平成26・28年度 委託研究開発成果報告書

I. 基本情報

事業名：(日本語)難治性疾患実用化研究事業
(英語)The Practical Research Project for Rare/Intractable Diseases

研究開発課題名：(日本語)福山型筋ジストロフィーの自然歴の把握と病状を反映するバイオマーカーの検索
(英語)Grasping the natural history of Fukuyama type muscular dystrophy and searching for biomarkers reflecting the medical condition

研究開発担当者：(日本語)戸田 達史
所属役職 氏名：(英語)Tatsushi Toda

実施期間：平成26年6月2日～平成29年3月31日

分担研究：(日本語)研究の総括および臨床評価・実験データ評価
開発課題名：(英語)Research management and clinical and experiment data evaluation

研究開発分担者：(日本語)戸田 達史 神戸大学・大学院医学研究科・教授
所属役職 氏名：(英語)Tatsushi Toda, Professor, Kobe University Graduate School of Medicine.

分担研究：(日本語)臨床評価・遺伝子解析・検体の収集・分子生物学的実験・データ解析
開発課題名：(英語)Investigating serum biomarkers in Fukuyama muscular dystrophy

研究開発分担者：(日本語)池田 真理子 神戸大学・大学院医学研究科・特命准教授
所属役職 氏名：(英語)Mariko Taniguchi-Ikeda, Associate Professor, Kobe University Graduate School of Medicine.

分担研究：(日本語)(1)FCMDの自然歴の把握 (2)FCMDの運動機能評価法の開発 (3)FCMD患者のインピーダンス法による筋量測定 (4)FCMDにおけるCT, MRIの経時的変化

開発課題名：(英語)(1)Natural history data of motor function in Fukuyama congenital muscular dystrophy patients (2)Developing reliable and valid motor function scales for Fukuyama congenital muscular dystrophy. (3)A new...
index of muscle development and disease progression for patients with Fukuyama congenital muscular dystrophy, using bioelectrical impedance analysis: an Observation Study(4)Longitudinal study on skeletal muscle imaging in patients with FCMD Fukuyama congenital muscular dystrophy

Study development participants (Japanese) Ishigaki Keiko, Senior Lecturer, Department of Pediatrics, Tokyo Women's Medical University, School of Medicine.

Duties and roles: (English) Keiko Ishigaki, Senior Lecturer, Department of Pediatrics, Tokyo Women's Medical University, School of Medicine.

Study development participants (Japanese) Ishigaki Keiko, Senior Lecturer, Department of Pediatrics, Tokyo Women's Medical University, School of Medicine.

Duties and roles: (English) Keiko Ishigaki, Senior Lecturer, Department of Pediatrics, Tokyo Women's Medical University, School of Medicine.

Study development participants (Japanese) Ishigaki Keiko, Senior Lecturer, Department of Pediatrics, Tokyo Women's Medical University, School of Medicine.

Duties and roles: (English) Keiko Ishigaki, Senior Lecturer, Department of Pediatrics, Tokyo Women's Medical University, School of Medicine.

Study development participants (Japanese) Ishigaki Keiko, Senior Lecturer, Department of Pediatrics, Tokyo Women's Medical University, School of Medicine.

Duties and roles: (English) Keiko Ishigaki, Senior Lecturer, Department of Pediatrics, Tokyo Women's Medical University, School of Medicine.

II. Conclusion (General Research Report)

- Study development representatives by the report in the case of

Fukuyama congenital muscular dystrophy (FCMD) is an intractable disease, and detailed natural history research is almost nonexistent, making comprehensive disease monitoring and treatment assessment difficult. Furthermore, FCMD patients often become non-ambulatory and have concomitant intellectual disabilities, making accurate disease monitoring and objective treatment evaluation difficult.

Therefore, to accurately monitor the natural history and establish an accurate functional assessment system, we aimed to identify biomarkers that reflect disease status and treatment evaluation. In this study, we conducted (1) a retrospective-future natural history assessment and (2) genetic expression analysis and metabolomics to explore new biomarkers.

For FCMD, specifically, we examined microRNA expression in blood serum and identified microRNAs that are specifically upregulated in FCMD patients, particularly MiR206, which showed statistically significant upregulation. This provided an evaluation tool to be used in clinical evaluation.

