様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　２ 年　 ６ 月 １９ 日現在

機関番号：８２４０１
研究種目：若手研究
研究期間：2018～2019
課題番号：１８Ｋ１５５３１

研究課題名（和文）Identification of mtDNA mutations associated with bipolar-like behaviour in CaMKIIa-Polg1 mice using Ultra-Deep Next-Generation Sequencing

研究課題名（英文）Identification of mtDNA mutations associated with bipolar-like behaviour in CaMKIIa-Polg1 mice using Ultra-Deep Next-Generation Sequencing

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交付決定額（研究期間全体）：（直接経費）　　3,100,000円

研究成果の概要（和文）：ミトコンドリア遺伝子の変異は、一部の双極性障害（BD）患者で発見されています。ミトコンドリアは細胞のエネルギー生成単位であり、機能的なミトコンドリアがなければ、細胞自体が機能不全のリスクがあります。 ミトコンドリア遺伝子の変異は、ミトコンドリア内に存在する特定の種類のDNA、mtDNAに変異を引き起こすと考えられています。
Please see English version of 研究成果の概要 for the full description.

研究成果の学術的意義や社会的意義
The method we have implemented allows us to examine mtDNA from smaller tissue pieces than previously without the need for biasing approaches. Our brain-wide view of mtDNA mutations add new depth to mitochondrial research and better our understanding of the molecular mechanisms of bipolar disorder.

研究成果の概要（英文）：Mutations in mitochondrial genes have been found in some patients with bipolar disorder (BD). Mitochondria are the energy-producing units of the cell and without functional mitochondria the cell itself is at risk of dysfunction. Mutations in mitochondrial genes are thought to cause mutations in a specific type of DNA, mtDNA, that is present within mitochondria.
In this research project we implemented a method that allows us to look at all mutations in mtDNA from very small pieces of tissue, and thereby create a "brain-wide view" of mtDNA mutations. By analysing the brains of mice that have a mutation in a mitochondrial gene found in BD patients, we found that specific brain regions are very sensitive to have mutations. These brain regions have previously been found to be involved in BD. This indicates that mitochondria and mitochondrial regulation is different across brain regions and may contribute to neuropsychiatric disorders.

研究分野：Neuroscience

キーワード：mitochondria　mtdna　bipolar disorder　sequencing　epigenetics

１．研究開始当初の背景

Bipolar disorder is often found comorbid with mitochondrial disease, and bipolar disorder patients have an increased prevalence of deletions in their mitochondrial DNA (mtDNA). Few animal models are available to study bipolar disorder development, molecular mechanisms and genetics. However, mice that express a proof-reading deficient *Polg* in forebrain neurons only (CaMKIIα-*Polg* mice), thereby increasing the rate of mtDNA mutations, exhibits depressive-like behavioural changes in that their wheel-running activity is decreased in spontaneous reoccurring episodes [1]. These depressive-like episodes are associated with several other traits reminiscent of observations in human bipolar disorder patients such as higher corticosterone levels, disturbed sleep pattern, altered body temperature and increased weight [2].

Our lab has previously mapped mtDNA deletions in the brain of these mice, however, the approach used was based on *a priori* assumptions of the nature of the mtDNA deletions [2]. Advanced techniques to examine mtDNA mutations are inherently biased in that they rely on PCR-amplification of mtDNA [3-5]. To overcome this bias, high tissue input is required [6]. This limits the spatial resolution with which mtDNA mutations can be analysed (e.g. must be summed across an entire brain or liver). In neuropsychiatric disorders such as bipolar disorder, the causative regions are thought to be rather discrete and thus analysis of an entire brain of the *Polg* bipolar disorder-model mouse would likely mask any brain region-specific mtDNA mutation patterns. We therefore saw a need to implement a method that would allow us to overcome both of these obstacles.

