

# Parkin gene analysis of familial Parkinson's disease in Tokushima National Hospital of 2023-2024

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## Abstract

Parkin is a neuroprotective protein with many functions, including maintaining mitochondrial homeostasis. Recent evidence suggests that Parkin is recruited from the cytoplasm to damaged mitochondria with low membrane potential. We found that intracellular localization of Parkin changed with cellular growth phase. Parkin was preferentially localized in the mitochondria of cultured cells in the late log phase of growth. The mitochondria with large amounts of Parkin showed preserved membrane potentials even during treatment with carbonyl cyanide m-chlorophenylhydrazone. Elucidation of the cause and pathogenesis of Parkinson's disease whole, may lead to the development of new treatments, analysis of gene PARK2 wants to proceed to explore the case in the future.

**Keywords:** Parkin, familial Parkinson's disease, mitochondria biogenesis, PCR, biopsy

## Introduction

Parkinson's disease (PD) is a prevalent age-associated progressive neurodegenerative movement disorder primarily characterized by the death of nigrostriatal dopaminergic neurons and the presence of intracytoplasmic proteinaceous inclusions, termed Lewy bodies. Although PD is a sporadic disorder of unclear etiology, recent studies have demonstrated the importance of genetic contributions to PD that may provide insight into the mechanistic details of disease pathogenesis. Mutation of the gene encoding Parkin (PARK2) plays a major etiopathogenic role in autosomal recessive juvenile parkinsonism[1] PARK2 contains RING finger motifs and functions as a ubiquitin-protein ligase for protein degradation[2,3].

Recently, important insight has been obtained into the mechanism by which Parkin regulates mitochondrial homeostasis.

Parkin translocates from the cytoplasm to accumulate on depolarized mitochondria and promotes their degradation by autophagy[4]. Several studies have suggested that PTEN-induced putative kinase 1 (PINK1) is required for Parkin-mediated mitochondrial autophagy, wherein it recruits Parkin to dysfunctional mitochondria and promotes their degradation[5-7]. However, we and others have reported that Parkin can associate directly with mitochondria under basal conditions[8-10]. Recent studies have detected Parkin in the mitochondria of untreated cultured cells, although it is mainly present in the cytoplasm[5,10-12]. We previously reported that Parkin is localized in the mitochondrial matrix during proliferation and is rapidly released to the cytosol in differentiated or quiescent states. We also found that Parkin enhances mitochondrial transcription and replication in vitro and in vivo[9], which was confirmed by a recent study[10].

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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 uinity	blood drawing date	PARK2	biopsy
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 parkin 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.

### Parkin PCR

All PCR amplifications were performed in an TaKaRa PCR Thermal cycler (TaKaRa Inc., Japan) in standard mixtures of 25  $\mu$  L containing PrimeSTAR Max Premix ( $2 \times$ ) 12.5uL, 10 pmol of each primer 2.5uL, 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 1.7% agarose gel, stained with ethidium bromide and visualised on a UV transilluminator (ATTO Printgraph2M., Tokyo Japan).

## Results

As shown in Table1, we analyzed 3 patients (2male) 2023 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 mutations in the Parkin gene in the same part of people.

## Discussion

We reported that Parkin was present in both the cytoplasm and mitochondria at basal conditions and that its intracellular localization changes with growth phase. Parkin was mainly located in the cytoplasm from the lag growth phase to the early log phase, but a portion of Parkin appeared to be located in the mitochondria from the late log growth phase to the plateau phase. Unlike previous reports, its mitochondrial localization was not associated with reduced membrane potential during the log growth phase.

Elucidation of the cause and pathogenesis of Parkinson's disease whole, may lead to the development of new treatments, analysis of gene PARK2 want to proceed to explore the case in the future.

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