In an attempt to determine the pathologic state of parkin-deficient mice, we assessed the binding of D_1 and D_2 receptors by ex vivo autoradiography. We used the [^{11}C]SCH23390 and [^{11}C]raclopride ligands against the D_1 and D_2 receptors, respectively. Intriguingly, parkin deficiency was associated with marked elevation of both D_1 and D_2 receptors. Indeed, previous studies reported that although striatal D_2 binding increased in untreated patients with PD, adaptive postsynaptic mechanisms and treatment decreased this as the condition advanced (Ahlskog et al., 1991; Brooks et al., 1992; Ichise et al., 1999). In a study of AR-JP patients, Scherfler et al. (2004) reported a global decrease in D_2 receptor and argued for parkin genetic defect itself or susceptibility to receptor downregulation after long-term exposure to dopaminergic agents. Taking into account the results of the present study, we consider the latter scenario; i.e., long-term exposure to dopaminergic agents, to result in downregulation of D_2 receptor. The decrease in DA concentration in synaptic clefts in early-stage PD is expected to lead to increased expression of D_2. Goldberg et al. (2003) reported that DA actually increases extracellularly in knockout mice.

We next measured DA release in knockout mice. When endogenous DA binds to the D_2 receptor, it competes with the antagonist [^{11}C]raclopride. This process allows synaptic DA levels to be estimated indirectly from changes in D_2 receptor binding. Elevation of DA synaptic concentrations can be achieved in vivo by administration of DA releaser such as amphetamine. The level of binding index in parkin-deficient mice was significantly higher than that of control mice, indicating a low DA release capacity in knockout mice (Fig. 6C). A similar method showed a decrease in release capacity of DA in PD (Piccini et al., 2003). However, this level of alteration of DA release potential is not sufficient to cause atrophy and degeneration of DAergic neurons, thus explaining the lack of a clear phenotype in knockout mice. Indeed, the open field and rotorod tests showed no reduction in locomotor activity, as mentioned above.

Quantitative analysis showed no differences in dopamine levels in the striatum of mutant and wild-type mice, although higher DA concentrations were noted in the midbrain of knockout mice relative to the control. This regional difference may be due to either accumulation of DA consequent to the low level of DA release, or due to increased DA reuptake. Although the expression levels of DAT and VMAT were not different between the two types of mice, further research is necessary to determine their precise functions. It is well-known that [^{18}F]DOPA undergoes reuptake in DAergic neurons and metabolizes to [^{18}F]DA, which is indicative of the synthetic capacity of DA involved in PD (Dagher, 2001; de la Fuente-Fernandez and Stoessl, 2002). Likewise, L-[β-^{11}C]DOPA is also metabolized to form [β-^{11}C]DA. Using this method, the overall synthetic capacity of DA was significantly low in knockout mice compared to wild-type mice (Fig. 6B). Excess DA in neurons may induce down-regulation of this synthetic capacity. In this regard, abnormal reuptake of [^{18}F]DOPA was reported previously in AR-JP patients (Broussolle et al., 2000; Hilker et al., 2001; Portman et al., 2001; Khan et al., 2002; Thobois et al., 2003; Scherfler et al., 2004). In contrast, the levels of DOPAC and HVA in the ventral midbrain were apparently normal in our knockout mice. This finding may be dependent on the regulation of activities of various enzymes that metabolize catecholamines, which compensate the altered DA transmission in parkin deficiency.

In conclusion, we have shown in the present study the presence of low levels of DA release in parkin-deficient mice, suggesting that DAergic neurons could behave abnormally before neuronal death.