Mitochondria themselves are energy- and metabolite-producing organelles. The mitochondria-derived metabolites are used for maintenance of diverse cellular processes including regulation of gene expression by epigenetic modulation. Mitochondrial dysfunction can be induced by accumulation of mtDNA mutations, and dysfunctional mitochondria may not be able to provide the cell with the metabolites it requires for epigenetic regulation. We therefore wondered whether the mitochondrial dysfunction induced by expression of proof-reading deficient *Polg* would induce dysregulation of this process. The influence of loss of mtDNA, and through this mitochondrial dysfunction, on the epigenetic landscape has only been investigated in a cell culture model. Though this model does not recapitulate the biological complexity of the brain, it did show some highly interesting consequences of loss of mitochondrial function on the epigenetic regulatory mechanisms. To investigate this in an *in vivo* context of bipolar disorder, we wanted to take advantage of the ATAC-seq method, as it is plausible to perform with limited cell/nuclei input.

In addition, we were interested in the gene expression changes induced by mitochondrial dysfunction. We had previously profiled the gene expression changes using total RNA-sequencing of bulk tissue, where we performed the analysis in four different brain regions from *Polg*-mice and controls. However, we did not find any changes in gene expression in this study. We hypothesized that performing bulk RNA-sequencing was not adequate, as it may mask gene expression changes occurring in only a fraction of the cells. Based on staining of COX/SDH (i.e. staining the activity/functionality of mitochondria), we believe only about half of neurons exhibit overall cellular changes [2], why transcriptomic changes may easily be masked by bulk RNA-sequencing. We therefore saw a potential to gain more information about the consequences of mtDNA mutations on gene expression changes specifically in neurons with and without mitochondrial dysfunction in the *Polg* animals.

２．研究の目的

The molecular mechanisms of bipolar disorder are poorly understood. Modelling of bipolar disorder in animals is difficult and thus it is important to vigorously study the models available as well as develop methods that allows us to perform investigations of limited tissue dissections in order to make brain-wide maps of the consequences of bipolar-like phenotypes in the models which include mapping of mitochondrial dysfunction.

Here, we aimed to map mtDNA mutations as well as chromatin accessibility and RNA-seq in our bipolar disorder model mouse with the aim to understand how mitochondrial dysfunction may induce bipolar-like behavior on a molecular level. This work will provide new insights into cellular mechanisms that may contribute to bipolar

disorder in human patients.

３．研究の方法

In this study we used mice expressing a mutant version of the mitochondrial DNA polymerase, *Polg*, under the CaMKIIα promoter. We took advantage of a previous mtDNA deletion map we generated to decide on regions of interest and decided to investigate paraventricular thalamic nucleus (PVT), nucleus accumbens (NAc), (sensory) cortex, caudate putamen, dorsal raphe, and substantia nigra. These were our targets for mtDNA sequencing. For the study of mtDNA mutations we used bulk tissue. Due to the limited size of the regions of interest, we could not purify mitochondria and making whole cells from adult brain tissue is non-trivial. We therefore decided to use a bulk method, despite the problems that present.

After dissection of brain regions of interest, we purified total DNA and then enzymatically depleted nuclear DNA using an enzyme targeting free ends of DNA, thus leaving mtDNA, which is circular and thus have no free ends, intact. This mtDNA was then prepared for next-generation sequencing using a tagmentation-based approach, to ensure sequencing of the entire mtDNA molecule without bias towards molecules with or without deletions. This ensured an unbiased sequencing approach. The bioinformatic processing of the sequencing data was optimized for the analysis of deletions derived from circular DNA, the details of which will not be explained here.

