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# 科学研究費助成事業　　研究成果報告書

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研究課題名（和文）The roles of PIGA in epileptic encephalopathy and mental retardation

研究課題名（英文）The roles of PIGA in epileptic encephalopathy and mental retardation

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研究成果の概要（和文）：PIGAの3種の異なる神経細胞種特異的なKOマウス系統を確立した。結果、IKOとEKOでは、学習記憶能力の低下と、運動失調症が生じた他、どの系統でも、カイニン酸誘発発作が増加がみられ。まだ、すべての系統で、海馬の一部領域にてシナプス密度に変異が生じており、IKOとEKOでは、神経細胞の興奮/抑制バランスが変化していた。この結果からAAVによる海馬でのPIGAのレスキュー実験を行った。結果、IKOマウスとEKOマウスでカイニン酸誘発発作を部分抑制することに成功したことから、PIGA脳症が生じるメカニズムの一端に、海馬の一部領域でPIGAを介したシナプスの異常が大きく関わると結論づけられた。

研究成果の学術的意義や社会的意義
Patients with PIGA mutations suffer from intractable epileptic seizures and mental retardation.Our study provided important clues of molecular and circuit mechanisms underlying PIGA encephalopathy, and will help to develop effective gene therapy to treat brain disease caused by GPI-anchor deficits.

研究成果の概要（英文）：Global PIGA knockout causes embryo lethality. By using conditional knockout system, we successfully obtained mouse lines with the ablation of PIGA specifically in inhibitory neurons(IKO), in forebrain excitatory neurons(EKO) or in thalamus neurons(TKO).The behavioral, EEG, histology and electrophysiology analysis showed that IKO and EKO but not TKO mice performed poorly in learning and memory task. IKO and EKO demonstrated obvious ataxia. It is notable that the susceptibility to kainic acid induced seizures was significantly increased in all the three types of mutants. The synaptic density was changed in specific hippocampal subregions of all the mutants. There was a significant E/I balance change in IKO and EKO mice. In addition, AAV virus mediated PIGA expression in hippocampus could significantly suppress Kainic acid induced seizure in IKO and EKO. We elucidated that the synapse abnormality in specific subregions of hippocampus plays important roles underlying PIGA encephalopathy.

研究分野：神経分子病態学

キーワード：PIGA, epilepsy, mental retardation, ataxia, hippocampus, synapse, E/I balance, GPI-anchor

## １．研究開始当初の背景

The international focus on understanding human glycosylation(“sugar coding”) disorders has grown considerably in the last few years. Many disorders occur as a result of mutations in the genes responsible for synthesis and maturation of glycophosphatidylinositol (GPI) anchors, and some patients showed pronounced neurological impairment such as early onset epileptic encephalopathies(EOEE), mental retardation and multiple congenital anomalies-hypotonia-seizures syndrome . However, hitherto nothing has been done to reveal the mechanisms underlying the encephalopathy of the GPI anchor deficits.

On the other side, epilepsy or intelligence disability is inextricably a circuit-level phenomenon and cannot be understood outside this context. However, current available therapies for epilepsy and mental retardation target their symptoms rather than the dysfunction of specific circuits. It is thus urgent to identify discrete elements of neuronal circuits critical to epileptogenesis and intellectual disability.

Phosphatidylinositol glycan biosynthesis class A protein(PIGA) catalyzes the very first step of GPI anchor biosynthesis (Tarailo-Graovac et al., 2015). Our collaborator Prof. Naomichi Matsumoto identified multiple PIGA mutations in patients with serious EOEE.. Our previous work on a pair of GPI-anchored molecules, netrin-G1 and netrin-G2, has showed that the ablation of either molecule in mice resulted in serious deficits in learning and memory(Zhang Qi et al.,2016a&b, Matsukawa H&Zhang Qi et al.,2014). Mutations in human caused neurological diseases (Prosselkov P&Zhang Qi et al.,2016). Since PIGA deficit influences all the GPI-anchored proteins, and PIGA is expressed throughout the mouse brain with the highest expression in hippocampus. We hypothesize that PIGA knockout (KO) will produce a novel animal model with seizures and mental retardation. Based on this, we could investigate the histological, electrophysiological, and behavioral effects of GPI anchor deficit in vivo. In addition, circuit-specific rescue experiments will further identify the key neural circuits responsible for the neurological phenotypes underlying PIGA encephalopathy.

## ２．研究の目的

1)To obtain the conditional knockout mice with PIGA deficit specifically in forebrain excitatory neuronal population(EKO), in inhibitory neuronal population(IKO) or in thalamus neurons(TKO).

