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Dominant-negative effect of mutant valosin-containing protein in aggresome formation

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Abstract Lewy bodies (LBs) are the pathologic hallmark of Parkinson's disease. Recent studies revealed that LBs exhibit several morphologic and molecular similarities to aggresomes. Aggresomes are perinuclear aggregates representing intracellular deposits of misfolded proteins. Recently, valosin-containing protein (VCP) was one of the components of LBs, suggesting its involvement in LB formation. Here, we showed the localization of VCP in aggresomes induced by a proteasome inhibitor in cultured cells. Cells overexpressing mutant VCP (K524M: D2) showed reduced aggresome formation relative to those overexpressing wild-type and mutant (K251M: D1) VCPs. Our findings suggest that the D2 domain is involved in aggresome formation.

Keywords: Valosin-containing protein; Parkinson's disease; Lewy body; Aggresome

1. Introduction

Parkinson's disease (PD) is characterized histopathologically by the relatively selective loss of dopaminergic neurons in the substantia nigra and the presence of Lewy bodies (LBs) [1]. Recent studies suggest that LBs are related to aggresomes [2]. The latter are formed upon exhaustion of neuronal cell machinery responsible for the degradation of misfolded proteins [3]. Furthermore, aggresomes are formed at the microtubule (MT)-organizing center (MTOC) and are defined as pericentriolar membrane-free cytoplasmic inclusions that contain misfolded, ubiquitinated proteins ensheathed in a cage of the intermediate filament (IF) protein, vimentin [3,4].

Valosin-containing protein (VCP) is a 97-kDa protein and a member of type II ATPases associated with a variety of cellular activities (AAA), which are characterized by the presence of two conserved ATPase domains, also called AAA domains [5]. Recent studies revealed that VCP acts as a molecular chaperone in many apparently unrelated cellular activities [6]. Among these activities, VCP recognizes misfolded proteins such as polyglutamine [7]. Indeed, VCP is recognized in nuclear inclusion bodies of polyglutamine diseases [8]. Moreover, VCP is also recognized in LBs of PD [8,9].

Based on the above background, we postulated that VCP is involved in the formation of aggresomes. To test this, we examined the induction of VCP in aggresomes of cells treated with a proteasome inhibitor, MG132, and the role of VCP in aggresome formation.

2. Materials and methods

2.1. Plasmids and antibodies

We constructed pUHD10-3/VCP^{WT}-Myc, pCMV/FLAG-6c-VCP^{WT}, and pCMV/FLAG-6c-VCP$^{K251M/K524M}$ for this experiment. pUHD10-3/VCP^{WT}-Myc was digested and inserted into the *Xho*I site of the pcDNA3.1 vector (Invitrogen). pcDNA3.1/FLAG-VCP^{WT} was prepared by polymerase chain reaction (PCR) using appropriately designed primers with restriction site (*Bam*HI). The PCR product was inserted into the pcDNA3.1 vector. pcDNA3.1/FLAG-VCP^{K251M}, FLAG-VCP^{K524M} were prepared by using QuikChange Site-Directed Mutagenesis Kit (Stratagene). These point mutants lack two ATPases activity domains such as D1 and D2 [8]. pcDNA3.1/α-synuclein^{WT} and pcDNA3.1/FLAG-IκBα^{WT} were kind gifts from Drs. Suzuki and Chiba (The Tokyo Metropolitan Institute of Medical Science, Tokyo). We prepared FLAG-tagged α-synuclein to examine the interaction between VCP and α-synuclein.

The following antibodies were used in the present study; anti-VCP polyclonal antibody (Santa Cruz), anti-ubiquitin monoclonal antibody (Chemicon), anti-FLAG polyclonal antibody (Affiniti), anti-FLAG-HRP (Affiniti), anti-Myc monoclonal antibody (Santa Cruz), anti-vimentin monoclonal antibody (Sigma), anti-β actin monoclonal antibody (Sigma), anti-γ-tubulin monoclonal antibody (Sigma), and anti-Hsp70 antibody (BD Transduction Laboratories).

2.2. Cell culture and transfection

Kidney cell lines, HEK293 cells were grown in Dulbeccos modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin. Confluent cells were transfected with 9 μg Myc-vector, VCP^{WT}-Myc, 5 μg FLAG-α-synuclein and 2 μg FLAG-IκBα. At 24 h after transfection, the cells were lysed with 500 μl lysis buffer (150 mM NaCl, 50 mM Tris–HCl [pH 7.5], 1.0% nonidet-P40, 10% glycerol, 1 M dithiothreitol) and protease inhibitor cocktail; Complete Mini. The lysate was then centrifuged at 17000 × *g* for 15 min at 4 °C, and then 30 μl volume of the supernatant was used as the "lysate" for SDS–PAGE, while 450 μl volume of the supernatant was used for immunoprecipitation. For immunoprecipitation, 2 μg anti-Myc antibody was added to each 450 μl of the supernatant and the mixture was rotated for 3 h at 4 °C, then centrifuged at 17000 × *g* for 15 min. The supernatants were mixed with 20 μl protein G-Sepharose (Amersham Biosciences), rotated for 3 h at 4 °C, then

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centrifuged at 1800 × *g* for 5 min, washed three times and then mixed with 30 μl of the sample buffer. The samples were separated by SDS–PAGE (10–20% gradient gel) and transferred onto a PVDF membrane. Finally, detection was performed with anti-FLAG-HRP antibody (1:2000) and anti-Myc monoclonal antibody (1:2000).

2.3. Cell culture and immunological analysis

SH-SY5Y neuroblastoma cell and HEK 293 cells were grown in DMEM containing 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin. Confluent cells were treated with 10 μM MG132 (Sigma), proteasome inhibitor and dimethyl sulfoxide (DMSO; Sigma) (for control) for 0, 4, or 8 h. The cells were lysed with lysis buffer (150 mM NaCl, 50 mM Tris–HCl [pH 7.5], 1.0% nonidet-P40, 10% glycerol and cocktail: Complete Mini; Roche). The lysate was then centrifuged at 17000 × *g* for 15 min at 4 °C. Next, the supernatant was used as the "crude". We considered the presence of aggregation in the pellet fraction and accordingly the pellet was solubilized with a mixture of 6 M urea, 50 mM Tris–HCl [pH 7.5], and 5 mM 2-mercaptoethanol, and sonicated. We then added the sample buffer to the sonicated sample as the "pellet". The whole lysates were prepared using the same method. The samples were separated by SDS–PAGE and transferred onto a PVDF membrane. Finally, detection was performed with VCP polyclonal and ubiquitin monoclonal antibodies.

2.4. Immunohistochemistry

After growing on 35-mm dishes (with glass coverslips), SH-SY5Y or HEK293 cells were treated with 10 μM MG132 or DMSO for 24 h. The cells were fixed in 4% paraformaldehyde in PBS for 30 min and permealized with Triton-X 100 for 20 min. Then, the cells were blocked overnight at 4 °C with 4% normal goat serum in PBS, incubated overnight with anti-VCP and anti-ubiquitin, anti-vimentin, anti-γ-tubulin, anti-Hsp70, and anti-FLAG antibodies in each case, washed with 0.01% Triton-X 100, and incubated for 30 min with Alexa 543 nm anti-mouse antibody and FITC 488 nm anti-rabbit antibody. The coverslips were washed and mounted on one vectashield. Fluorescence images were obtained using a fluorescence microscope.

2.5. Cell viability

HEK293 cells were grown under the same conditions. Confluent cells were transfected with 5 μg of FLAG-vector, FLAG-VCP^{WT}, FLAG-VCP^{K251M}, FLAG-VCP^{K524M}, or FLAG-VCP$^{K251M/K524M}$. The next day, the cells were transferred to 96-well dishes. After 1 h, the cells were treated with 10 μM MG132 or DMSO, and the cell viability was analyzed the next day. We used the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] reduction assay kit (Dojindo). We repeated the same experiments four times. To confirm the efficiencies of expression level, transfected cells were lysed with lysis buffer and centrifuged. We then added the sample buffer to the supernatant samples and applied them on the SDS–PAGE. In addition, we performed Western blotting with anti-FLAG, anti-VCP and anti-β-actin antibodies.

3. Results and discussion

Recent studies showed that cells treated with MG132 form aggregates that resemble LBs [10]. We first investigated whether VCP exists in such aggregates. As shown in Fig. 1, VCP was found in an aggregate formed under MG132. We also found that VCP levels were increased in the insoluble fraction, similar to polyubiquitinated proteins under MG132 condition, but not DMSO (Fig. 2). This finding indicates that these aggresomes were detergent insoluble and that VCP and ubiquitinated proteins were components of this fraction. Under this condition, the supernatant VCP fraction did not decrease at the same time, suggesting upregulation of VCP in the detergent-insoluble fraction. On the other hand, no increase in the whole VCP fraction was detected, suggesting that the amounts of supernatant fractions were larger than the insoluble fractions.

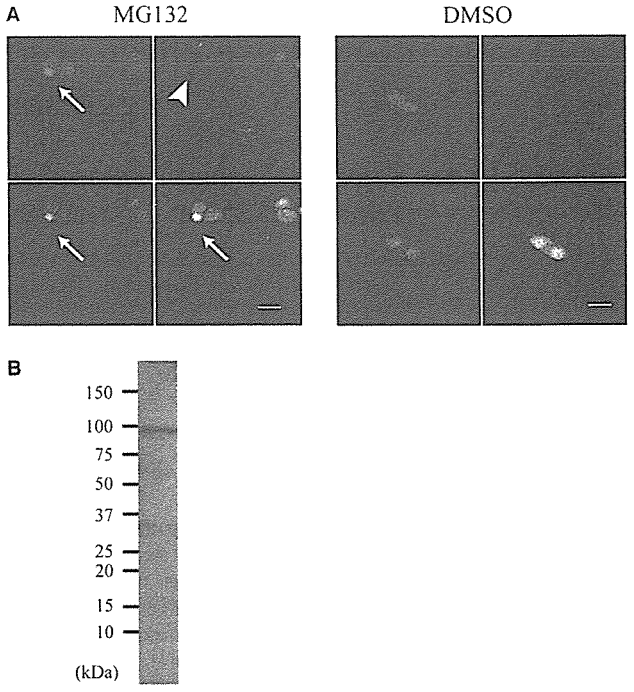

Fig. 1. Subcellular localization of endogenous VCP and ubiquitin in SH-SY5Y cells treated or untreated with a proteasome inhibitor. SH-SY5Y cells were treated with 10 μM MG132 (A) or DMSO (B) for 24 h. Cells were stained with anti-VCP and anti-ubiquitin antibodies. Nuclei were stained with DAPI (blue). FITC (green) and Alexa (red) correspond to VCP and ubiquitin, respectively. After treatment with MG132, both VCP and ubiquitin showed perinuclear accumulation and colocalization and appeared as clear protein aggregates (arrows). Nuclear torsion was observed (arrowhead). Scale bar = 20 μm.

