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Press Release

Keio University  
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## Researchers Decode the Pathology of Glial Cells in Muro Disease (Kii ALS/PDC), a Difficult-to-Treat Neurological Disorder in the Kii Peninsula, Paving the Way for iPS Cell-Based Drug Discovery

A research group has made significant strides in understanding Muro disease—a prevalent condition in the Kii Peninsula of Japan that manifests as an amyotrophic lateral sclerosis (ALS) and Parkinson’s disease dementia complex. The group included Professor Hideyuki Okano (currently director of the Keio University Regenerative Medicine Research Center) and Project Assistant Professor Nicolas Leventoux (currently a research scientist at RIKEN) from the Department of Physiology at the Keio University School of Medicine, Assistant Professor Satoshi Morimoto (currently project associate professor at the Keio University Frontier Research & Education Collaborative Square [K-FRECS]), Guest Professor Yasumasa Kokubo from the Mie University Graduate School of Regional Innovation Studies, and colleagues. By using induced pluripotent stem cell (iPS cell) models<sup>1</sup> derived from Muro disease patients, the team successfully created astrocytes<sup>2</sup>—cells believed to play a crucial role in the disease—and revealed a significant decrease in the expression of *CHCHD2*,<sup>3</sup> a gene and protein critical for mitochondrial function. Further, they identified a decline in the neuroprotective functions of these astrocytes, developed methods to restore these functions, and confirmed abnormalities in *CHCHD2* in astrocytes within the brains and spinal cords of actual patients.

For over 300 years since Muro disease was first documented, its cause has remained unknown. In a major step forward, researchers have employed patient-derived iPS cell models and advanced cell differentiation techniques to elucidate critical aspects of the disease’s pathology, paving the way for iPS cell-based drug discovery.<sup>4</sup>

This breakthrough sheds light on the previously enigmatic Muro disease—an illness for which no animal disease models even existed—significantly advancing our understanding and opening new avenues for developing treatment.

These research findings were published online on May 16, 2024, in the renowned scientific journal *Acta Neuropathologica*, which focuses on the pathology and etiology of neurological disorders.

### 1. Main Points of Research

- Muro disease (Kii ALS/PDC), a serious neurodegenerative disorder prevalent in the Kii Peninsula of Japan, manifests in patients as amyotrophic lateral sclerosis (ALS),<sup>5</sup> Parkinson’s disease symptoms,<sup>6</sup> or dementia.

- In Muro disease, abnormalities occur in various types of neurons and glial cells in the brain and spinal cord, suggesting that astrocytes, which are distributed throughout the brain and spinal cord, play a crucial role in its development.
- This research successfully established induced iPS cells from patients' blood cells and efficiently differentiated them into astrocytes using a novel method.
- Analysis of these patient-derived astrocytes revealed a significant decrease in the expression of *CHCHD2*, a gene and protein critical for mitochondrial function. This was accompanied by a reduction in mitochondria, abnormalities in cristae<sup>7</sup> morphology, and a decline in the neuroprotective functions of the astrocytes.
- Furthermore, by introducing genes to boost the expression of *CHCHD2*, the researchers were able to partially restore the function of patient-derived astrocytes. They also identified small molecules that improve mitochondrial function and promote the recovery of the astrocytes' functionality.
- Finally, examination of astrocytes in the brains and spinal cords of deceased patients revealed a reduction in the expression of the *CHCHD2* protein.

## 2. Background of Research

The southern coast of the Kii Peninsula, facing the Kumano-nada Sea of the Pacific Ocean, was known as “Muro of the former Kii Province” until the Edo period. In the Kozagawa River region, which flows through the central part of this area, locals had long pointed to what they called *Koza no ashinae-byo* (lit. “Koza atrophy of the leg”). At the end of the Meiji era, Kinnosuke Miura, a pioneer of neurology in Japan, noted a high incidence of ALS along the southern coast of the Kii Peninsula from Kii to Kisei.

Furthermore, Kiyoshi Kimura and Yoshiro Yase of the Department of Psychiatry at Wakayama Medical University discovered that *Koza no ashinae-byo*, which was endemic in Muro, was in fact ALS, and identified the Kii Peninsula's southern coast as a high-incidence area, comparable to Guam and West New Guinea.

