and Brugada syndrome (BrS).

1. Deep sequencing of LQTS: Professor Tanaka's group in Tokyo Medical Dental University (TMDU) have collected 624 samples of genotype-negative LQTS probands un collaboration with the clinical investigators in this group, and he has finished the deep sequencing in 583 probands. Based on the *in silico* analysis, he obtained some preliminary results that mutations are clustered at the promoter regions of LQTS genes.
2. Functional analysis of new LQTS genes using iPS technology: Dr. Makiyama's group in Kyoto University in collaboration with Professor Makita in Nagasaki Universty established iPS-derived cardiomyocytes (iPS-CM) from a patient with LQTS carrying a mutation in calmodulin gene *CALM*2, and functionally analyzed the molecular mechanisms underlying the novel LQTS entity, LQT14. Furthermore, they showed that the allele-specific ablation using the CRISPR/Cas9 technology restored the normal action potentials in the iPS-CM of the LQTS patient (Yamamoto *et al*, Hum Mol Genet, in press).
3. Exome of BrS: Professors Makita, Yoshiura, and Dr. Ishikawa in Nagasaki University have performed collected DNAs from 363 symptomatic BrS in collaboration with the clinical investigators in this group, and carried out exome in 296 of them. They are performing *in silico* analysis to look for novel genes responsible for BrS. They have also started "buden test", a rare variant association study to identify genetic risk for BrS by comparing the rare variations between exome data of BrS (n=296) and control Japanese (in-house; n=370).
4. GWAS of BrS: Professor Tanaka in TMDU, and Professor Makita and Dr. Ishikawa in Nagasaki University have

started GWAS to identify genetics risks for sudden death in BrS. Since the previous BrS GWAS implicated common variants in BrS-type ECG (Bezzina and Makita *et al*. Nat Genet, 2013), they explored if they can identify genetic risks for SCD in BrS by the GWAS among symptomatic and asymptomatic BrS. By genotyping symptomatic BrS (n=436), asymptomatic BrS (n=321), and control (n=1,154), they identified a new SNP associated with symptoms located at the chromosome 19, in additions to the 3 previously identified SNPs. They are in the process of replication studies using DNA samples of other Asian and Caucasia populations.

5. Stratification SCD in BrS: Although *SCN5A* is the most prevalent gene responsible for BrS, it's mutations have not been implicated in the prediction of the prognosis of SCD or lethal arrhythmias in BrS. To evaluate the significance of *SCN5A* mutations in BrS, Professor Shimizu has started a Japanese multicenter BrS registry project about 10 years ago, in which many investigators of this AMED study have participated from the beginning. Based on a long-term follow-up of 415 Japanese BrS probands, they have confirmed that *SCN5A* mutation carriers have experienced the first cardiac event at a younger age than the *SCN5A*-negative subgroup. These results reinforce the importance of genetic testing in BrS (Yamagata *el al*. Circulation, in press).

III. 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 9 件、国際誌 147 件）

国内誌

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151. Variants Of Aldehyde Dehydrogenase 2 And Alcohol Dehydrogenase Genes Are Associated With Atrial Fibrillation. ポスター. Nakano Y, Ochi H, Tomomori S, Matsumura H, Tokuyama T, Sairaku A, Motoda C, Chayama K, Chen C, Mochly-Rosen D, Kihara Y. American Heart Association 2016. 2016/11/12, 国外.
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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＝
3. 不整脈ってなんでなるの？　林　研至　第81回日本循環器学会学術集会「市民公開講座」2017/3/20, 国内.
4. 不整脈による突然死,遺伝子でリスク判定. 中野由紀子. 日本経済新聞.13:2016.2.8(月)
5. 心房細動 新手術を導入,広島大病院が「ホット・バルーン法」,従来より体の負担少なく. 中野由紀子. 中国新聞. 29:2016.7.10(日)
6. 市民公開講座(不整脈について). 中野由紀子.中国新聞.社:2016.12.13(火)
7. 不整脈による突然死を起こすブルガダ症候群(ポックリ病)の発症リスクが低減する遺伝子を発見. 中野由紀子.プレスリリース,2016.1.
8. Ⅰ 隣人が倒れたら,Ⅱ 急性心筋梗塞と狭心症,Ⅲ 不整脈と突然死,Ⅳ 大動脈解離と大動脈瘤破裂,Ⅴ エコノミークラス症候群(急性肺血栓塞栓症). 知っておきたい心臓病の救急. 木原康樹,日髙貴之,土肥由裕,福田幸弘,栗栖智,中野由紀子. 1-25,2016.9.医師会での配布

（４）特許出願

なし