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Leucine-Rich Repeat kinase 2 G2385R variant is a risk factor for Parkinson disease in Asian population

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To assess the effect of genetic factors on sporadic Parkinson disease, we performed a case–control study of a variant (G2385R) in *Leucine-Rich Repeat kinase 2* among the Japanese population. The G2385R (c.7153G>A) variant was reported as a risk factor for sporadic Parkinson disease in the Chinese population from Taiwan and Singapore. Genotyping was conducted in 448 Parkinson disease patients and 457 healthy controls. The frequency of A allele in Parkinson disease was significantly higher than in the control ($P=1.24\times10^{-4}$, odds ratio 2.63, 95% confidence interval 1.56–4.35). Our results suggest that the G2385R variant is a risk factor for sporadic Parkinson disease in the Asian population. *NeuroReport* 18:273–275 © 2007 Lippincott Williams & Wilkins.

Keywords: *Leucine-Rich Repeat kinase 2*, risk factor, single nucleotide polymorphisms

Introduction

Parkinson disease (PD) is one of the most frequent neurodegenerative diseases characterized by resting tremor, rigidity, bradykinesia, and postural instability. PD is thought to be a multifactorial disease caused by a combination of aging, environmental, and genetic factors. Although the majority of patients of PD are of sporadic type, some genes have been identified as a monogenic causative gene by molecular genetic studies for familial PD [1–6]. *Leucine-Rich Repeat kinase 2* (*LRRK2*) has been identified as a causative gene associated with autosomal dominant familial PD [7,8]. To date, many pathogenic substitutions in *LRRK2* have been identified in familial and sporadic PD [9]. The G2385R variant (c.7153G>A) in *LRRK2* was reported recently as a risk factor for sporadic PD in the Chinese population from Taiwan and Singapore [10,11]. This variant was identified originally as putative pathogenic mutation in a small Taiwanese PD family and was not found in Caucasians [12]. Thus, it is possible that the G2385R variant is a risk factor in Asian sporadic PD. To test this hypothesis, we conducted a case–control study to evaluate the association between the G2385R genotype and the risk for PD in the Japanese population.

Methods

Subjects and genomic DNA

Genomic DNA was isolated from 448 sporadic PD patients and 457 controls of the Japanese population by a standard protocol (Table 1). All PD patients had no family history of PD. PD patients with *parkin* or *PTEN-induced putative kinase 1 (PINK1)* mutation were not included in the study. Diagnosis of PD was adopted by the participating neurologists and was established on the basis of the United Kingdom Parkinson's Disease Society Brain Bank criteria [13]. This study was approved by the ethics committee of Juntendo University School of Medicine. All individuals gave an informed and signed consent form.

Genotyping

Exon 48 of *LRRK2* from each individual was amplified by polymerase chain reaction (PCR) using the primers and protocol described by Zimprich *et al.* [8]. The PCR products were sequenced directly using the BigDye Terminators v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California, USA). The reverse PCR primer was used as sequencing primer.

Statistical analysis

Statistical analysis included the Hardy–Weinberg equilibrium test, χ^2 test, Fisher's exact test, odds ratio and its 95% confidence interval (95% CI), using SNPAlyze v5.1 software (Dynacom, Chiba, Japan). The *t*-test was performed using JMP 6.0 (SAS Institute Japan, Tokyo, Japan). In all statistical analyses, *P* values of 0.05 or less were considered statistically significant.

Results

We analyzed the frequency of the c.7153G>A (G2385R) substitution in 448 patients and 457 controls. Genotypes of the controls and patients were concordant with Hardy–Weinberg equilibrium. The frequency of A allele in the patients was significantly higher than in the controls ($P=1.24\times10^{-4}$, odds ratio 2.63, 95% CI 1.56–4.35, Table 2). We also detected homozygous substitution for the G2385R variant in two patients; however, we detected only the heterozygous substitution in the controls. Concerning the age at onset, the G2385R carriers were somewhat older than the noncarriers in total patients and in those <50 years of age. In contrast, the age at onset was not significantly different between carriers and noncarriers aged ≥50 years (Table 3). The disease duration was not significantly different between carriers and noncarriers (data not shown).