Subsequent research indicated that ALS in the Kii Peninsula (Kii ALS) was characterized neuropathologically by the frequent presence of Alzheimer's neurofibrillary tangles (NFTs)<sup>8</sup> in the central nervous system, making it similar to ALS found in other high-incidence areas.

Although the incidence of ALS was thought to have drastically decreased in the 1980s, Shigeki Kuzuhara, the founding professor of Neurology at Mie University, discovered in 1990 that there were still a significant number of ALS patients living in the region. Moreover, the same communities experienced a high incidence of Parkinsonism-dementia complex (PDC), a disease prevalent in Guam (Kii PDC). Today, Kii ALS and Kii PDC are considered the same disease neuropathologically and are collectively referred to as “ALS/PDC”.

Despite over 300 years passing since the disease was first documented, the causes of its high incidence remain unclear, with both environmental and genetic factors yet to be determined (Kuzuhara S, et al. Ann Neurol 2001, Kuzuhara S. Rinsho Shinkeigaku 2021).

In recent years, a group led by Hideyuki Okano at Keio University has established a novel drug discovery approach, including a platform for pathological analysis and iPS cell-based drug discovery for diseases with unidentified genetic causes and pathological backgrounds (Fujimori K et al. *Nature Medicine* 2018, Okano H and Morimoto S. *Cell Stem Cell* 2022, Morimoto S, et al. *Cell Stem Cell* 2023).

### 3. Research Design and Findings

Based on the background described above, a collaborative research effort between Keio University (Project Assistant Professor Nicolas Leventoux, Assistant Professor Satoshi Morimoto, Professor Hideyuki Okano, and others at the time of the research) and Mie University (Guest Professor Yasumasa Kokubo) led to the establishment of iPS cells from the blood cells of five Kii ALS/PDC patients.

Furthermore, the researchers successfully differentiated astrocytes, which are crucial in the pathology of Kii ALS/PDC, from these patient-derived iPS cells using a novel technique (Figure 1).

The patient-derived astrocytes tended to exhibit the morphology of activated astrocytes<sup>9</sup>, suggesting some abnormalities in the cells themselves (Figure 1). RNA-seq analysis<sup>10</sup> of these cells revealed a significant decrease in the expression of the gene which encodes *CHCHD2*, a protein vital for mitochondria (an organelle that supports cells from the inside) to function. Additionally, the patient-derived astrocytes showed decreased and abnormally agglutinated *CHCHD2* protein (Figure 1), fewer mitochondria as well as cristae abnormalities, decreased glutamate reuptake capacity<sup>11</sup>, and reduced activity (lower oxygen consumption rate). Consequently, the neuroprotective effect of these patient-derived astrocytes on neurons was diminished. Moreover, the study confirmed that treatment with elamipretide, a drug that improves mitochondrial function, or restoring *CHCHD2* gene expression could improve glutamate reuptake capacity.