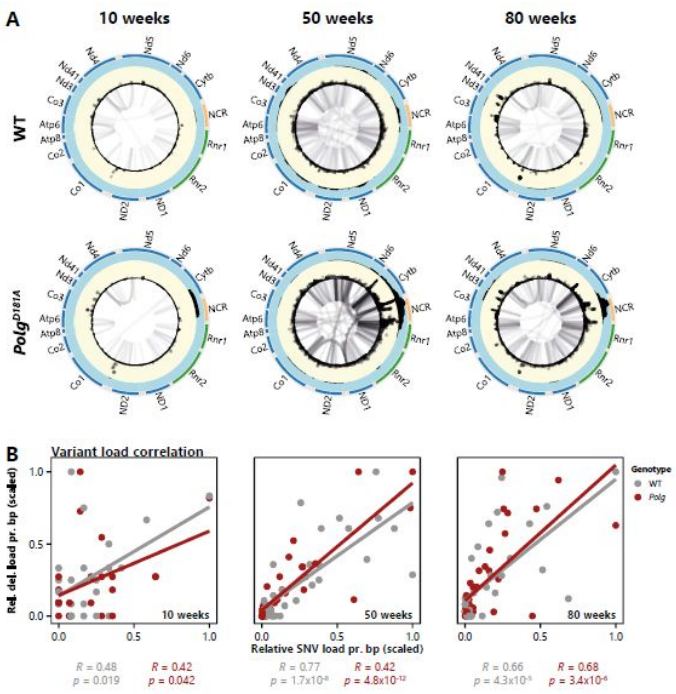
We were able to evaluate the behavioral state of the *Polg* mice as either depressive-like or euthymic using constant wheel running activity measurements for six months. This method is labor-intensive and time consuming. We therefore chose to only use this for mice to be used for the RNA-sequencing analysis. During the constant wheel-running measurements we could identify an episode of depressive-like behavior as it happens, thus enabling us to sample mice in and out of depressive-like episodes. PVT was our main region of interest for influence of the bipolar-like behavior in the *Polg* mice. After sampling, we stained PVT tissue section for mitochondrial function and used laser capture micro dissection to selectively investigate neurons with or without dysfunctional mitochondria both in and out of depressive episodes. Using this approach, we aimed to form a detailed transcriptomic view of the changes associated both with depression-like episodes and with mitochondrial dysfunction.

To investigate the chromatin accessibility, we sampled PVT from *Polg* mice that had not been used for wheel-running behavioral analysis. Initially, we tried to perform ATAC-seq on laser micro dissected neurons, however, that proved unsuccessful. We were therefore not able to discriminate between neurons with and without mitochondrial dysfunction for this analysis. Instead, we sampled the entire PVT and performed fluorescence activated cell sorting (FACS) of nuclei to only get neuronal nuclei using NeuN-staining. We believe that though this will pool nuclei from two cellular states (with and without mitochondrial dysfunction), it will still be possible to identify strong changes in chromatin accessibility.

４．研究成果

Part 1 – Mapping of the mtDNA mutation landscape across the brain of Polg mice.

We mapped the mtDNA mutation spectrum across PVT, NAc, cortex, caudate putamen, dorsal raphe and substantia nigra in mice at 10, 50 and 80 weeks-of-age for both WT and *Polg* mice.



Our data showed that all examined brain regions in WT mice showed an aging-dependent accumulation of mtDNA single nucleotide variants (SNVs) and deletions. This aging-dependent mtDNA mutation accumulation was increased in specific brain regions in the *Polg* mice. We found that PVT and NAc are clear mutational *hotspots* in this bipolar disorder mouse model. Interestingly, PVT and NAc have previously been highlighted as causative brain regions in bipolar disorder and this work connects brain region-specific mitochondrial susceptibility to mitochondrial mutations to bipolar-like symptoms in our mouse model. In addition, we identified another class of mitochondrial mutations in the form of duplications, which were only present in *Polg*-mice. This indicates that mutations in the mitochondrial DNA polymerase has the ability to induce mtDNA mtuations that may not be present under other conditions.

In the figure on the previous page, the data of mtDNA sequencing has been summarized. In part A, the mtDNA the presense of duplications (blue region), SNVs (yellow region) and deletions (lines spanning the inner part) across the mtDNA (genes on mtDNA are shown on the outside). In part B, we show a clear correlation of the genes in which there is a high load of deletions and SNVs at all ages in both WT and *Polg* mice.

This work has been submitted for publication.