In the next step, we investigated the localization of endogenous VCP in aggresomes. VCP formed a single large perinuclear aggresome-like structure and was co-localized with ubiquitin. Nuclear torsion was also noted (Fig. 1). Then we characterized the structure of these aggresomes to determine whether it is similar to that of typical aggresomes [3]. As shown in Fig. 3A, VCP-positive aggresomes were surrounded by vimentin in MG132-treated cells. Aggregates of misfolded proteins that escape degradation are targeted and accumulate in the MTOC. Subsequently, aggresomes are formed in the MTOC [3]. First, we showed that VCP-positive aggresomes co-localized with γ-tubulin, a marker of MTOC (Fig. 3B). Second, aggresomes are also abundant in chaperones such as Hsp70 [11]. We observed that Hsp70 co-localized with VCP in such aggresomes (Fig. 3C). Finally, we investigated the effect of inhibition of microtubule dynamics using an anti-mitotic agent, nocodazole, on the formation of these aggresomes. Co-incubation of cells with 10 μM nocodazole and MG132 resulted in inhibition of aggresome formation as evident in VCP and vimentin staining (Fig. 3D). These results indicate that the VCP-positive aggregates in SH-SY5Y cells are typical aggresomes.

We next examined the involvement of VCP in aggresome formation. We prepared FLAG-vector, FLAG-VCP^{WT}, FLAG-VCP^{K251M}, FLAG-VCP^{K524M}, and FLAG-VCP$^{K251M/K524M}$. The latter three vectors encode proteins lacking the function of two ATPase domains, D1 and D2, respectively. Considering the transfection efficiency, we used

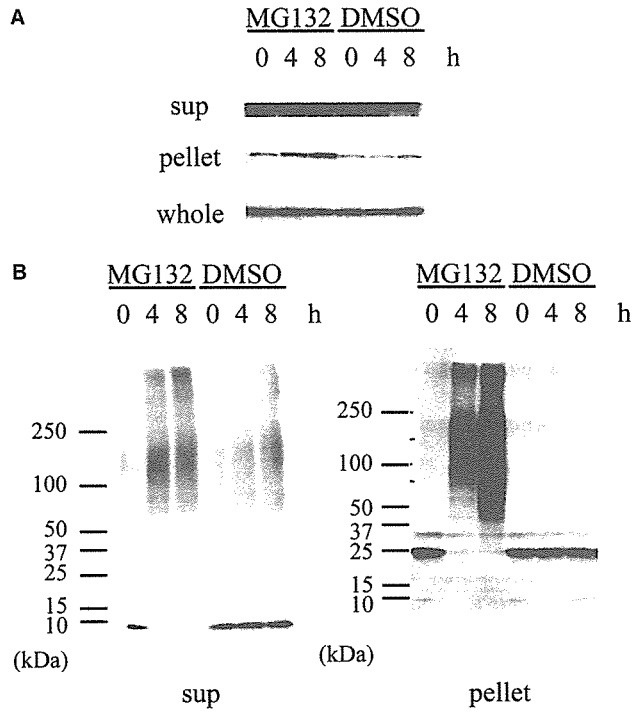

Fig. 2. Endogenous VCP moved to insoluble fraction similar to polyubiquitin under treatment with proteasome inhibitor. Cells were treated with 10 μM MG132 and DMSO for 0, 4 and 8 h. While VCP was identified in the supernatant fraction (A), it moved to the insoluble fraction (A) similar to polyubiquitin in MG132-treated cells (B).

HEK293 cells instead of SH-SY5Y cells. In this regard, the mechanisms of aggresome formation have been well studied in HEK293 cell lines [3]. The proportions of aggresomes-containing FLAG-VCP^{K524M}- and FLAG-VCP$^{K251M/K524M}$-transfected HEK293 cells were significantly lower than others (Fig. 4A). However, there was no significant difference in cell viability among FLAG-VCP^{K524M}-, FLAG-VCP$^{K251M/K524M}$-transfected HEK293 cells and cells transfected with other plasmids before treatment with MG132, as FLAG-VCP^{K524M} and FLAG-VCP$^{K251M/K524M}$ mutants tended to induce cell loss. In contrast, treatment with MG132 significantly influenced the viability of cells overexpressing FLAG-VCP^{K524M}, FLAG-VCP$^{K251M/K524M}$, and others. Considering the enhancement effects of MG132, we calculated the ratio of cell viability under DMSO and MG132, e.g., the ratio of FLAG-VCP^{WT}/FLAG-vector (under MG132) and FLAG-VCP^{WT}/FLAG-vector (under DMSO). There was no significant difference among the transfected cells. Thus, cell death of HEK293 cells with different expression vectors was similar under MG132 (Fig. 4B). Furthermore, the expression levels of exogenous FLAG-vectors were almost equal, suggesting that the transfection efficiencies of the vectors are similar. Indeed, the ratios of endogenous VCP to transfected FLAG-VCPs were almost 1:1 (Fig. 4C). These results suggest that the D2 domain of VCP is required for the formation of aggresomes rather than for cell death. Hirabayashi et al. [8] reported that cell viability was significantly reduced in mutant D2 VCP-transfected cells. In the previous report, the increased ratio of transfected mutant D2 VCP to wild-type VCP was inversely associated with cell viability. The discrepancy may be due to the different expression level of mutant VCP.

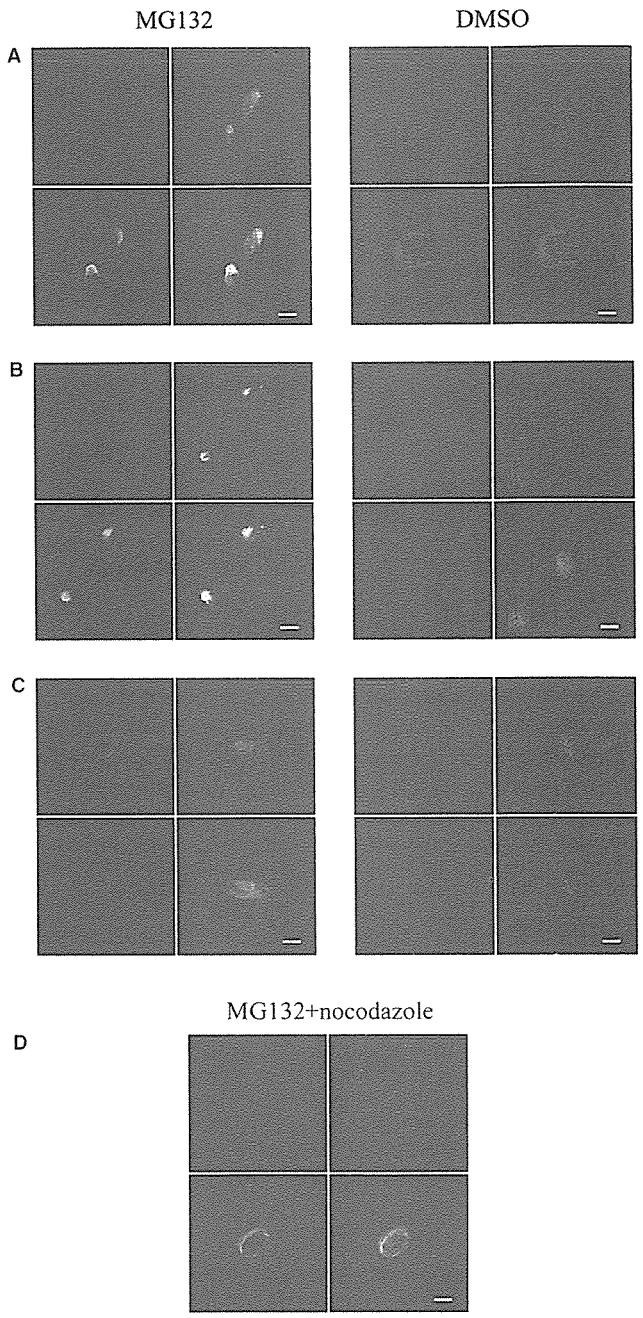

Fig. 3. VCP-positive aggregates are aggresomes. SH-SY5Y cells were treated with 10 μM MG132 or DMSO for 24 h, then stained with anti-VCP (A–D), anti-vimentin (A, D), anti-γ-tubulin (B) or anti-Hsp70 antibodies (C). Nuclei were stained with DAPI (blue). FITC (green) represents VCP and Alexa (red) represents vimentin (A, D), γ-tubulin (B) or Hsp70 (C). In MG132-treated cells, VCP-positive aggregates are surrounded by vimentin (A). Co-localization studies with anti-VCP and γ-tubulin antibodies showed VCP-positive aggresomes co-localized with γ-tubulin in MG132-treated cells (B). Hsp70 also co-localized with VCP-positive aggresomes (C). Inhibition of microtubule dynamics by antimitotic agent, nocodazole, was associated with inhibition of VCP and vimentin staining in MG132-treated cells (D; nocodazole and MG132). Scale bar = 20 μm.

