

Gene analysis of Parkinson's disease in Tokushima National Hospital

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Received 26 February 2016; received in received from 27 February 2016; accepted 6 March 2016

Abstract

Parkin is a neuroprotective protein with many functions, including maintaining mitochondrial homeostasis. The pivotal role that parkin plays in maintaining neuronal survival is underscored by our current recognition that parkin dysfunction represents not only a predominant cause of familial Parkinsonism but also a formal risk factor for the more common, sporadic form of Parkinson's disease. The importance of genetic contributions to PD is that they may provide insight into the mechanistic details of disease pathogenesis. We performed gene analysis of parkin in Tokushima National Hospital. The present report describes the results for the last year. We analyzed 14 patients (7 male, 7 female) in 2015. Their age was 64.1 ± 14.9 (mean \pm SD) years old, (male 60.0 ± 17.0 , female 68.8 ± 11.5). Onset age was 64.1 ± 16.2 (male 59.8 ± 21.9 , female 62.2 ± 11.9). Eleven cases had a family history of the disease. Consanguineous marriage was observed in five cases. We did not find mutations in the parkin gene in any of the cases. Elucidation of the cause and pathogenesis of Parkinson's disease whole, may lead to the development of new treatments. Analysis of the PARK2 gene wants to proceed to explore the case in the future.

Key words: Parkin, 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, called 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 parkinsonism¹. PARK2 contains RING finger motifs and functions as a ubiquitin-protein ligase for protein degradation [2,3]. We carried out gene analysis of parkin in Tokushima National Hospital. The present report describes the results for the last one year.

Materials and Methods

Subjects and Sample Collection

We analyzed the parkin gene in patients with familial Parkinson's disease. The subjects were patients with Parkinson's disease in Tokushima National Hospital. 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. Total DNA in PBMCs was extracted using the QIAamp DNA Blood Mini Kit (QIAGEN Inc., Germany) following the manufacturer's instructions.

Parkin PCR

All PCR amplifications were performed in a TaKara PCR Thermal cycler (TaKaRa Inc., Japan) in standard mixtures of 25 μ L containing 1 \times PCR buffer, 10 pmol of each primer, 2.5 nM dNTP, 2.5 U of Taq DNA polymerase. The PCR program included one incubation at 95 $^{\circ}$ C for 5 min and 40 amplification cycles (95 $^{\circ}$ C for 60 s, 53 $^{\circ}$ C for 60 s and 72 $^{\circ}$ C for 60 s), followed by one final extension incubation of 7 min at 72 $^{\circ}$ C. The PCR products were separated on a 1.7% agarose gel, stained with ethidium bromide and visualized on a UV transilluminator (ATTO Printgraph2M., Tokyo Japan).

Results

As shown in Table1, we analyzed 14 patients (7 male, 7 female) in 2015. Their age was 64.1 \pm 14.9 (mean \pm SD) years old, (male 60.0 \pm 17.0, female 68.8 \pm 11.5). Onset age was

64.1 \pm 16.2 (male 59.8 \pm 21.9, female 62.2 \pm 11.9). Eleven cases included a family history of the disease. Consanguineous marriage was observed in five cases. We did not find mutations in the parkin gene in any of the cases.

Discussion

Elucidation of the cause and pathogenesis of Parkinson's disease whole has led to the development of new treatments. Recently, important insights have 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 autophagy⁴. Several studies have suggested that PTEN-induced putative kinase 1 (PINK1) is required for parkin-mediated mitochondrial autophagy. This 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].

We reported that parkin was present in both the cytoplasm and mitochondria at basal conditions and that its intracellular localization changes with the growth phase. Parkin was mainly located in the cytoplasm from the lag growth phase to the early log phase, but some 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 the PARK2 gene wants to proceed to explore the case in the future.

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Table 1. Summary of patients with Parkinson's disease examined in the present study.

NO	Sex	birthdate	Age 2016/2/26	age of onset	disease	family history	consangui nity	blood drawing date	PARK2	Klokin 1	biopsy
330	F	1961/4/22	53	52	PD	-	+	2015/1/9	no	no	no
331	F	1943/2/26	72	61	PD	-	+	2015/1/29	no	no	no
332	F	?	?	-	-	-	-	2015/2/19	no	no	no
333	M	1953/11/25	61	60	-	+	-	2015/3/17	no	no	no
334	M	1942/7/5	73	71	-	+	+	2015/3/26	no	no	no
335	M	1944/2/4	72	71	PD	+	-	2015/5/28	no	no	no
336	F	1940/12/29	75	69	PD	+	-	2015/8/11	no	no	no
337	M	1953/1/14	63	-	-	+	?	2015/10/5	no	no	no
338	M	1972/7/10	43	22	PD	+	-	2015/10/6	no	no	no
339	M	1984/5/28	31	-	なし	+	-	2015/11/16	no	no	no
340	M	1938/9/30	77	75	PD	+	-	2015/12/17	no	no	no
341	F	1955/1/18	61	59	PD	+	+	2015/12/22	no	no	no
342	F	1949/3/31	66	50	PD	+	?	2016/1/28	no	no	no
343	F	1929/12/10	86	82	PD	+	+	2016/2/9	no	no	no