2) To analyze the seizure related phenotype and intellectual ability of the different types of PIGA conditional knockout mice.

3)To elucidate the cellular and physiological mechanisms underlying PIGA encephalopathy from a circuit level.

4). To explore the efficiency of gene therapy targeting on the neuropathology caused by PIGA deficit.

## ３．研究の方法

1) The EKO, IKO and TKO conditional knockout lines were obtained by breeding PIGA floxed mouse line with Emx-Cre line, Vgat-Cre line and PKCD-Cre line respectively.
2) The behavioral and EEG analysis were used to investigate the seizure related phenotype and intellectual ability of EKO, IKO and TKO lines.
3) Histology and electrophysiology analysis were used to analyze the general brain structure, neuron and synaptic changes.
4) Virally expressing PIGA in candidate circuits of conditional KO mice was used to observe the rescue effects.

## ４．研究成果

1) By using conditional knockout system, we observed much more striking phenotypes than what we expected. Since PIGA is an X chromosome linked gene, we could get deletion of PIGA in the entire specific cell population in male mouse, and we could get a deletion in half of the specific cell population due to x-inactivation in female mouse. This special opportunity allowed to us to study the phenotypes of the conditional knout-out lines in a fine detail. ①Deletion of PIGA in all inhibitory neurons resulted in developmental abnormality and embryo death.②Deletion of PIGA in all forebrain excitatory neurons resulted in embryo lethality. ③Deletion of PIGA in half inhibitor neurons(IKO) or half forebrain excitatory neurons(EKO) or all thalamus neurons(TKO) didn't cause embryo death and the mutants could grow normally to adults.

2) The behavioral and EEG studies clearly demonstrated that IKO and EKO but not TKO mice performed poorly in learning and memory task. IKO and EKO also showed obvious ataxia. It is notable that the susceptibility to kainic acid induced seizures was significantly increased in all three types of mutants.

3) Histology and electrophysiology analysis showed that the synaptic density was changed in specific hippocampal subregions of all the mutants. There was a significant E/I balance change in the hippocampus of IKO and EKO mice.

4) In addition, AAV mediated PIGA expression in hippocampus could significantly suppress Kainic induced seizure in IKO and EKO. Therefore we realized all the research aims set up at the beginning and we even made more interesting discoveries about the roles of PIGA in inhibitory and excitatory neurons in brain development. Especially, the system established here allowed us to identify the critical neural circuits involved in epilepsy and mental retardation in PIGA deficient mice, and will absolutely be very helpful to develop the gene therapy strategy to treat the intractable epilepsy and mental retardation patients who suffer from the dysfunction of GPI anchors.

５．主な発表論文等

〔雑誌論文〕　計6件（うち査読付論文　6件／うち国際共著　6件／うちオープンアクセス　6件）

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| １．著者名<br>Zhang Qi、Kobayashi Yuki、Goto Hiromichi、Itohara Shigeyoshi | ４．巻<br>- |
| ２．論文標題<br>An Automated T-maze Based Apparatus and Protocol for Analyzing Delay- and Effort-based Decision Making in Free Moving Rodents | ５．発行年<br>2018年 |
| ３．雑誌名<br>Journal of Visualized Experiments | ６．最初と最後の頁<br>- |
| 掲載論文のDOI（デジタルオブジェクト識別子）<br>doi: 10.3791/57895 | 査読の有無<br>有 |
| オープンアクセス<br>オープンアクセスとしている（また、その予定である） | 国際共著<br>該当する |

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| １．著者名<br>Masuda Akira、Sano Chie、Zhang Qi、Goto Hiromichi、McHugh Thomas J、Fujisawa Shigeyoshi、Itohara Shigeyoshi | ４．巻<br>9 |
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| ３．雑誌名<br>Biorxiv | ６．最初と最後の頁<br>- |
| 掲載論文のDOI（デジタルオブジェクト識別子）<br>doi.org/10.1101/229856 | 査読の有無<br>有 |
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| 掲載論文のDOI（デジタルオブジェクト識別子）<br>doi.org/10.1101/139444 | 査読の有無<br>有 |
| オープンアクセス<br>オープンアクセスとしている（また、その予定である） | 国際共著<br>該当する |

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| 1．著者名<br>Zhang Qi、Kobayashi Yuki、Goto Hiromichi、Itohara Shigeyoshi | 4．巻<br>138 |
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| 1．発表者名<br>Kandasamy LC, Kato M, Matsumoto N, Takeda J, Itohara S, Ogawa S, Young LJ, Zhang Q |
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2019年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

| | 氏名<br>（ローマ字氏名）<br>（研究者番号） | 所属研究機関・部局・職<br>（機関番号） | 備考 |
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