As reported previously [8,9], VCP is localized in LBs of PD patients. Thus, it is possible that VCP interacts with α-synuclein within LBs. However, wild-type VCP did not interact with α-synuclein while IκBα as a positive control interacted with

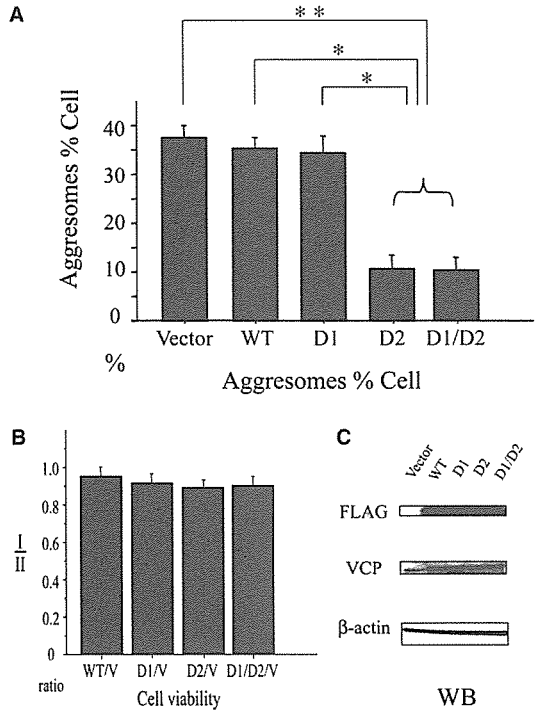

Fig. 4. Overexpression of VCPs affects aggresome formation. (A) HEK293 cells were transfected with FLAG-vector, FLAG-VCP^{WT}, FLAG-VCP^{K251M}, FLAG-VCP^{K524M}, or FLAG-VCP$^{K251M/K524M}$ vectors. The cells were then treated with MG132 after 24 h and harvested at 48 h after transfection. Anti-FLAG antibody and anti-vimentin antibody were used for detection of aggresome-containing transfected cells. Data are mean ± S.D. of the proportion of aggresome-containing cells. $^{*}P < 0.005$, $^{**}P < 0.01$, by Student's paired *t*-test. (B) Cell viability was analyzed using MTT assay. There was no significant difference among the ratios of each overexpression FLAG-VCP^{WT}, FLAG-VCP^{K251M}, FLAG-VCP^{K524M}, and FLAG-VCP$^{K251M/K524M}$ cells. (I = FLAG-VCP^{WT}, FLAG-VCP^{K251M}, FLAG-VCP^{K524M}, or FLAG-VCP$^{K251M/K524M}$/FLAG-vector [under MG132 treatment]), II = FLAG-VCP^{WT}, FLAG-VCP^{K251M}, FLAG-VCP^{K524M}, or FLAG-VCP$^{K251M/K524M}$/FLAG-vector [under DMSO treatment]. (C) Wild-type and mutant FLAG-VCPs and FLAG-vector were only expressed in HEK293 cells. Immunoblotting was performed with anti-FLAG, anti-VCP and anti-β-actin antibodies. The ratio of endogenous VCP to transfected FLAG-VCPs was 1:1.

VCP, as reported previously (data not shown) [12]. The results suggest that VCP and α-synuclein do not interact directly with each other.

VCP consists of two ATP binding sites: D1, which exhibits a heat-enhanced ATPase activity, and D2, which exhibits ATPase activity. D1 is responsible for the formation of stable hexamers while D2 has a major ATPase activity [6,13,14]. The ATPase activity of VCP is modulated by various environmental factors, similar to molecular chaperones. In the present study, fewer cells with aggresomes were noted among the cells transfected with D2 mutant VCP. In contrast, the proportion of aggresomes-containing cells was not significantly different between wild-type VCP- and mock-transfected cells, suggesting that endogenous VCP is involved in aggresome formation. Therefore, the D2 mutant VCP reduced the frequency of aggresomes as a dominant negative effect. On one hand, the lack of increment in wild-type VCP suggests that there is some rate-control process in aggresome formation. For example, dynein motor protein contributes to aggresome formation, and this step is thought to be a physically a rate-controlling step [3,15,16]. The dominant negative effect may be related to the inhibition of the nuclear-to-cytosol translation of VCP under MG132 treatment.

A recent study concluded that aggresome is important in LB formation [10], based on three reasons. The first is that LB consists of various proteins involved in the ubiquitin-proteasome system such as ubiquitinated proteins, proteasome subunits, parkin, α-synuclein, synphillin-1, Hsp70, and Hsp40 [11,17–19]. The second is the perinuclear localization, which is similar to LBs [2,10]. The third is the morphological similarity in the halo and core structures of LBs. The finding that VCP contributes to aggresome formation may enhance our understanding of the biogenesis of LBs.

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Clinical Heterogeneity of α-Synuclein Gene Duplication in Parkinson's Disease

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Objective: **Recently, genomic multiplications of α-synuclein gene (*SNCA*) have been reported to cause hereditary early-onset parkinsonism. The objective of this study was to assess the frequency of SNCA multiplications among autosomal dominant hereditary Parkinson's disease (ADPD).** ***Methods:*** **We screened 113 ADPD probands and 200 sporadic PD cases by quantitative polymerase chain reaction and confirmed *SNCA* multiplications by flurorescence in situ hybridization (FISH) and comparative genomic hybridization array.** ***Results:*** **Two families (two patients from Family A and one from Family B) with SNCA duplication were identified among ADPD patients. Even though they had the same *SNCA* duplication, one patient had dementia. Because there was exactly the same difference between the regions originated from each patient, the finding suggests that the phenotype of *SNCA* multiplication may be also influenced by the range of duplication region. We also detected asymptomatic carriers in the families of both patients. Interestingly, the penetrance ratio was 33.3% (2/6) in one kindred, indicating that the ratio was very much lower than expected.** ***Interpretation:*** **These two newly identified Japanese patients with *SNCA* duplication and the five previously identified American and European families with *SNCA* triplication or duplication mutations indicate that the incidence of *SNCA* multiplication may be more frequent than previously estimated.**

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Parkinson's disease (PD) is the second most common neurodegenerative disorder next to Alzheimer's disease (AD). Although the exact cause for PD remains to be elucidated, genetic factors could contribute to the pathogenesis of PD. Indeed, six causative genes and four chromosomal loci for familial PD (FPD) have been identified.[1–13] *α-Synuclein*, *UCH-L1*, and *LRRK2* have been identified as causative genes for autosomal dominant forms of FPD (ADPD), whereas *parkin*, *PINK1*, and *DJ-1* have been identified as causative genes for autosomal recessive forms of FPD (ARPD).[1,10,14] The presence of several causative genes and loci for FPD indicates that the pathogenic mechanisms of sporadic PD are also multifactorial. Studies of FPD are important as they enhance our understanding of nigral neuronal death. Furthermore, it has been proposed that the gene products for FPD are components of common pathways in sporadic PD. As testament, missense mutations such as A30P,[15] E46K,[16] and A53T,[9] in the N-terminal of α-synuclein gene (*SNCA*) have been linked to a rare form of FPD, and α-synuclein subsequently was confirmed to be a major component of Lewy bodies (LBs) and Lewy neurites, the pathological hallmark of sporadic PD and dementia with LBs (DLB).[17] Based on large population-based studies, missense mutations of *SNCA* are infrequent.[18] In particular, the SNCA A53T mutations identified in patients with FPD originate from a single founder. To date, SNCA A30P and E46K mutations have been found in only one family each, suggesting that missense mutations are a very rare cause of parkinsonism. Recently, *SNCA* multiplications in FPD haves been re-

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ported in two families with genomic triplication and in three families with duplications.[11–13,19] These findings suggest that overproduction of α-synuclein is one of the most important factors in FPD. In general, unequal intrachromosomal crossovers that result from misalignment of two homologous flanking sequences may account for genomic multiplications as well as deletions. The *SNCA* multiplications mutations, triplications, and duplications found in five unrelated patients probands with FPD are de novo within each kindred.[11–13,19] Affected individuals within the Iowa kindred, with *SNCA* genomic triplication, have fulminant, early-onset disease with a phenotype ranging clinically and pathologically from PD to diffuse LB disease (DLBD).[20] In contrast, *SNCA* duplication families have later onset disease and a longer duration to death, and neither cognitive decline nor dementia are prominent. Therefore, overproduction of wild-type α-synuclein (*SNCA*) may result in phenotypes of PD, PD with dementia (PDD), and DLBD, suggesting that regulation of α-synuclein protein levels is central to the cause of these phenotypes. In summary, the phenotype may be dependent on copy numbers of *SNCA*.

In this study, to gain further insight into the role of this multiplication, we assessed a series of 113 PD patients with autosomal dominant mode of inheritance and 200 sporadic PD patients for multiplication at this locus.

Subjects and Methods

Patients

This study consisted of 113 patients with ADPD and 200 patients with sporadic PD. Diagnosis of PD was adopted by the participating neurologists and the diagnosis was established based on the United Kingdom Parkinson's Disease Society Brain Bank criteria.[21] The mean age at onset of the 56 male and 57 female index patients with ADPD was 66.0 ± 9.5 (±SD), and that of the 81 male and 119 female patients with sporadic PD was 64.7 ± 10.0 (±SD). All patients were of Japanese origin. The study was approved by the ethics review committee of Juntendo University. Blood samples for genetic analysis were collected after obtaining informed consent from each patient and 17 unaffected relatives. None had mutations in *parkin*, *PINK1*, or *DJ-1*. We could not detect heterozygous exon deletions of such recessive genes by quantitative analysis in the patients studied. In addition, none had mutations in exon 41 in *LRRK2*.

Gene Dosage Analysis for SNCA

DNA was prepared using standard methods. The mutation screening was performed as described previously.[22] Semiquantitative multiplex polymerase chain reaction (PCR) of genomic DNA samples was performed using a real-time PCR method to detect the dosage of *SNCA* (ABI Prism 7700 sequence detector; Applied Biosystems, Foster City, CA). As the first step, we targeted exon 3 of *SNCA* to screen the gene dosage of *SNCA*. β-*Globin* gene was amplified as an endogenous reference. In addition, we used a DNA sample from the Iowa family (patients had triplication of *SNCA*) as a positive control. The primer and TaqMan MGB probe sequences used in this study are described in Table 1. PCR was conformed with PCR universal master mix using 25ng of genomic DNA, 900nM primers, and 250nM probes (β-globin is 50–200nM) in a total reaction volume of 50μL. PCR cycling conditions were 95°C for 10 minutes, 95°C for 15 seconds, and 60°C for 1 minute (40 cycles). Values between 0.4 and 0.6 were considered as heterozygous deletion, between 0.8 and 1.2 as normal, between 1.3 and 1.7 as heterozygous duplication, and greater than 1.8 as triplication.

In the second step, we performed semiquantitative analysis on exons 1/2, 4, 6, and 7 for the patients found to carry multiplication of this gene in the first step. All the sequences of this gene are shown in Table 1.

