Mutation in the gene analysis of parkin and Klokin 1 in Tokushima National Hospital

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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. Elucidation of the cause and pathogenesis of Parkinson's disease whole, may lead to the development of new treatments, analysis of gene PARK2 and Klokin 1 want to proceed to explore the case in the future.

Keywords: Parkin, Klokin 1, 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 insights into the mechanistic details of the 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]. We found a novel protein, named Klokin 1, that carries parkin to the mitochondria. Klokin 1, a splicing variant of human chondroitin polymerizing factor (ChPF) potentially attenuated cellular apotosis with or without parkin [4]. In the present study, we analyzed.

Materials and Methods

We analyzed the parkin gene and the Klokin 1 gene in patients with familial Parkinson's disease. The subjects were patients with d Parkinson's disease who vivited to Tokushima National Hospital. They were juvenile-oncet 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

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using the QIAamp DNA Blood Mini Kit (QIAGEN Inc., Germany) following the manufacturer's instructions.

parkin & Klokin 1PCR

All PCR amplifications were performed in a TaKara PCR Thermal cycler (TaKaRa Inc Japan) in standard mixtures of 25μL containing PCR buffer, pmol of each primer, 2.5 nM dNTP, 2.5 U of Taq DNA polymerase, The PCR program included one incubation at 95˚C for 1min and amplification cycles (for 95˚C for 60s, 53˚C for 60s, and 72˚C for 60s). There were seven followed by one final extension incubation of 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. The primer design is shown in Table 1 [1-3].

Biopsy

Skin biopsies were obtained from one patient and one control biopsy. A punch biopsy of 4mm was performed in the forearm skin of some patients. We washed the obtained skin with 70% ethanol and PBS three times. We scraped the sample to 1mm2 on a laboratory dish. The skin sample was left in a 6cm dish. Four ml of Rat Fibroblast Growth Medium (Cell Application San Diego, CA) was added and cultured at 37˚C in a CO_2 incubator

Table 1. Primer pairs used in the present study

Parkin

Primer sequences and sizes of expectd PCR products				
Exon	primer	Forward(5'-3')	Reverse(5'-3')	product size(bp)
1	EX1	GCGCGGCTGGCGCCGCTGCGCGCA	GCGGCGCAGAGAGGCTGTAC	112
2	EX2	ATGTTGCTATCACCATTTAAGGG	AGATTGGCAGCGCAGGCGGCATG	308
3	EX3	ACATGTCACTTTTGCTTCCCT	AGGCCATGCTCCATGCAGACTGC	427
4	EX4inner	AGGTAGATCAATCTACAACAGCT	CAGGGTCAAGGTGAGCGTTGCCTGC	121
4	EX4outer	ACAAGCTTTTAAAGAGTTTCTTGT	AGGCAATGTGTTAGTACACA	261
5	EX5	ACATGTCTTAAGGAGTACATTT	TCTCTAATTTCCTGGCAAACAGTG	227
6	EX6	AGAGATTGTTTACTGTGGAAACA	GAGTGATGCTATTTTTAGATCCT	268
7	J-17inner	GAGCCCCGTCCTGGTTTCC	CCACACAAGGCAGGGAGTAGCCAA	137
7	J-17outer	TGCCTTTCCACACTGACAGGTACT	TCTGTTCTTCCATTAGCATTAGAGA	239
8	EX8	TGATAGTCATAACTGTGTGTAAG	ATCGTCTCATTAGCGTCTATCTT	206
9	EX9	GGGTGAAATTTGCAGTCAGT	AATATAATCCCAGCCCATGTGCA	278
10	EX10	ATTGCCAAATGCAACCTMTGTC	TTGGAGGAATGAGTAGGGCATT	165
11	EX11	ACAGGGAACATAAACTCTGACC	CAACACACCAGGCACCTTCAGA	303
12	EX12	GTTTGGGAATGCGTGTTTT	AGAATTAGAAAATGAAGGTAGACA	255

Klokin 1

Primer sequences and sizes of expectd PCR products				
Exon	primer	Forward(5'-3')	Reverse(5'-3')	product size(bp)
1	EX1	TGACACGTCGGACAGCTGCTGCGGCCG	GCGCGCTTCCCGGCCGCTGCCCAG	640
2	EX2	TACAGAGAAGACGTGACCTGCCAG	TGTATCTCCAACCCTTTGCCT	757
3	EX3	TCAGACTGCAACTCCACAACAGGT	AACTTGTCATTTCTCTGGGCTCTC	370
4	EX4-1	ATCCTGAGTGGCACCTACTGCCTA	AGGCTTGGAAATGCATGGGAAAGA	964
4	EX4-2	ATCTACTCTCCAAAGAAGCACCCGC	CACGCCTGCCTACCTTTTCAAAAC	1000

Table 2. Summary of the subjects

NO	Sex	birthdate	Age (2013/3)	age of onset	disease	family history	consanguinity	blood drawing date	PARK2	Klokin 1	biopsy
269	F	1942/8/6	74	73	PD	-	-	3014/5/7	no	no	
270	M	1939/8/12	54	48	PD	-	-	2014/5/9	no	no	
271	F	1960/12/2	48	45	PD	-	-	2014/9/27	deletion Exon3	no	●
272	F	1965/4/3	79	74	PD	-	-	2014/10/5	no	no	
273	M	1934/4/1	47	41	PD	-	-	2014/10/30	no	no	
274	M	1944/12/3	69	?	PD	-	-	2014/11/20	no	no	
275	F	1946/8/23	67	59	PD	+	-	2014/2/17	no	no	
276	F	?	?	?	?	?	?	2014/2/17	?	?	
277	M	1943/2/25	71	65-66	PD	+	-	2014/2/20	no	no	
278	F	1949/10/21	64	60-61	PD	+	-	2014/3/7	no	no	

Table 3. A patient with parkin gene deletion

Age	Sex		sample	
		PARK2 mutation	PBMC	fibrobrast blood drawing date
48	F	deletion Exon3	○	●（2014/3/5）

Results

We analyzed ten patients (four men, women six) this year. Their age was 63.67 ±11.46 (mean±SD) years old (male 60.25 ±11.64, female 66.4±11.84). Onset age was 58.25±12.55 years old (male 51.5±12.62, female 62.3±11.88). Three cases included family history of the disease. The approved cases were not in a consanguineous marriage. The Exon3 mutation of the parkin gene was found in only one case (Figure 1). A skin biopsy was performed on this patient. Human fibroblastic primary culture was performed (Table 3). The Klokin 1 genetic mutation was not found with any of the cases.

Discussion

Elucidation of the cause and pathogenesis of Parkinson's disease whole, may lead to the development of new treatments. The clinical phenotype of homozygous or compound heterozygous PARK2 cases is usually indistinguishable from early onset idiopathic PD, with slowly progressive Levodopa-responsive disease often requiring lower equivalent doses with frequent late motor complications. Atypical and later onset cases are described with prominent dystonia, hyperreflexia and early complications. Interestingly, some cases seem responsive to nicotine [5]. We are going to conduct a genetic analysis of patients with various forms of Parkinson's disease in future.

References

1. Kitada T, Asakawa S, Hattori N, et al. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature. 1998;392: 605–608.

2. Imai Y, Soda M, Inoue H, et al. An unfolded putative transmembrane polypeptide, which can lead to endoplasmin reticulum stress, is a substrate of Parkin. Cell. 2001; 105: 891–902

3. 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

4. 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

5. Lohmann E, Periquet M, Bonifati V, et al. How much phenotypic variation can be attributed to the parkin genotype? Ann Neurol. 2003;54:176–185