Gene analysis of familial Parkinson’s disease in Tokushima National Hospital

Yukiko Kuroda, Ph.D. #1, Masako Sogo, Ph.D. #1, Kazuyuki Kawamura, M.D. #2, Takao Mitsui, M.D. #1,#2

#1. Department of Clinical Research, Tokushima National Hospital, National Hospital Organization, 1354 Shikiji, Kamojima, Yoshinogawa, Tokushima 776-8585 Japan

#2. Department of Neurology, Tokushima National Hospital, National Hospital Organization, 1354 Shikiji, Kamojima, Yoshinogawa, Tokushima 776-8585 Japan

Received 26 February 2015; received in revised form 27 February 2015; accepted 6 March 2015

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 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]. 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. We found a novel protein—Klokin 1—that transports Parkin to
the mitochondria. Klokin 1, a splice variant of
human chondroitin polymerizing factor
(ChPF), may attenuate cellular apoptosis
with or without Parkin [13].

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 visited to
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 & 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 CO2 incubator.

Table 1. Primer sequences and sizes of expected PCR products

<table>
<thead>
<tr>
<th>Exon</th>
<th>primer</th>
<th>Forward(5'-3')</th>
<th>Reverse(5'-3')</th>
<th>product size(bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EX1</td>
<td>GGGCGCTGGCCGCTGGGCGCA</td>
<td>GGGCGAGGAGGCTGAC</td>
<td>112</td>
</tr>
<tr>
<td>2</td>
<td>EX2</td>
<td>ATGTGGTACACATTTAGGGA</td>
<td>AGATGGCAGCGGAGGCCAGT</td>
<td>308</td>
</tr>
<tr>
<td>3</td>
<td>EX3</td>
<td>AATGTCATCTTTTGTCCCT</td>
<td>AGGGCTATGGCCATGGCGTC</td>
<td>427</td>
</tr>
<tr>
<td>4</td>
<td>EXinner</td>
<td>AGGTAGATCAATCTCAACAGCT</td>
<td>CAGGGTGTAAGGAGCTGCTG</td>
<td>121</td>
</tr>
<tr>
<td>5</td>
<td>EXouter</td>
<td>AAGCTTCTTAAGGAGTTTCTTGT</td>
<td>AGGGCAATGGGTAGAACA</td>
<td>261</td>
</tr>
<tr>
<td>6</td>
<td>EX5</td>
<td>ACATGTCTCTAGAAGGATACATT</td>
<td>TCCTCAATTTCTGGCAGAAGATG</td>
<td>227</td>
</tr>
<tr>
<td>7</td>
<td>EX6</td>
<td>ATGAGATTTTATCTGGAAACA</td>
<td>GAGTGAAGCTATTTTAGTCTT</td>
<td>268</td>
</tr>
<tr>
<td>8</td>
<td>J-1inner</td>
<td>TGGCCGCTGTTCGGCC</td>
<td>CCAACAGAGGAGGAGATGCAA</td>
<td>137</td>
</tr>
<tr>
<td>9</td>
<td>J-17outer</td>
<td>GGCTCTCCACACGCTAGTACT</td>
<td>TCTGTCCTCCATCATGATAGA</td>
<td>239</td>
</tr>
<tr>
<td>10</td>
<td>EX8</td>
<td>TGGATGTCATATCTGGTGAAG</td>
<td>ATCGTCTACATTAGCTTCT</td>
<td>206</td>
</tr>
<tr>
<td>11</td>
<td>EX9</td>
<td>GGTGAATTGTCGTACAGT</td>
<td>AATATACCCAGAGATGTC</td>
<td>278</td>
</tr>
<tr>
<td>12</td>
<td>EX10</td>
<td>ATGGCCAAAGACCTCTCTGTC</td>
<td>TGGAGGAGATAATGAGGCT</td>
<td>165</td>
</tr>
<tr>
<td>13</td>
<td>EX11</td>
<td>GCAGGGAAGATACTCTGACC</td>
<td>CAAACACAGACACTTCAAGA</td>
<td>303</td>
</tr>
<tr>
<td>14</td>
<td>EX12</td>
<td>GGGGGAAGCTGCGTCTT</td>
<td>AGAATTAGAAATGAGATAGA</td>
<td>255</td>
</tr>
</tbody>
</table>

Klokin 1

<table>
<thead>
<tr>
<th>Exon</th>
<th>primer</th>
<th>Forward(5'-3')</th>
<th>Reverse(5'-3')</th>
<th>product size(bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EX1</td>
<td>TGGACAGTGGGAGACCTGGGTCGCGC</td>
<td>GGGGCTCTCCGCGGCTGGGCGCAG</td>
<td>640</td>
</tr>
<tr>
<td>2</td>
<td>EX2</td>
<td>TACAGAAGAGATCTGACCTGCA</td>
<td>TGCAATCCACCCTTGGCTC</td>
<td>757</td>
</tr>
<tr>
<td>3</td>
<td>EX3</td>
<td>TGGACAGTGGGACCTCCGACAGT</td>
<td>AACTGTGCTTTCTGCGCTGTC</td>
<td>370</td>
</tr>
<tr>
<td>4</td>
<td>EX4-1</td>
<td>ATGGCGATGGGACCTCCGACAGT</td>
<td>AGGGTCTGGAATGCTGGGAAAGA</td>
<td>964</td>
</tr>
<tr>
<td>5</td>
<td>EX4-2</td>
<td>ATCTACTCTCCCAAGAGGCCACCCGC</td>
<td>CACGGCTGCTACCTCTTCCAAGAC</td>
<td>1000</td>
</tr>
</tbody>
</table>
Table 2. Summary of the subjects

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

Table 3. A patient with parkin gene deletion

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>PARK2 mutation</th>
<th>sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>F</td>
<td>deletion Exon3</td>
<td>○</td>
</tr>
</tbody>
</table>

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 [14]. We are going to conduct a genetic analysis of patients with various forms of Parkinson’s disease in future.

References