Fluorescence In Situ Hybridization Analysis

We used two-color standard fluorescence in situ hybridization (FISH) and prophase FISH for metaphase and interphase. FISH analyses were performed as described previously,[23] using a BAC located around the region of interest. The location of each bacterial artificial chromosome (BAC) was archived by the database of UCSC (http://genome.ucsc.edu) or NCBI (http://www.ncbi.nlm.nih.gov). Two BAC contigs representing the region at 4q21-22. BACs RP11-17p8 and RP11-61407 were used as probes. BAC RP11-17p8 locates at site of centromere of chromosome 4, and BAC RP11-61407 locates at site of telomere of the same chromosome. PR11-61407 contains *SNCA*, suggesting that the signal of this clone shows the copy numbers of *SNCA*. The distance between the two BAC clones was approximately 1.4Mb. Probes were labeled with biotin-16-dUTP or digoxigenin-11-dUTP by nick-translation (Roche Diagnostics, Tokyo, Japan). The copy number of the region was assessed according to the hybridization patterns observed on both metaphase and interphase chromosomes. We established Epstein–Barr virus (EBV)–transformed lymphoblastoid cell line as described previously.[24]

Multiplication (duplication) Region Using Comparative Genomic Hybridization Array and Gene Dosage Technique

The triplication region in Iowa family is between 1.61 and 2.04Mb and contains 17 annotated or putative genes. A recently constructed high-density comparative genomic hybridization (CGH) array, designated MCG Whole Genome Array-4500,[25] which contains 4532 BAC/P1-artificial chromosome (PAC) clones covering the entire genome at intervals of approximately 0.7Mb, was used for CGH array analysis. This array is suitable for detecting the size of the multiplication if the size is greater than 0.7Mb. Hybridizations were performed as described previously with minor modifications.[26,27] In brief, test and reference genomic DNAs from the patient's lymphoblastoid cells and normal lymphocytes, respectively, were labeled with Cy3- and Cy5-dCTP (Amersham Biosciences, Tokyo), respectively, precipitated together with ethanol in the presence of Cot-1 DNA, redissolved in a hybridization mix (50% formamide, 10% dextran sulfate, 2 × standard saline citrate [SSC], and 4% sodium dodecyl sulfate [SDS], pH 7.0),

Table 1. Sequences of Primer and TaqMan Probes Used in the This Study

		Forward Primer	Reverse Primer
β-globin	—	5′-TGGGCAACCCTAAGGTGAAG-3′	5′-GTGAGCCAGGCCATCACTAAA-3′
ABCG2	Exon 1	5′-GGAAGGTCCGGGTGACTCA-3′	5′-GGAGGCAGCGCTTTAACAAT-3′
—	Exon 2	5′-GTGTCACAAGGAAACACCAATGG-3′	5′-AGCTCCTTCAGTAAATGCCTTCAG-3′
DFKZ	Exon 7	5′-CTGGACCACTTACTGGTGAAAGC-3′	5′-CACTGTGCCTGGCCAAATT-3′
FAM13A1	Exon 12	5′-GAAGAGGACCTAACTCCCAGGAT-3′	5′-TTCTCAAGTTGGGAACCAAAACTCT-3′
LOC345278	Exon 8	5′-GTTGGCTGGGCCAATCTCT-3′	5′-TGGTCTTAGCTGAAGGCCAGTT-3′
SNCA	Exon s1/2	5′-CCTTCAAGCCTTCTGCCTTTC-3′	5′-CGAATGGCCACTCCCAGTT-3′
—	Exon 3	5′-TTCCAGTGTGGTGTAAAGAAATTCAT-3′	5′-CCTTGGCCTTTGAAAGTCCTT-3′
—	Exon 4	5′-CAGCAATTTAAGGCTAGCTTGAGACT-3′	5′-CCACTCCCTCCTTGGTTTTG-3′
—	Exon 6	5′-TATGCCTGTGGATCCTGACAAT-3′	5′-TCAGCTTGGACTCCTACCTCAGA-3′
—	Exon 7	5′-TCTTTGCTCCCAGTTTCTTGAGA-3′	5′-TGGAACTGAGCACTTGTACAGGAT-3′
MMRN1	Exon 1	5′-ATCAAACTCTCACATCCAC-3′	5′-CACCTGCTGAGGGTGTGAGA-3′
—	Exon 5	5′-CAGGCAATGAAACTGACTCTTCTG-3′	5′-CTTCTAGGGAGGAGTAAGTGTTCCT-3′
—	Exon 6	5′-GTTTCAATAGCAGCCCAGCAAAA-3′	5′-CAGTCAAAGTGGGCCGATTCT-3′
—	Exon 8	5′-GCTTCATATACCCCAAGAACTGGAA-3′	5′-GCACTAAATGACTCGATGGTGTACT-3′
KIAA1680	Exon 1	5′-TTAAATAACGCAGCTGGACTCTGT-3′	5′-TTAAATAACGCAGCTGGACTCTGT-3′
—	Exon 2	5′-GGCCACAATGATTCTACCTCTCA-3′	5′-CCGTAAGTTCTGTTGTTGTCTTTGT-3′
—	Exon 3	5′-AGCTCAGGTAGCACAGGTAAACG-3′	5′-TGGTGGAAGCTAATGGAAGGA-3′
—	Exon 4	5′-CCATTTCGTGAAGGAAGATTTATAGAG-3′	5′-TCCCTGCAGTGCCTTCTGA-3′
—		MGB probe	
β-globin	—	5′-CTCATGGCAAGAAAGTGCTCGGTGC-3′	
ABCG2	Exon 1	5′-CCCAACATTTACATCCTT-3′	
—	Exon 2	5′-CCGCGACAGCTTCCAA-3′	
DFKZ	Exon 7	5′-ACCATGCAAAAGAAAT-3′	
FAM13A1	Exon 12	5′-AAGCAACACACTCCCC-3′	
LOC345278	Exon 8	5′-CAGAAGCTGACTCTCA -3′	
SNCA	Exons 1/2	5′-ACCCTCGTGAGCGGA-3′	
—	Exon 3	5′-AGCCATGGATGTATTC-3′	
—	Exon 4	5′-TGTCTTGAATTTGTTTTTGTAGGC-3′	
—	Exon 6	5′-AGGCTTATGAAATGCC-3′	
—	Exon 7	5′-TGCTGACAGATGTTC-3′	
MMRN1	Exon 1	5′-ACTTGACCACTCCTTCTGCTTTCT-3′	
—	Exon 5	5′-CACAGTCAAAGAAATATTG-3′	
—	Exon 6	5′-CTTGCACCAAAACAAAC-3′	
—	Exon 8	5′-TCCAAGATACGGAATTCTA-3′	
KIAA1680	Exon 1	5′-TCCCCTTCTCGGCTGTTG-3′	
—	Exon 2	5′-ATGTCCCTCAATTCTG-3′	
—	Exon 3	5′-AGGAGCATATTCCG-3′	
—	Exon 4	5′-AGACTGCGATCCTC-3′	

and denatured at 75°C for 8 minutes. After 40-minute preincubation at 42°C, the mixture was applied to array slides and incubated at 50°C for 10 minutes, 46°C for 10 minutes, and 43°C for 60 hours in a hybridization machine, GeneTAC (Harvard Bioscience, Holliston, MA). After hybridization, the slides were washed once in a solution of 50% formamide, 2 × SSC (pH 7.0) for 10 minutes at 50°C and 1 × SSC for 10 minutes at 42°C, respectively, and then scanned with a GenePix 4000B (Axon Instruments, Foster City, CA). The acquired images were analyzed with GenePix Pro 4.1 imaging software (Axon Instruments). Fluorescence ratios were normalized so that the mean of the middle third of $\log_2$ ratios across the array was zero. The average values for each clone were within the thresholds of 0.2 and −0.2 (log2ratio), and the mean ± 2 SD values of all clones were within the range of 0.4 and −0.4 (log2ratio). The thresholds for copy number gain and loss were set at log2 ratios of 0.4 and −0.4, respectively.

We picked up the locus region between *ABCG* and *KIAA1680* of approximately 1.6 to 2.0 Mb. To identify the region of duplication spanning *SNCA*, we performed semiquantitative PCR on target genes including *ABCG*, *DFKZ*, *FAM13A1*, *LOC345278*, *MMRN*, and *KIAA1680* using the same methods. The sequences of all primer and probe sets are shown in Table 1.

Haplotype Analysis

To determine whether the same haplotype was shared between our probands with *SNCA* multiplication, we performed haplotype analysis in patients with *SNCA* duplication from unrelated families. We used four microsatellite markers including *D4S2361*, *D4S2505E* (located within *SNCA*), *D4S2380*, *D4S1647*, and *D4S421*.

Results

Gene Dosage Analysis for α-Synuclein

Using semiquantitative PCR to detect gene dosage, we did not find patients harboring *SNCA* multiplication