Discussion

In this study, we observed the *LRRK2* G2385R variant in 11.6% (52/448) of sporadic PD patients. So far, many putative pathogenic mutations have been reported including the G2385R. We detected G2385R in both patients and controls (22/457: 4.8%, Table 2); thus, this variant is not a pathogenic mutation, but a single nucleotide polymorphism. These results were similar to the allele frequencies in the Chinese [10,11]. It is estimated that mutations of *LRRK2* are the most frequent among the causative genes for autosomal dominant familial PD so far. Indeed, only one mutation (G2019S) accounted for ~6.6% of familial PD and ~1.6% of sporadic PD in Caucasians [14–16]. Interestingly, the frequency of the G2019S mutation is ~40% in the familial PD of North African Arabs [17] and ~30% in the familial PD of Ashkenazi Jews [18], whereas the G2019S mutation is a much less common mutation in Asians [19,20]. It is likely that some differences of genetic background exist among Caucasians, North African Arabs, Ashkenazi Jews, and Asians. Although G2385R has been detected only in Asian population, some risk variations in PD such as α-synuclein would be found in not only Asians but also all ethnic groups [21–24].

Among patients with age at onset <50 years, the G2385R carriers were somewhat older than noncarriers. This might indicate that G2385R has no influence on early-onset PD, and that PD of patients with early-onset might be influenced by other genetic and/or environmental factors. In addition, there were no differences in any clinical features including age at onset among carriers with homozygous or heterozygous G2385R substitution and noncarriers. Although the G2385R might increase the risk of development of PD, it does not seem to have a clear effect on modifying the symptoms or worsening the progression of the disease.

The amino-acid G2385 is located in the WD domain of LRRK2. This domain is known to bind various proteins [9]. The WD domain of LRRK2 appears to play an important role in neuronal cells. Indeed, oxidative-stress-induced cell death was more enhanced by the overexpression of G2385R variant than wild-type LRRK2 using culture cells [11]. More studies are needed to understand the functional significance of the substitution of glycine to arginine.

Conclusion

In this study, we identified that the G2385R variant in *LRRK2* is a risk for PD in Japanese population. To combine with the result of Chinese population [10,11], this variant increases the risk of PD in Asian population. So far, multiple genomic loci have been identified as susceptibility loci for PD [25], suggesting that many genes have a synergistic influence on the development of PD.

Table 1 Age characteristics of individuals

	Patients	Controls
Total sample, *n* (%)	448 (100)	457 (100)
Male, *n* (%)	217 (48.4)	240 (52.5)
Female, *n* (%)	231 (51.6)	217 (47.5)
Age at onset (years)[a]	50.7 ± 14.6 (5–89)	—
Male[a]	49.1 ± 14.8 (5–89)	—
Female[a]	52.2 ± 14.2 (7–82)	—
Age at sampling (years)[a]	59.4 ± 13.8 (15–93)	43.8 ± 16.0 (21–98)
Male[a]	57.8 ± 14.7 (15–93)	43.8 ± 14.5 (23–92)
Female[a]	60.9 ± 12.7 (22–88)	43.9 ± 17.5 (21–98)

[a]Data are mean ± SD (range).

Table 3 Comparison of age at onset of PD patients

Age at onset (years)	Carriers (*n*)	Noncarriers (*n*)	*P*-value
<50	42.5 ± 5.8 (17)	37.1 ± 9.4 (180)	0.003
≥50	59.9 ± 7.0 (33)	61.6 ± 7.8 (209)	0.24
Total	54.0 ± 10.6 (50)	50.3 ± 14.9 (389)	0.03

Data are mean ± SD.
Patients without information about age at onset (two of carriers and seven of noncarriers) were excluded from this analysis.
PD, Parkinson disease.

