

# Gene analysis of the Parkin-related gene Klokin 1 in Tokushima National Hospital of 2023-2024

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## Abstract

We found that the parkin protein is closely related to mitochondria in our research on PARK 2 [1-3]. That is, Parkin reported that it localizes to mitochondria in proliferating cells and promotes mitochondrial transcription and replication.

We found that the parkin protein localizes to the mitochondria of proliferating cells and promotes transcription and replication of mitochondrial genes [4].

On the other hand, since parkin protein does not have a mitochondrial translocation signal, it is assumed that an unknown protein that binds to parkin protein and transports it to mitochondria is involved in the translocation of parkin protein to mitochondria. We reported the existence of a novel protein, Klokin 1, which binds to the parkin protein [5]. Elucidation of the cause and pathogenesis of Parkinson's disease whole, may lead to the development of new treatments, analysis of gene Klokin 1 wants to proceed to explore the case in the future.

**Key words:** Parkin, Klokin 1, mitochondria biogenesis, PCR,

## Introduction

Parkinson's disease is one of the typical neurodegenerative diseases, and most of them occur sporadically. In this disease, selective degeneration of substantia nigra dopamine-producing cells is observed, and it is assumed that mitochondrial dysfunction of the cells is involved as a pathological condition. However, the cause and mechanism of the mitochondrial disorder remain unknown. On the other hand, in recent years, the causative genes of hereditary Parkinson's disease have been discovered one after another, and attempts are being made to elucidate the pathophysiology of sporadic Parkinson's disease through their analysis. Parkin was discovered as the causative gene (PARK 2) for the most frequently occurring familial Parkinson's disease [4,6]. The parkin protein has ubiquitin ligase (E3) activity, and

mutation of the parkin gene causes a decrease in E3 activity, and the accumulation of harmful substrates causes selective denaturation of substantia nigra dopamine-producing cells[7]. The introduction was estimated. In our research on hereditary Parkinson's disease, especially PARK 2[1-3], we found that the parkin protein is closely related to mitochondria. That is, Parkin reported that it localizes to mitochondria in proliferating cells and promotes mitochondrial transcription and replication[4]. We discovered a novel protein that binds to parkin and transports it to mitochondria, which also has no mitochondrial migration signal, and named it Klokin 1[5]. Klokin 1 is a splicing variant of chondroitin polymerizing factor (ChPF), and it was revealed that ChPF has the effect of saccharifying parkin together with another splicing variant, ChPF $\Delta$ 996[5].

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Table 1 Patients with Parkinson's disease who underwent genetic testing from April 2023 to March 2024

NO	Sex	birthdate	Age 2023/12/31	age of onset	disease	family history	consang unity	blood drawing date	Klokin 1
394	F	1974/1/19	49	?	PD?	+	-	2023/8/17	no
395	F	1947/12/10	76	66	PD	-	+	2023/9/19	no
396	M	1948/3/6	75	75	PD	-	+	2023/12/28	no

## Materials and Methods

### Subjects and Sample Collection

We analyzed the Klokin 1 gene and in patients with familial Parkinson's disease. The subjects were patients with Parkinson's disease who visited to Tokushima National Hospital and referral patients from other facilities. They were juvenile-onset patients and/or had a family history of the disease or were in a consanguineous marriage. Healthy volunteers were used as subjects.

### Ethics committee

This study was carried out with the approval of the Tokushima National Hospital Ethical Review Board.

### DNA Isolation

Peripheral blood mononuclear cell (PBMC) was isolated from fresh whole blood with heparin using QIAGEN DNA Blood Maxi Kit (QIAGEN Inc., Germany) following the manufacturer's instructions.

### Klokin 1 PCR and Sequence

All PCR amplifications were performed in an TaKaRa PCR Thermal cycler (TaKaRa Inc., Japan) in standard mixtures of 25  $\mu$ L containing 2x GC Buffer 112.5uL, dNTP Mixture 4uL, 10 pmol of each primer 1.25uL, template <100ng, up to 25uL. The PCR program included one incubation at 95 °C for 5min and 40 amplification cycles (95 °C for 60 s, 53 °C for 60 s and 72 °C for 60 s), followed by one final extension incubation of 7 min at 72 °C. The PCR products were separated on a 1.8% agarose gel, stained with ethidium bromide and visualised on a UV transilluminator (ATTO Printgraph2M,

Tokyo Japan) . After that, single band was cut out using the QIAGEN Gel Extraction Kit (QIAGEN Inc., Germany) and a direct sequence was performed following the manufacturer's instructions.

## Results

As shown in Table1, we analyzed 3 patients (1male,) 2024 3month present. Their age was  $66.66 \pm 15.31$  (mean  $\pm$  SD) years old. Consanguineous marriage was observed in 2 out of 3 cases. We did not find the exon deletions and point mutations in the Klokin 1 gene in the same part of people.

## Discussion

It is certain that parkin also functions as a ubiquitin ligase (E3) in terms of gene structure, and that its mutation can cause ER stress. However, it remains highly controversial whether parkin gene deficiency is caused by decreased E3 activity in parkin. Recent findings indicate that parkin gene deficiency has mitochondrial disorders, that parkin has a non-specific ameliorating effect on mitochondrial disorders, and that parkin is the causative gene of hereditary Parkinson's disease that causes other mitochondrial disorders. It has been suggested that it is also related to transcripts. Analysis of the gene Klokin 1 that transports Parkin to mitochondria may lead to elucidation of the cause and etiology of Parkinson's disease as a whole and development of new therapeutic methods, and we would like to continue investigating cases in the future.

## References

1. Kunishige M, Mitsui T, Kuroda Y, et al  
Expanding phenotype and clinical heterogeneity in patients with identical mutation of the parkin gene. *Eur Neurol*, 2004;51: 183-185.
2. Kuroda Y, Mitsui T Parkin gene related neuronal multisystem disorder. *J Neurol Neurosurg Psychiatry*, 2002;72: 419-420.
3. Kuroda Y, Mitsui T Parkin gene related neuronal multisystem disorder. *J Neurol Neurosurg Psychiatry*, 2002;72: 419-420.
4. Kuroda Y, Mitsui T, Kunishige M, et al  
Parkin enhances mitochondrial biogenesis in proliferating cells. *Hum Mol Genet*, 2006;15: 883-895.
5. Kuroda Y, Sako W, Goto S, et al. Parkin interacts with Klokin1 for mitochondrial import and maintenance of membrane potential. *Hum Mol Genet*. 2012; 21: 991-1003.
6. Kitada T, Asakawa S, Hattori N, et al  
Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature*, 1998;392: 605-608.
7. Shimura H, Hattori N, Kubo S, et al  
Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. *Nat. Genet*, 2000;25: 302-305.