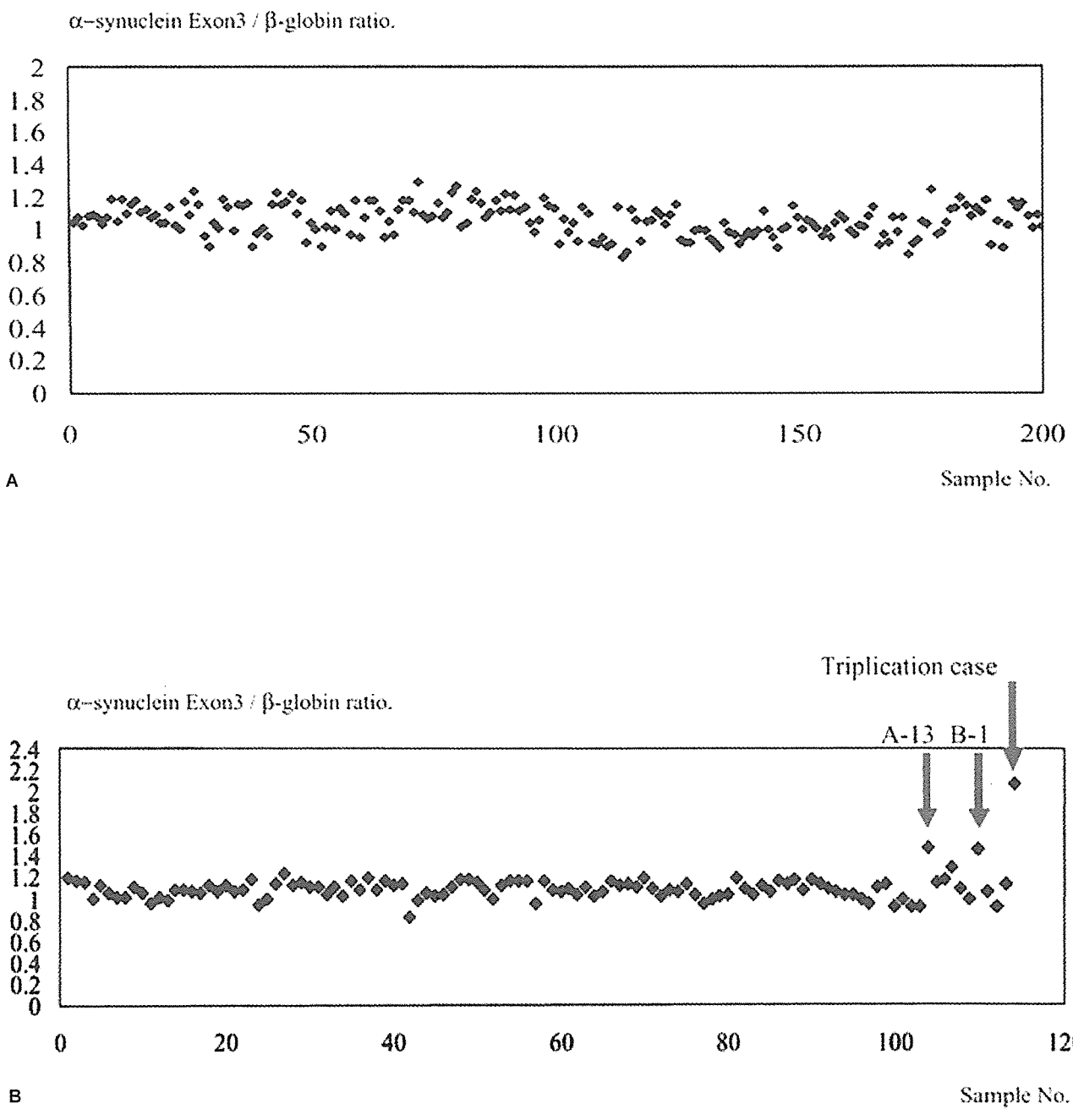

Fig 1. The ratio of α-synuclein exon 3, used as a target gene, to β-globin, used as a reference gene, as determined by semiquantitative real-time polymerase chain reaction in: (A) 200 patients with sporadic PD (the ratio ranged from 0.8 to 1.3, suggesting that single SNCA *copy exists in one allele, and (B) 113 patients with autosomal dominant hereditary Parkinson's disease. Note the two cases of duplication ratio (the ratio is 1.46 in one patient and 1.48 in the other), and the single Iowa family triplication case with a ratio of 2.07.*

among 200 sporadic cases (Fig 1A) but detected two index patients (A-13 and B-1) with potential *SNCA* duplications among 113 autosomal dominant pedigrees using exon 3 of *SNCA* (Fig 1B). To confirm the entire region of the α-synuclein gene was multiplied, we performed the exon dosage analysis including exons 1/2, 4, 6, and 7. We confirmed duplication of this gene in two patients. Thus, we were able to confirm that two families (Families A and B) were ADPD with *SNCA* duplication. In Family A, two patients with duplication had typical PD whereas five carriers were asymptomatic (Fig 2A). In Family B, one patient had duplication of the *SNCA* gene; two members were carriers (see Fig 2B).

FISH analysis also confirmed the *SNCA* duplication in the two index patients (Fig 3A, B). Figure 3 shows

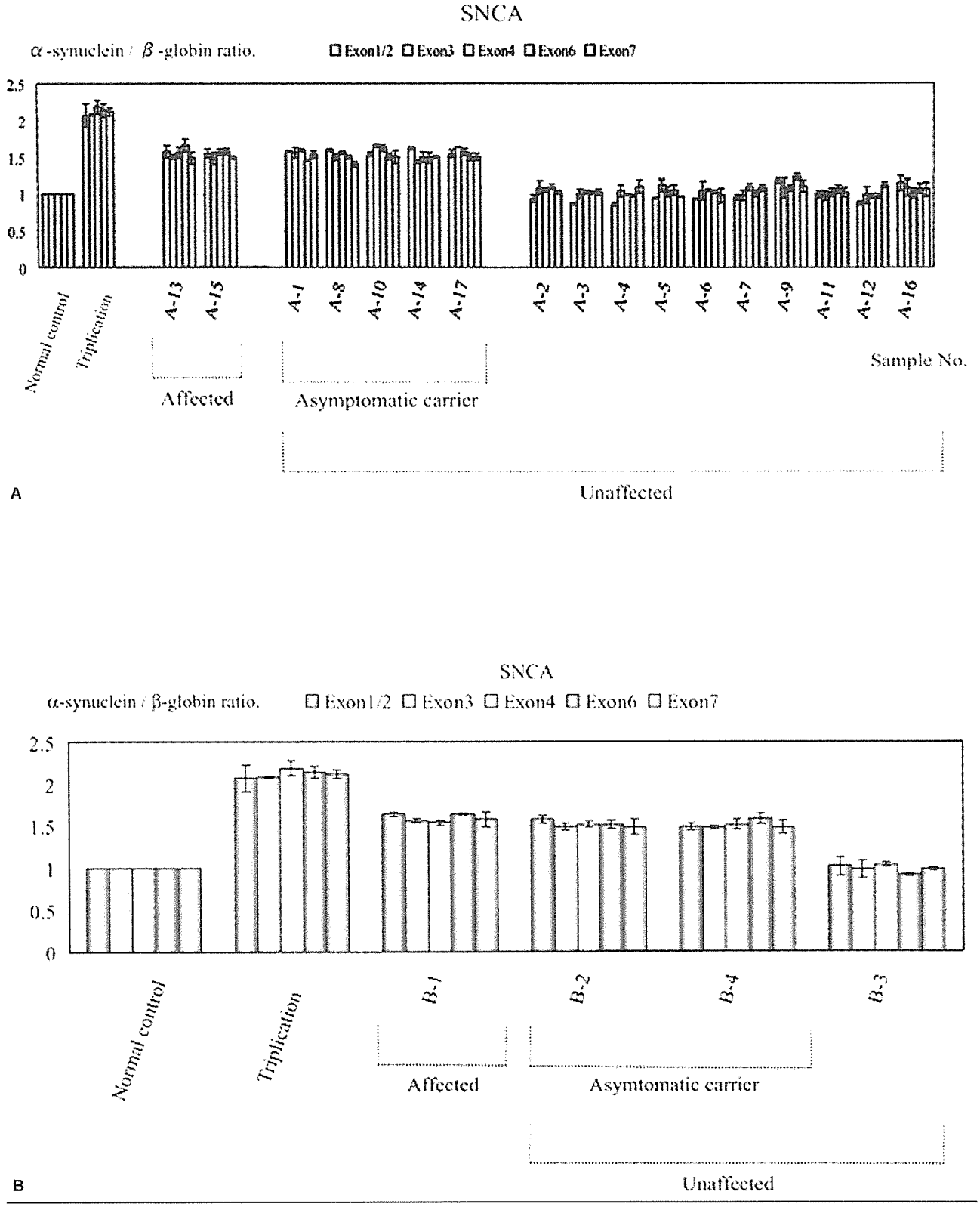

Fig 2. Results of screening for SNCA *multiplications for exons 1 to 7 in Family A (A). We detected two patients with* SNCA *duplication and five asymptomatic carriers in this family (a penetrance ratio of 33.3%) and (B) Family B. We detected three patients with* SNCA *duplication in four family members.*

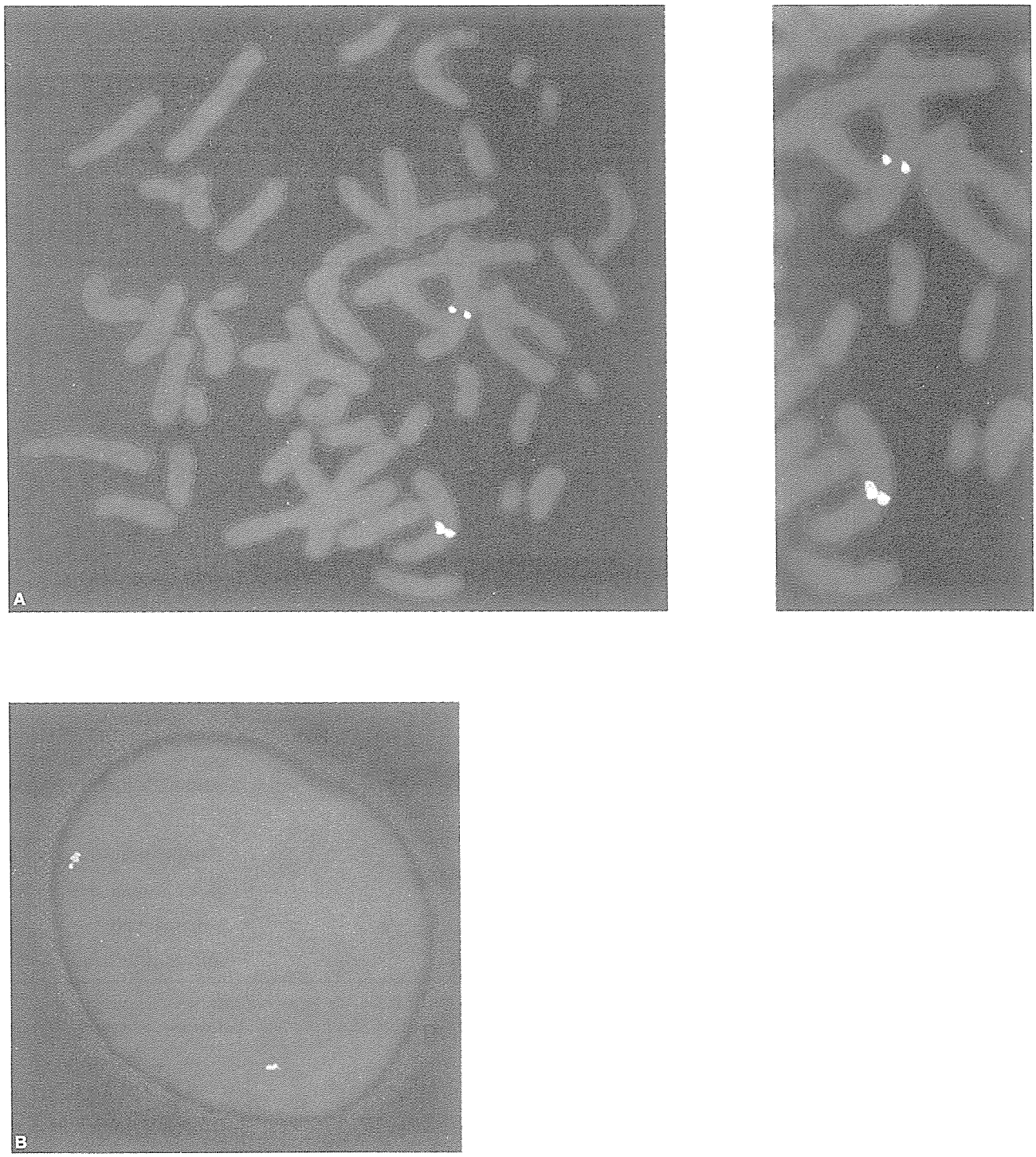

Fig 3. (A) Schematic representation of fluorescence in situ hybridization assay of metaphase chromosomes from Epstein–Barr virus (EBV)–transformed lymphocytes derived from Patients A-13 and B-1. We used BACs RP11-17p8 for normal control sample (shown in green and located 1.4Mb centromeric to SNCA, *left panel) and RP11-61407, which included the* SNCA *shown in red on chromosome region 4q21-22 (right panel). These pictures show clearly disproportional segregations compared with the normal control. (B) Standard one-color FISH of the interphase, using BACs RP11-61407. Note the two disproportional signals.*

the representative results of FISH analysis of interphase and metaphase chromosomes from EBV-transformed lymphocytes derived from Patients A-13 and B-1. We detected tight apposition of the metaphase chromatids compared with signals of BAC RP11-17P8 located 1.4Mb centromeric to *SNCA*. The intensity of the sig-