Table 2 Association analysis of *LRRK2* G2385R variant

	Genotype, *n* (%)			Allele, *n* (%)			
	G/G	G/A	A/A	G	A	χ^{2} [a]	*P*-value[a]
Patients (*n*=448)	396 (88.4)	50 (11.2)	2 (0.4)	842 (94.0)	54 (6.0)	14.74	1.24×10^{-4}
Controls (*n*=457)	435 (95.2)	22 (4.8)	0 (0)	892 (97.6)	22 (2.4)		

LRRK2, *Leucine-Rich Repeat kinase 2*.
[a]Compared with the control.

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Molecular mechanisms of nigral neurodegeneration in Park2 and regulation of parkin protein by other proteins

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Summary. Most of the patients with Parkinson's disease (PD) are sporadic. However, Since identification of monogenic forms of PD, the contribution of genetic factors to the pathogenesis of sporadic PD is proposed as one of major risk factors. Indeed, this is supported by the demonstration of the high concordance in twins, increased risk among relatives of PD patients in case control and family studies. Thus, the functional analysis for the gene products for familial PD provides us a good hint to elucidate the pathogenesis of nigral degeneration. For example, although α-synuclein is involved in a rare dominant form of familial PD with dopa responsive parkinsonian features, this molecule is a major component of and Lewy bodies (LBs). In contrast, Park2 (parkin-related disease) is the most frequent form among patients with young-onset PD. However, Park2 brains generally lack the formation of LBs. In the other word, parkin responsible for Park2 is essential for the formation of LBs. Thus, both α-synuclein and parkin are speculated to share a common pathway. Here, we reviewed the parkin function and molecular mechanisms of Park2.

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder primarily caused by selective dopaminergic cell loss in the midbrain substantia nigra pars compact. However, the exact cause of PD is still unknown. Since identification of monogenic form of PD, the functions of gene products for familial PD (FPD) have provided us good information for studying the mechanisms underlying neurodegeneration in PD. To date, eleven loci have been mapped and among them, six causative genes have been identified as causative genes in familial PD, which have significantly enhanced our understanding of the genetic mechanisms of not only FPD but also sporadic PD. Among the forms of FPD, the causative gene, *parkin* of AR-JP, representing the most prevalent form of familial PD (Kitada et al., 1998), is of a special interest, because it is liked to ubiquitin-proteasome system (UPS) as an E3 ubiquitin-protein ligase (Shimura et al., 2000), which covalently attaches ubiquitin to target proteins, designating them for degradation by the 26S proteasome (Pickart et al., 2001). These findings suggest that accumulation of the parkin substrate(s) due to loss-of-function of parkin induces loss of dopaminergic neurons. Thus, Park2 is caused by failure of proteolysis mediated by UPS (Dawson and Dawson, 2003). Since then, our knowledge of the substrate(s) for parkin has expanded, and indeed at present various putative substrates, such as