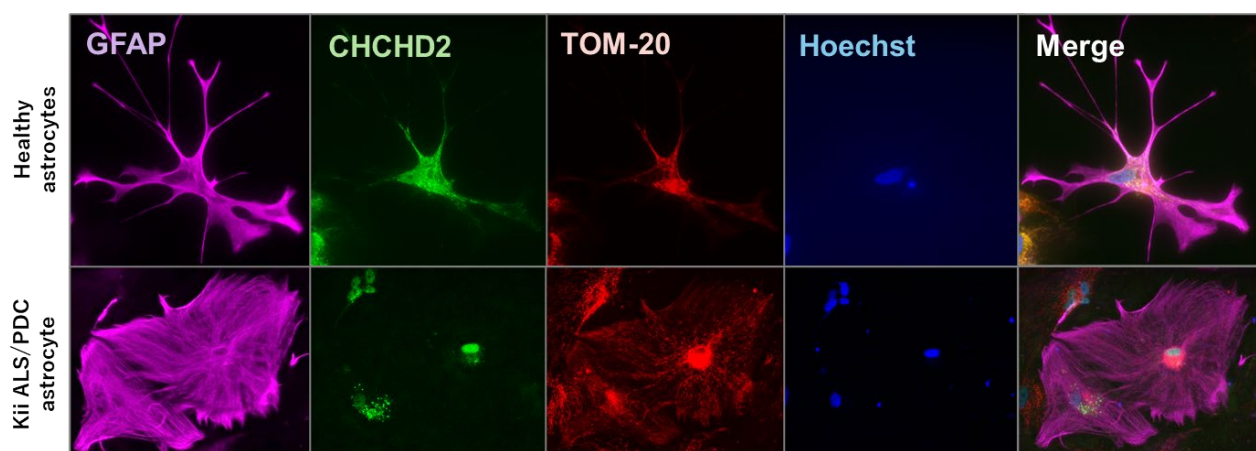


Figure 1. Abnormalities in astrocytes and *CHCHD2* protein and mitochondria derived from iPS cells of healthy individuals and patients

Significantly, examination of autopsy brains and spinal cord samples from deceased patients revealed that, similar to the changes observed in iPS cell-derived astrocytes, *CHCHD2* protein levels were reduced in astrocytes in the motor cortex and anterior horn of the spinal cord (regions affected in ALS and Kii ALS; Figure 2).

Figure 2. Quantification of decreased *CHCHD2* protein in astrocytes in patient brains

This study has demonstrated that astrocytes derived from patient iPS cells can replicate the pathological conditions occurring in the brains and spinal cords of patients (Figure 3). This finding indicates that disease-specific iPS cells are highly effective models for studying diseases like Kii ALS/PDC, the causes of which—including apparent genetic abnormalities—remain unknown. Such models are particularly valuable for investigating sporadic diseases<sup>12</sup>, which are challenging to approach using animal models. Furthermore, researchers believe that the treatment methods targeting mitochondria that showed efficacy in iPS cell-derived astrocytes provide crucial insights for iPS cell-based drug discovery.