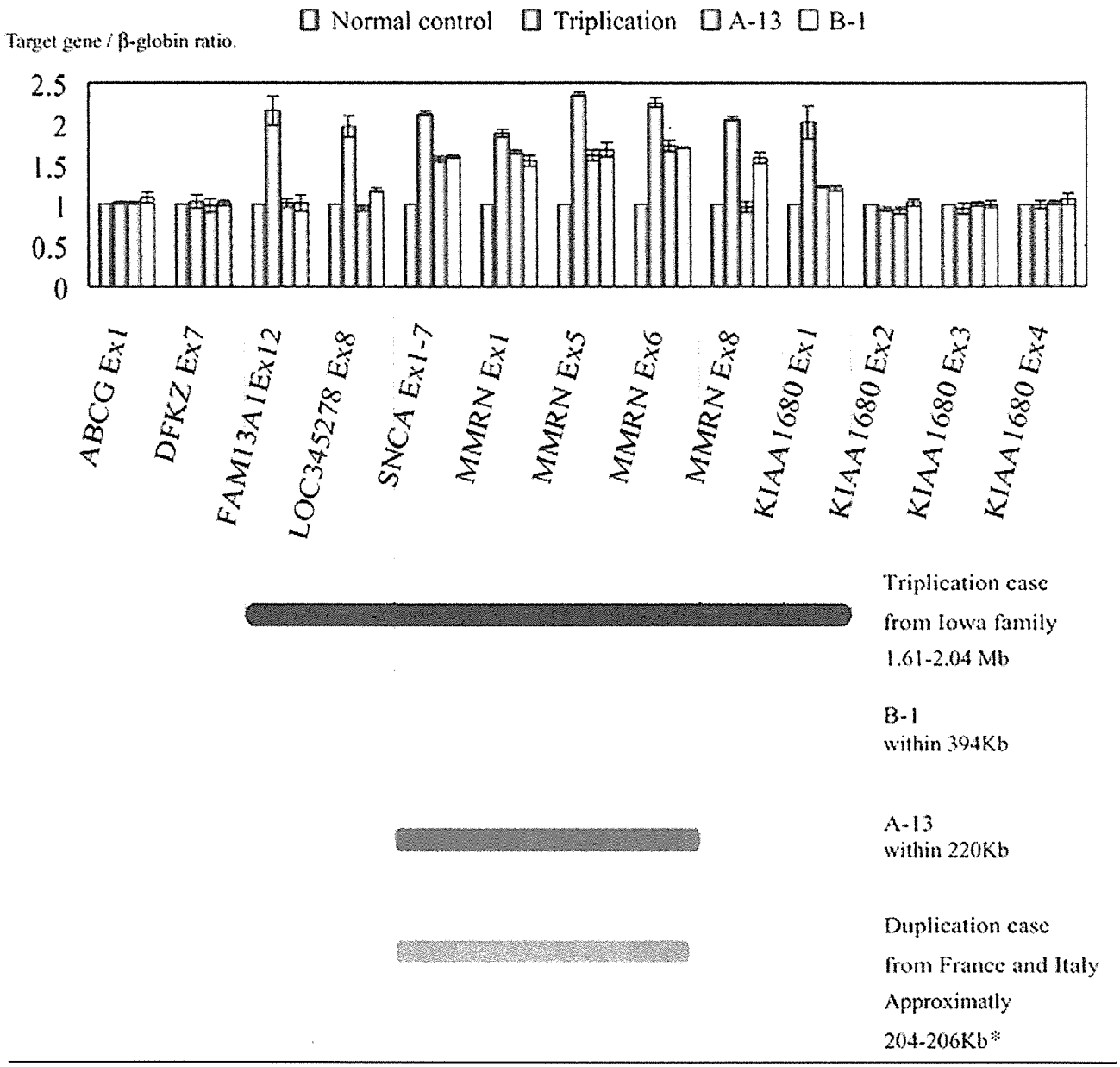

Fig 4. Identification of the region of SNCA *duplication between* ABCG *to* KIAA1680 *by using real-time semiquantitative polymerase chain reaction method. The different duplication region appears on* MMRN1 *Exon 8. *Duplication case as reported in Ibanez et al.*[13]

nal suggests *SNCA* duplication in these two patients. When considered together with the results of gene dosage analysis, we were able to confirm *SNCA* duplication. We did not observe two separate signals between BACs RP11-17P8 and PR11-61407, suggesting that the size of the duplication region is less than 1.4Mb. CGH array analysis showed that the specific elevation ratio could not be detected because the *SNCA* region could not be directly included in BAC probes used in MCG Whole Genome Array-4500. However, this BAC-based array contains BACs RP11-49M7 and RP11-17p8 that are close to 5′ or 3′ sites of *SNCA*, respectively. Alternatively, this finding indicates that the *SNCA* duplication region is less than 0.7Mb based on information archived by the database of UCSC (http://genome.ucsc.edu) and NCBI (http://www.ncbi.nlm.nih.gov). Although MCG Whole Genome Array-4500 covers the entire genome, no specific multiplication or deletions existed in other regions apart from 4q21-22. Identification of the *SNCA* duplication region was carefully assessed by gene dosage analysis for flanking genes around *SNCA* (Fig 4). The length of *SNCA* duplication of Patient A-13 spanned all of *SNCA* and part of *MMRN1* such as exons 1 to 6. In

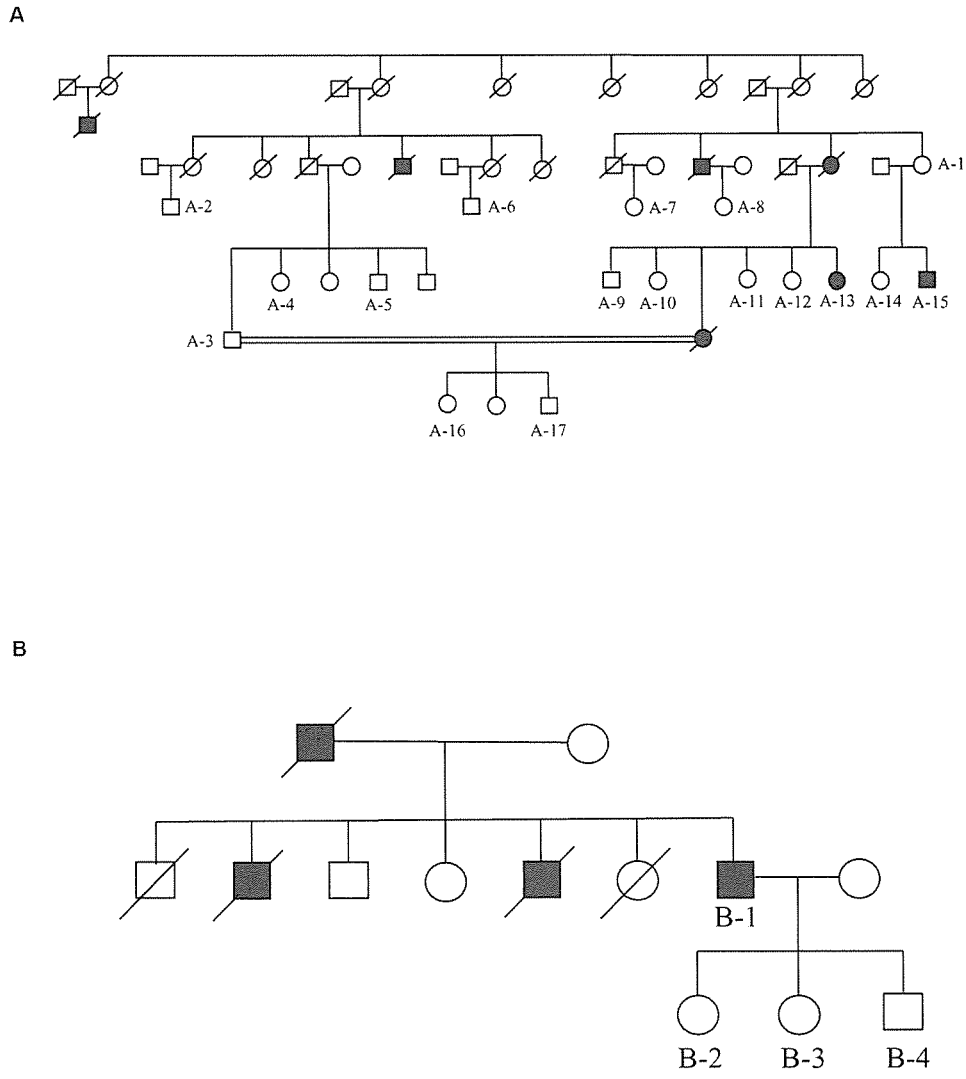

Fig 5. (A) Pedigrees of Patient A-1 with Parkinson's disease (PD) showing four generations. Black boxes represent affected patients. Symbols with numbers represent family members who were examined clinically by neurologists and from whom blood samples were collected. In 17 members, two patients were affected and five members (A-1, A-8, A-10, A-14, A-17) were carriers. Among seven carriers with SNCA *duplication, the ages of all carriers except for A-17 were beyond the mean age at onset of patients with* SNCA *duplication. Thus, the penetrance ratio was 33.3% (two patients/six asymptomatic carriers). (B) Pedigree of Patient B-1 with PD showing three generations. Symbols are as for Figure 6A. In four members, one patient was affected, and two members were carriers.*

contrast, the duplication region of Patient B-1 spanned all of *SNCA* and *MMRN1*. In addition, the regions of both patients did not span *LOC345278* and in Patient B-1, no duplication of *KIAA1680* was observed. Thus, the length of the duplication of Patient A-13 was shorter than that of Patient B-1, suggesting that the different lengths of the duplications differ by approximately 100 to 200kb. Furthermore, these two families have different allele sizes in microsatellite markers, suggesting that SNCA duplication is also de novo (data not shown). Clinical data, including the results of neuroimaging such as magnetic resonance imaging (MRI) and single-photon emission computed tomography (SPECT) and [^{123}I] meta-iodobenzylguanidine (MIBG) myocardial scintigraphy, are described below.