In the future, we aim to establish a complete natural history database to include FCMD, as well as to extend this research to other rare diseases.
明し、治験の評価項目としての基準を作成した。また福山型先天性筋ジストロフィー患者の精神運動発達のマイルストンに関する情報を収集し、将来の運動機能の程度を予測する、予測式構築の可能性についての知見が得られた。最高到達運動能ごとに定頸、座位の平均時期に大きな差が認められ、定頭獲得時期により最高到達運動能の大よその予測は可能であった（Neuromuscul Disord, 2016）。粗大運動評価尺度（Gross motor function measure：GMFM）が臨床試験主要評価項目として用いられると考えている。

またメタボローム解析により、福山型先天性筋ジストロフィーの病状を表現できる代謝物バイオマーカー候補を見出すことができた。

Since Fukuyama congenital muscular dystrophy (FCMD) was an incurable disease, there are few studies on detailed natural history of patients, so it is difficult to grasp the disease condition and evaluate the treatment. Also, many patients with FCMD are unable to walk and intellectual developmental delay is also accompanied, so it is difficult to grasp the patient's correct disease condition and objectively evaluate therapeutic evaluation in the clinical trial. Therefore, it is indispensable to grasp the natural history of FCMD, to construct an accurate motor function evaluation system, and to identify biomarkers that reflect disease condition and treatment evaluation. Therefore, in this research, we (1) grasped the natural history including motor function evaluation, muscle mass measurement, image diagnosis, and developmental function evaluation, retrospectively and prospectively and (2) searched new biomarkers using gene expression analysis and metabolomics.

We have investigated for finding circulating biomarkers which is specific for Fukuyama muscular dystrophy. We have identified that microRNA 206, 133, and 16 are especially high in patients serum compared to normal controls. We have also checked if there are such biomarkers to predict cardiac function in FCMD patients. We also identified that in FCMD, some microRNAs which are specific to cardiac muscle (i.e. miR377 and 22) have distinct expression profiles compared to those of normal control serum. These results might contribute to predict disease progression in Fukuyama muscular dystrophy patients.

Our study reevaluated the natural history of genetically diagnosed Fukuyama congenital muscular dystrophy patients, focusing on motor development and its deterioration. We retrospectively studied the medical records of 57 genetically diagnosed FCMD patients followed at our university from 1995. Age at which head control and sitting were achieved on average for each phenotype was delayed with severity of phenotype.

To confirm the validity of GMFM for the assessment of FCMD, 41 FCMD patients were recruited into the study. The GMFM scores correlated significantly with two previously used motor scales, and the time-dependent change in the GMFM scores was consistent with the natural course of the disease. The inter-rater reliability, which was assessed in four physiotherapists blinded to each other, was excellent. We concluded that GMFM was a useful and valid motor function measure for FCMD.

We evaluated whether it would be possible to assess growth and development in patients with FMCD using BIA The subjects were 21 FCMD patients who were being followed up on our
university. Our results showed that the qualitative index of the lower legs tended to decrease both before and after the age of eight, suggesting that replacement by fat in the lower legs may start from birth, which is characteristic of FCMD in which replacement by fat starts in the lower legs and extends up to the thighs. In addition, the upper arm qualitative index increased significantly until the age of eight, reflecting the usual clinical course of FCMD in which the upper limb function is better preserved than lower limb function.

We found high-intensity changes on skeletal muscle MRI in some young patients with FCMD patients without changes on T1-weighted imaging (T1WI). In this study, we evaluated the longitudinal changes on skeletal muscle MRI in FCMD patients focused on STIR and T1WI. Skeltal muscle MRI in 18 patients with FCMD (2 -13 years) were analyzed by two radiologists. We characterized the specific patterns of disease progression in FCMD patients. High intensity on STIR proceeded fatty replacement detected by T1WI at early stages, especially under 5 years.

Medical records of patients with FCMD were retrospectively reviewed. Patients with higher speech skill were able to attain better motor functions. Our study indicates that intellectual disability in FCMD patients potentially affect their motor developmental milestones.

First, the metabolome analysis system using gas chromatography/mass spectrometry was established, and then we performed the metabolome analysis for the plasma of the mice that were the animal model for Fukuyama type congenital muscular dystrophy. However, we could not find the effective biomarker candidates in the mouse plasma. Next, we performed the metabolome analysis for the plasma of the patients with Fukuyama type congenital muscular dystrophy. As a result, some metabolite biomarker candidates were discovered, and the relationship between metabolite biomarker candidates and pathological conditions of Fukuyama type congenital muscular dystrophy could be confirmed.

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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 1 件、国際誌 21 件）


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9. Genomewide analysis and molecular targeting therapy for neurological diseases. 口頭，Tatsushi Toda, nature conference · Genomic Variations in Precision Medicine, 2015/5/17 （国外）

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