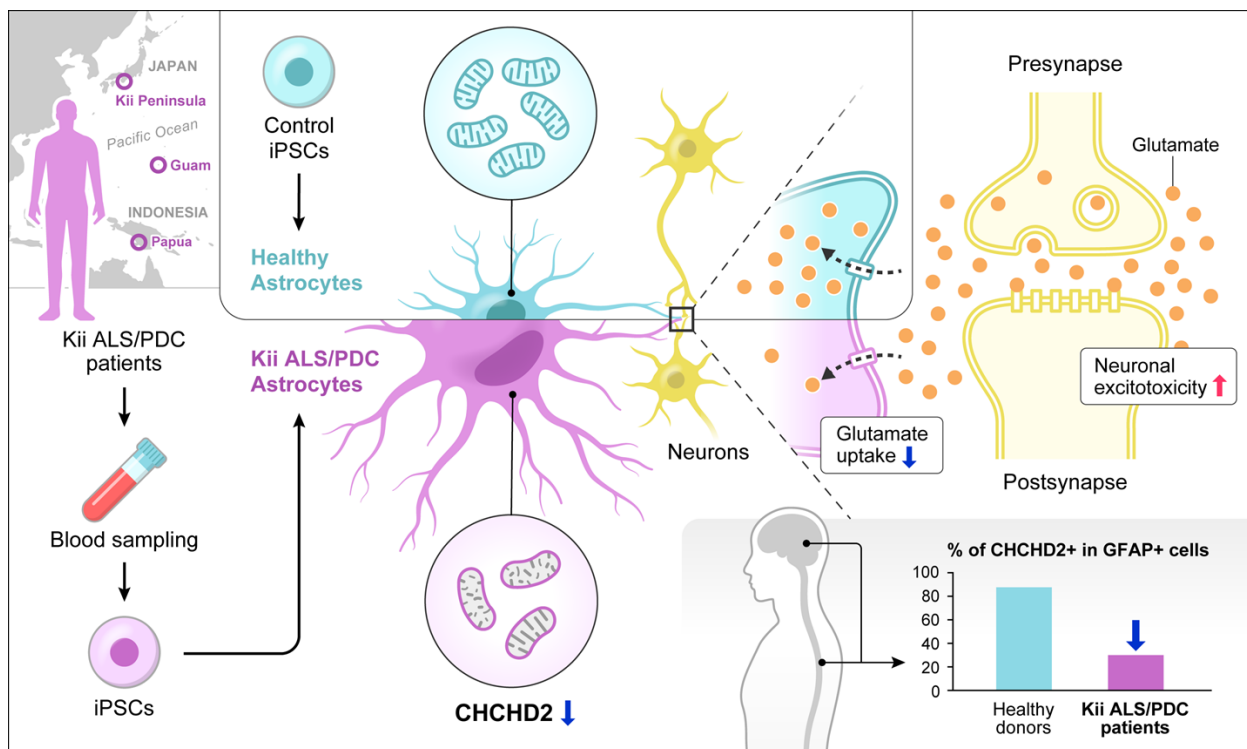


Figure 3. Overview of research findings

The university extends its deep appreciation to the patients and their families who cooperated with this research in the pursuit of overcoming the disease.

### 5. Special Notes

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#### Details of Journal Article

Title: Aberrant *CHCHD2*-associated mitochondriopathy in Kii ALS/PDC astrocytes

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#### Glossary and References

\*1 Induced pluripotent stem (iPS) cells: pluripotent stem cells generated by introducing specific factors into somatic cells. These human-induced pluripotent stem cells can model diseases by taking on the complete genetic information of the patient.

\*2 Astrocytes: a type of glial cell found in the brain and spinal cord. They primarily support and protect nervous tissue, while also filling gaps between neurons, supplying nutrients, eliminating metabolic waste, and regulating the electrical and chemical environment of neurons.

\*3 *CHCHD2*: a protein involved in mitochondrial function, crucial for the process of oxidative phosphorylation, which generates ATP (adenosine triphosphate). Mutations in this gene can affect mitochondrial function and are known to be associated with Parkinson's disease and amyotrophic lateral sclerosis (ALS).

\*4 iPSC cell-based drug discovery: a new drug development method that uses patient iPSC cell models,

rather than traditional animal models, to develop drugs and treatments.

\*5 Amyotrophic lateral sclerosis (ALS): a neurodegenerative disease characterized by the selective degeneration of motor neurons, leading to muscle paralysis, difficulties swallowing, and respiratory failure. It typically results in a fatal outcome within 2–4 years, and there is currently no cure.

\*6 Parkinson's disease symptoms: the four main symptoms associated with Parkinson's disease are bradykinesia (slowed movement), tremors (muscular), rigidity (stiffness of the limbs), and postural instability (difficulty maintaining balance).

\*7 Cristae: the folded parts of the inner mitochondrial membrane. Mitochondria, the energy-producing centers of the cell, have a double membrane structure consisting of an outer and inner membrane, with the inner membrane containing essential proteins for electron transport and ATP synthesis.

\*8 Alzheimer neurofibrillary tangles (NFTs): one of the pathological characteristics of Alzheimer's disease, these are abnormally-shaped protein aggregates primarily composed of tau protein, found inside neurons.

\*9 Activated astrocytes: astrocytes that are more active than normal and are involved in various physiological processes. They may become activated in response to brain injury or inflammatory diseases, proliferating, secreting inflammatory cytokines, promoting angiogenesis, and supporting neuronal regeneration.

\*10 RNA-seq analysis: a molecular biology technique for comprehensive and quantitative analysis of gene transcripts (mRNA) expression.

\*11 Glutamate reuptake capacity: the ability of nervous system cells to reabsorb glutamate. Glutamate is one of the most abundant excitatory neurotransmitters in the central nervous system, facilitating communication between neurons when released into the synaptic cleft. After its release, reuptake by neural cells controls the stimulation of postsynaptic cells and regulates excitatory signals. Reduced glutamate reuptake in astrocytes leads to excessive accumulation of glutamate in the synaptic cleft, causing excitotoxicity, and resulting in neuronal damage or death. Proper regulation of glutamate reuptake is therefore crucial for maintaining normal neural function.

\*12 Sporadic diseases: diseases whose causes are unknown or have no specific identifiable cause. This term is used when the cause of symptoms or the disease is not understood.

\*Please direct any requests or inquiries to the contacts listed below in advance of any press coverage.

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