Family A

We collected DNA samples from 17 members of this family, including three affected and 14 unaffected members (Fig 5A). Among the three affected members, one patient (A-2) had no *SNCA* duplication. In addition, the age at onset of parkinsonism was 74 years. Moreover, L-dopa responsiveness was not excellent. Although MRI examination was not available, we considered that the cause of PD in this patient was not duplication but rather vascular parkinsonism based on

neurological findings. The mean age at onset of the disease was 43 years. The parents of A-16 and A-17 were close relatives. Five asymptomatic carriers were recognized by genomic analysis. No parkinsonism was observed in these asymptomatic carriers based on clinical neurological examination by two expert neurologists (K.N. and N.H.). The youngest age at onset was 38 years including the deceased patient (50 years old at onset). Thus, age 43 years was the cutoff age in this family. Considering this point, the penetrance ratio was 33.3% (2/6).

Patient A-13

The age of onset was 48 years. The initial symptom in Patient A-13 was rigidity and bradykinesia. She responded well to L-dopa. Six years after commencement of treatment with L-dopa, she developed drug-induced dyskinesia, which subsequently showed marked resolution. No tremor at rest has yet been noted. During the day, clinical assessment indicated Hohen and Yahr stage III. No dementia has developed yet and she has no symptoms related to autonomic nervous system dysfunction. Brain MRI study showed no abnormal mass or ischemic changes (Fig 6A) and ^{123}I-IMP SPECT study showed no evidence of hypoperfusion. However, the H/M ratio of MIBG myocardial scintigraphy was less than that of the normal control (A-13; early: 1.4, late: 1.24; see Fig 6D, E).

Patient A-15

The age at onset was 38 years. This patient was the cousin of Patient A-13. The initial symptom was gait disturbance with frequent falls. Tremor and autonomic nervous dysfunction were not seen. He was diagnosed with depression during the course of the disease, but neither dementia nor cognitive deterioration was prominent. The clinical course of this patient was similar to that of Patient A-13. Although this patient responded to L-dopa, he showed excellent response to anticholinergic agents such as trihexyphenidyl hydrochloride rather than L-dopa. In addition, the patient developed psychosis at 43 years of age.

Family B

DNA samples were collected from four members of Family B (see Fig 5B). Among the two generations, the number of affected member was four including three deceased members, and the unaffected members were three including two carriers with *SNCA* duplication. The age of asymptomatic carriers (B-2, B-3, and B-4) was younger than 35 years at the time of collection of DNA samples. Thus, it is difficult to speculate whether these carriers will develop PD in the future.

Patient B-1

The age at onset was 47 years. In the early stage, he responded to L-dopa; however, at 58 years of age, the disease was evaluated as stage III. Moreover, the gait disturbance and bradykinesia worsened and he suffered from cognitive dysfunction a few years later. Since 61 years of age, he has found it difficult to communicate with others and started gradually to develop abnormal behavior. Mini-Mental State Examination score was 17/30 at 61 years of age. At 62 years, his gait disturbance and hallucination worsened. At 64 years, he spent most of the day on the bed and required tracheostomy because of repeated episodes of aspiration pneumonia. Brain MRI showed moderate dilation of Sylvian fissure and atrophic changes in the temporal lobe on both sides. There was no evidence of ischemic changes or abnormal mass (see Fig 6B). A 99m-Tc-ECD SPECT study showed hypoperfusion predominantly on both frontotemporal lobes (see Fig 6C). The H/M ratio of MIBG myocardial scintigraphy was reduced (B-1; early: 1.40, late: 1.24).

Subject B-4

Subject B-4 was mentally retarded and had autism and generalized seizure. Since 1 year of age, he could not speak and was diagnosed with mental retardation by a pediatrician. At 12 years of age, he started to speak a few words and was sometimes observed to have sudden outburst of rage. At 15 years, he developed generalized seizure. EEG showed spiking waves predominantly localized to the right frontal lobe. Brain computed tomography scan showed no abnormal densities or other signs. No parkinsonism has been noted so far.

Table 2 summarizes the clinical features of these cases, including the results of neuroimaging and MIBG scintigraphy.

Discussion

Several recent studies suggest *SNCA* multiplications are a rare cause of PD, PDD, and DLBD.[22,28,29] In this study, we detected *SNCA* duplication in PD patients from 2 of 113 unrelated Japanese families with autosomal dominant parkinsonism. Thus, the incidence of *SNCA* multiplication may be more frequent than previously estimated. To our knowledge, the Iowa family and a single family of Swedish-American descent have been reported previously to have *SNCA* triplication.[11,19] In addition, two French families and one Italian family with *SNCA* duplication have been reported.[12,13] Taken together with this study, a total of seven families with *SNCA* multiplication, including triple and double *SNCA* copies, have been reported worldwide.

For all patients with *SNCA* duplication reported here, including patients of Family A, the phenotype was indistinguishable from idiopathic PD and no other

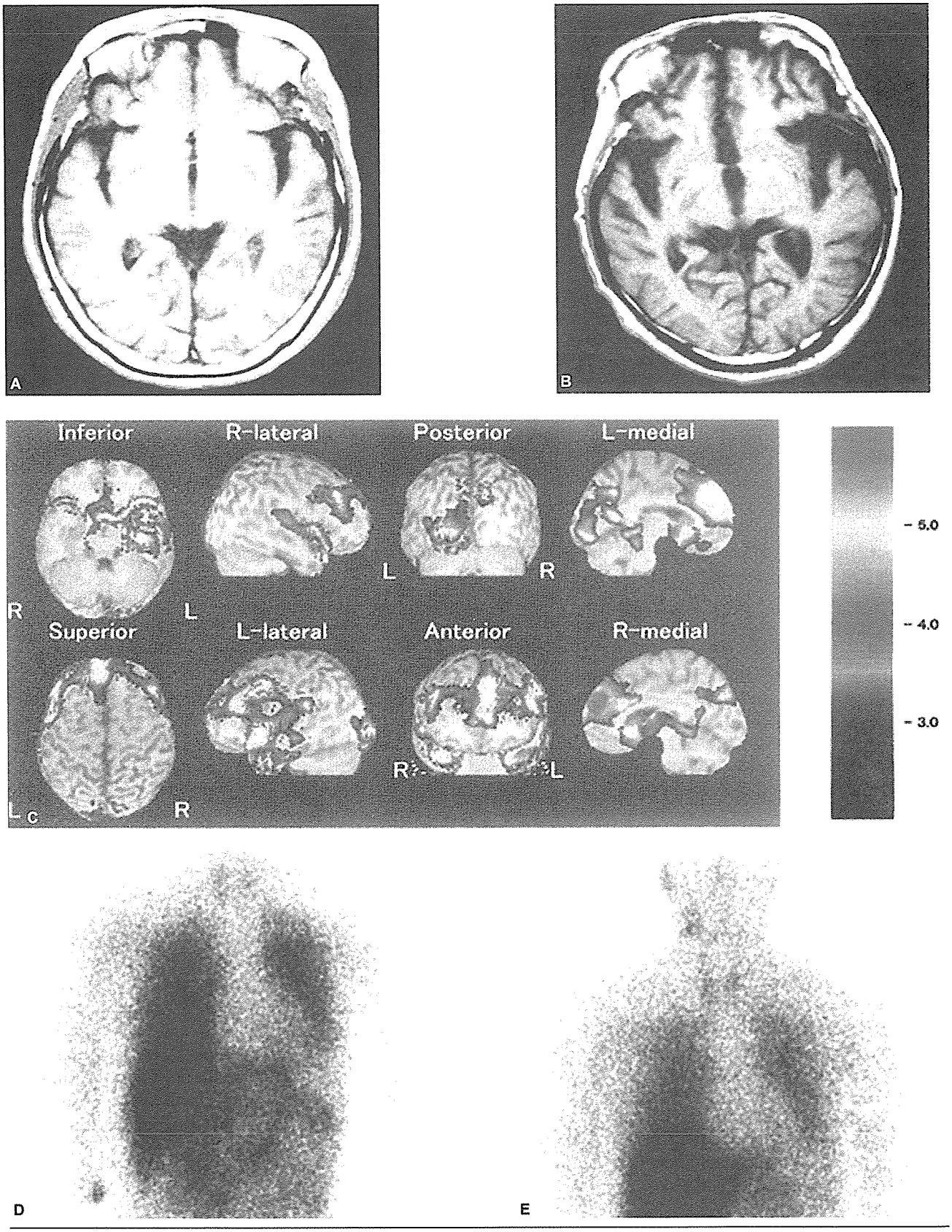

Fig 6. (A) Brain magnetic resonance imaging (MRI) T1 wedge study of Patient A-13. No abnormal masses or ischemic changes were evident. (B) Brain MRI T1 wedge study of Patient B-1. Note the dilation of Sylvian fissure and atrophic changes in both temporal lobes. (C) ^{123}I-IMP SPECT study of Patient B-1. Note the hypoperfusion of both frontotemporal lobes and medial-occipital lobes. (D, E) [^{123}I]meta-iodobenzylguanidine (MIBG) myocardial scintigraphy (D; early, E; late) of Patient A-13. The H/M ratio was reduced in this patient.