In addition to this work on the *Polg*-mice, we expanded our analysis to include another bipolar disorder model mouse, the *Ant1* conditional knock out mouse. Though not a part of this funding directly, the work on the *Polg* animals enabled us to further our work to this model using the laboratory and bioinformatic workflow developed. This work is currently in preparation for submission.

Part 2 – Transcriptomic changes associated with mitochondrial dysfunction and bipolar-like behavioral changes.

We performed total RNA-sequencing of neurons (as evaluated by Nissl staining) with or without mitochondrial dysfunction (as evaluated by COX activity staining) isolated from PVT by laser micro dissection using the Leica LMD7. As the number of neurons with mitochondrial dysfunction is minimal in PVT from wild-type mice, this type of cell was only collected from the *Polg*-animals. In effect, our dataset thus contains five different conditions of PVT neurons.

Data analysis is currently ongoing. However, the current state of analysis shows that several hundreds of genes are dysregulated in response to proof-reading deficient *Polg* expression whether the mice experience a bipolar-like episode or not and independent of mitochondrial functionality. Surprisingly, we found that downregulated but not upregulated genes are extensively shared between all groups. From this data, we cannot know whether upregulation of a specific transcriptomic profile in response to proof-reading deficient *Polg* expression increases the possibility that a mouse will have bipolar-like episodes or whether this specific transcriptomic profile is only seen during the episode. The current analysis supports a view where regulation of specific mitochondrial regulatory functions are altered specifically in PVT neurons with mitochondrial dysfunction during a depressive-like episode.

Part 3 – Chromatin accessibility changes in bipolar disorder model mice.

We performed ATAC-seq in PVT from WT and *Polg* mice after FACS sorting of nuclei that were positive for NeuN (i.e. neuronal nuclei). Initial analysis has not showed any changes in chromatin accessibility between these two genotypes. We have therefore decided to extend the analysis to perform ChIP-sequencing of neuronal nuclei from PVT as well as other brain regions of interest.

Performing ChIP-sequencing is quite difficult with limited cell/nuclei, such as what we have available in our setup. From one PVT, we are able to isolate 2000 to 6000 neuronal nuclei, depending on the quality of nuclei preparation and FACS efficiency. This is far below the input required for almost all ChIP-sequencing methods. Recently, a new method was published termed RELACS [7]. Using RELACS, ChIP-sequencing of ~1000 nuclei is feasible, at least for histone marks and highly abundant DNA interacting proteins (such as CTCF). Cells with depletion of mtDNA due to expression of dominant negative *Polg* show loss of specific histone marks, but not an overall deregulation [8].

Based on this data, we want to map changes to acetylation of specific histone marks. The RELACS method is close to being implemented in the laboratory. Using RELACS for ChIP-sequencing analysis of histone modifications in neurons from the *Polg*-mice, we aim to make the first dataset showing the *in vivo* changes to the epigenetic landscape as a consequence of mitochondrial dysfunction in a brain region- and neuron-specific manner.

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５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計4件（うち招待講演　0件／うち国際学会　1件）

１．発表者名 Emilie Kristine Bagge
２．発表標題 PCR-free method reveals a highly brain region-specific mtDNA mutation spectrum in CaMKIIα-Polg mice
３．学会等名 Neuro2019, Annual meeting of the Japan Neuroscience Society（国際学会）
４．発表年 2019年

１．発表者名 Emilie Kristine Bagge
２．発表標題 PCR-free method reveals a highly brain region-specific mtDNA mutation spectrum in CaMKIIα-PolgD181A mice
３．学会等名 Cold Spring Harbor Asia, Mitochondria and Metabolism in Health and Disease
４．発表年 2019年

１．発表者名 Emilie Kristine Bagge
２．発表標題 Mitochondrial replication instability induces a highly brain region-specific mtDNA mutation spectrum
３．学会等名 Luncheon Meeting of the All-RIKEN Aging Project
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２．発表標題 PCR-free method reveals a highly brain region-specific mtDNA mutation spectrum with aging in CaMKIIα-PolgD181A mice
３．学会等名 Meeting of the All-RIKEN Aging Project
４．発表年 2020年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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