Table 2. Clinical Features of Four Affected Patients in Two Unrelated Pedigrees

	A Family			B Family
Feature	A-13	A-15	A-2	B-1
Age (yr)	57	43	77	65
Age at onset (yr)	48	38	74	47
Disease duration (yr)	10	6	4	19
Initial symptom	Rigidity	Rigidity	Bradykinesia	Bradykinesia
Bradykinesia	+	+++	++	+++
Rigidity	+++	+++	++	+++
Resting tremor	−	−	−	−
Postural instability	−	+	+	−
UPDRS	10/108	32/108	27/108	−
MMSE	30/30	30/30	17/30	17/30
L-Dopa response	+++	+	−	+
SNCA duplication	+	+	−	+

UPDRS = Unified Parkinson's disease rating scale; MMSE = Mini-Mental Status Examination.

clinical features such as dementia were present, in contrast with families with *SNCA* triplication. Notably, dementia was observed in one patient of Family B. Therefore, it is important to screen PDD or DLB for *SNCA* multiplications. However, the age of onset of PD in the patient with dementia was older than that of Iowa patients (36.0 ± 10.5 years) and the patient of Swedish-American family (31 years).[19] Moreover, the age at onset of Japanese patients was similar to those of other families with *SNCA* duplication (48.4 ± 15.0 years). In addition, the asymptomatic carrier, B-2, had epilepsy, which has been reported in one French PD patient.[13] In addition, autism was observed in the same patient, although no clear parkinsonism was evident. Patient B-1 had dementia, in contrast with previously reported cases with *SNCA* duplication, although the duration of the disease was longer (18 years) compared with reported cases of *SNCA* duplication. In addition, dementia only appeared after 14 years of diagnosis of parkinsonism. Therefore, *SNCA* duplication may be a risk factor for development of dementia.

Within each kindred the SNCA multiplication is a de novo mutation. The 4q21 genomic duplication in Patient B-1 included all of *SNCA* and *MMRN1*, whereas the duplicated region in Patient A-13 contained all of *SNCA* but only part of *MMRN1*. The *SNCA* triplication in the Iowa family also contains *MMRN1*, suggesting that overexpression of MMRN1 plays a role in cognitive deficit.

However, northern blotting analysis indicates a paucity of expression for *MMRN1* in neurons.[30] It therefore is unlikely that the effects of *MMNR1* are related to the development of dementia. *MMRN1* more likely plays a role in hemostasis and if vasogenic factors, including platelets and endothelial cells, are involved in dementia, *MMRN1* overexpression may still contribute to the dementia phenotype.

Previous studies reported the association of cardiac denervation and parkinsonism caused by *SNCA* gene triplication.[31] Low H/M ratios by [^{123}I]MIBG myocardial scintigraphy were reported in patients with sporadic PD.[32,33] In contrast, the H/M ratio was not decreased in patients with *parkin* mutations who lacked LBs in the autopsied brains.[34] In this regard, this finding is similar in patients with *SNCA* multiplication.

This study showed that the disease penetrance of Family A was 33.3%. The current ages of the asymptomatic carriers in this family are beyond the mean age at onset of patients. Thus, the difference may be caused by the *SNCA* expression levels between patients and asymptomatic carriers. Considering the multiple copies of *SNCA*, the expression level could be important. Indeed, double expression level of this protein compared with the normal brain was identified in Iowa family with *SNCA* triplication.[19] In addition, several haplotypes in the promoter region of SNCA including the sequence repeat element Rep1 were shown to associate with increased risk for sporadic PD.[35,36] However, whether the promoter alleles are risk factors for the development of PD is currently controversial.

Recently, Mueller and colleagues reported that single nucleotide polymorphisms located within the 3′side of exons 5 and 6, but not promoter polymorphism, correlated significantly with PD.[35] However, the functional association between PD and the associated region of SNCA remains unclear. In our study, the presence of asymptomatic carriers indicated that not only *SNCA* dosage but also another genetic variability in *SNCA* may be a risk factor for the development of PD.

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パーキンソン病の遺伝子異常

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要　旨

家族性パーキンソニズムではPARK1-11までの遺伝子座がマップされ，そのうちα-*synuclein*，*parkin*，*UCH-L1*，*DJ-1*，*Nurr1*，*LRRK2/dardarin*の原因遺伝子が同定された．そして現在その遺伝子産物の機能解析が進められている．最近になり常染色体劣性遺伝性パーキンソニズム（ARPD）の新たな原因遺伝子として*PINK1*が報告された．*PINK1*遺伝子変異は日本とイタリアを中心に報告されているが，*parkin*遺伝子変異に次いで頻度の高い遺伝子異常であることが指摘されている．その臨床型は，発症年齢は平均32歳，発症時のジストニアや睡眠効果など*PARK2*に見られる特徴的な臨床症状は目立たず，むしろ孤発型パーキンソン病（PD）との類似性が指摘されている．PINK1の機能は未だ不明だが，ミトコンドリア機能異常との関連が指摘されている．孤発型PDには，ミトコンドリア電子伝達系の機能低下が重要な病態であるとする多くの確証が存在する．このことは遺伝性と孤発型が共通したメカニズムによって惹起されることを意味している．まだ報告例は少ないが，*PINK1*遺伝子の同定によりパーキンソン病の分子遺伝学的研究はさらなる展開を迎えた．〔日内会誌　94：775～781，2005〕

Key words：若年性パーキンソン病，*parkin*，*pink1*

はじめに

孤発性パーキンソン病（PD）は遺伝的素因，環境素因の関与する多因子疾患と考えられている．これまでに酸化ストレスやミトコンドリア機能障害の関与などが指摘されているが，依然一次的要因の解明には至っていない．一方で遺伝性パーキンソン病(FPD)における研究には，めざましいものがある．PDの多くは孤発性であるが，5～10%が家族内発症の認める遺伝性である．孤発性，家族性いずれにおいても黒質神経細胞の変性をきたす点は共通しており，FPDの原因遺伝子の同定そしてその機能解明が，孤発性PDへの病態解明の手がかりとなり得よう．本稿では，最近報告された*PINK1*遺伝子異常を含め原因遺伝子の明らかとなっているFPDを中心に解説したい．

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1．家族性パーキンソン病の分類と臨床型

現在のところPARK1～11まで遺伝子座がマップされ，そのうち6つの原因遺伝子が同定されている．常染色体優性遺伝性パーキンソン病（ADPD）では，*SNCA*，*UCHL-1*，*Nurr1*，*LRRK2/dardarin*が，常染色体劣性遺伝性パーキンソン病（ARPD）では，*parkin*，*DJ-1*，また最近になり新たに*PINK1*が同定された（表1）．PARK10はPDの疾患感受性遺伝子としてマップされている．

上記の遺伝子のうち，日本人PD患者では*parkin*遺伝子変異のみが報告されていたが，最近になり*PINK1*遺伝子異常をもつ日本人ARPD患者が存在することが判明した．日本は島国であり近

表1. 家族性パーキンソン病の分類

遺伝子シンボル	座位	遺伝形式	原因遺伝子	Lewy小体	発症年齢	臨床的特徴
SNCA	4q21	優性	*α synuclein*	+	46 ± 13	一部に痴呆を認める
PARK2	6q25.2-27	劣性	*parkin*	−	40 <	本文参照
PARK3	2p13	優性	?	+	36 ～ 89	一部に痴呆，姿勢時振戦を認める
PARK4	4q21-22	優性	*α synuclein*	+	24 ～ 48 歳	痴呆，本態性振戦，前頭葉徴候
UCHL1	4p14	優性	*UCH-L1*	?	50 歳前後	孤発型に似る
PARK6	1p35-36	劣性	*PINK1*	?	32 ± 7	本文参照
PARK7	1p36	劣性	*DJ-1*	?	27 ～ 40	本文参照
PARK8	12p11.2-q13.1	優性	*LRRK2/dardarin*	±	56 ～ 62 歳	孤発型に似る
PARK9	1p36	劣性	?	?	11 ～ 16 歳	痴呆，錐体路症状，眼球運動障害
PARK10	1p32	感受性遺伝子	?	?	65.8 歳	孤発型パーキンソン病
NR4A2	2q22-23	優性	*Nurr1*	?	45 ～ 67 歳	孤発型に似る
PARK11	2q36-37	優性	?	?	58 歳	孤発型に似る

親婚が多かったという地理的歴史的背景もあり，常染色体劣性若年性パーキンソニズム（ARPD）が多い．第二次世界大戦以後，人口の移動に伴い近親婚率は減少しているが，日常外来でARPDに遭遇することは決して稀ではないと思われる．後述するが，*parkin*遺伝子変異の家系が最も頻度的に多いことは世界的規模においても間違いない．一方，原因遺伝子が不明な家系が少なからず存在することも事実であり，更に原因遺伝子が増えると予想される．単一遺伝子異常で発症するFPDが，最低でも11型存在することは黒質神経細胞死の機序は，より複雑であり，孤発型の臨床症状の多彩から予想してもPDは多様性の高い疾患群であると言える．

1）*SNCA*

1997年から1998年にかけてヨーロッパのADの家系においてA53T，A30Pの2つの点変異が報告された．A53Tはイタリア，ドイツ，ギリシアの数家系において認めており，共通の祖先から生じる創始者効果が示唆される．臨床症状はやや経過が早く一部に痴呆を認める．その後スペインの家系において新たな点変異E46Kが認められ，さらにPARK4としてマップされていたIowa家系にてtriplicationによる変異が報告された．剖検脳においてmRNAレベルでの過剰発現が示され，遺伝子の高発現がその病態に関わっていることが証明されている[1]．Multiplicationについては追試により4例報告があるが，痴呆を伴わず孤発型と類似した臨床症状を呈する症例もあり興味深い[2]．これまで日本を含めアジアからの報告はなく，ヨーロッパに限局した比較的頻度の少ない変異である可能性があるが，multiplicationの発生機序がランダムであることから，世界中に分布している可能性がある．α-シヌクレインはLewy小体の主要構成成分であり，PDにおけるLewy小体形成や選択的細胞変性にどのように関与しているかメカニズムの解明の糸口となり得る重要な蛋白質である．

2）*parkin*

1973年にYamamuraらによりその臨床型が発表され，その後連鎖解析により6q25.2-27に遺伝子座が決定され，1998年我々と慶応大清水教授との共同研究により遺伝子単離に至った[3]．一連を世界に先がけて本邦が報告したものであり，本邦に比較的多い病型である．マイクロサテライトマーカーを含む広範囲な欠失が遺伝子を単離する糸口となったが，欠失は本邦にて多い．外国ではミスセンス変異及びmicrodeletionが多い．後述する特徴的臨床症状の他に多彩な表現型の報告があり，遺伝子異常のタイプと表現型