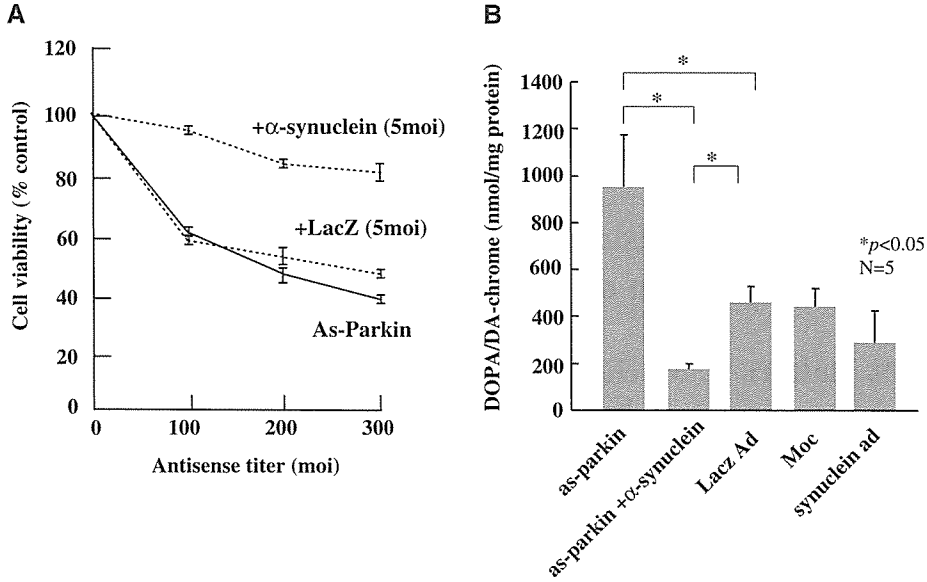

Fig. 1. α-Synuclein inhibits parkin knockdown-induced apoptosis and accumulation of DOPA- and DA-quinones. **A** Effects of overexpression of wild α-SN on as-parkin induced deterioration of cell viability. Differentiated SH-SY5Y cells were treated for 48 hours with as-parkin adenovirus. Cells were coinfected with LacZ and α-SN adenovirus (5 moi) and at the 150 moi titers of as-parkin adenovirus. The cell viability was measured and represented. **B** Cellular level of DOPA/DA-chromes. After the differentiated SH-SY5Y cells were treated for 36 hours with as-parkin, wild α-SN, LacZ and adenoviruses, cellular DOPA/DA-chromes were measured. Note the profound decrease of DOPA/DA-chromes in α-SN-expressing SH-SY5Y cells. Data are the mean ± SEM of 10 determinations. $^{*}P < 0.05$ versus control group (Tukey's multiple t test)

CDCrel-1, synphilin-1, α-SN22 (*O*-glycosylated form of α-SN), Pael-R etc, have been identified, but the pathophysiological role of parkin is still poorly understood (see review Hattori and Mizuno, 2004). Furthermore, null mice have no phenotypes for PD although several changes including dopamine metabolism have been reported so far. However, a direct link between these factors and dopaminergic cell death has not yet been reported. The important question of why dopaminergic neurons in the SN are particularly vulnerable to the loss-of-function effect of parkin remains to be determined. Although parkin is expressed ubiquitously in the brain, the pathologic findings of Park2 brains show severe neuronal loss with gliosis in the SN and mild neuronal loss in the locus coeruleus (LC), suggesting that the pathological change of Park2 brain is mainly in the SN. To investigate such selective neuronal loss, we established a good *in vitro* model by parkin knock down using full length antisense parkin.

Molecular mechanisms of Park2

Recently, two groups independently reported the generation of model mice lacking the *parkin* gene, which display certain abnormalities of dopamine metabolism (Itier et al., 2003; Goldberg et al., 2003). However, these parkin knockout mice had only subtle phenotypes exhibiting grossly normal brain morphology. In contrast, full-length human parkin antisense knocked-down endogenous parkin protein in differentiated human neuroblastoma cells (SH-SY5Y), 12 to 36 hours after infection and reduced cell viability (Machida et al., 2005). In addition, control β-galactosidase expressing adenoviruses could not knockdown

parkin and failed to affect cell viability. Thus, this system itself using adeno virus is not cytotoxic to the culture cells. The specificity of the antisense effect was confirmed by the result of co-infection of sense *parkin* expressing adenovirus, which abrogated reduction of cell viability. On the other hand, *parkin* antisense had no effect on cell viability of HeLa cells, suggesting that *parkin* antisense exert a specific effect on the cell viability of differentiated SH-SY5Y cells. Thus, this *in vitro* model is a powerful tool for elucidating the several issues as mentioned before.

Although *in vitro* system could induce the cell loss, why do parkin knockout mice lack abnormalities like AR-JP patients? One plausible explanation is the presence of a putative molecule(s) that suppresses the defect induced by loss-of-function of parkin, and such molecule(s) present abundantly in the brain should be linked to the pathogenesis of PD. Here we propose that α-SN is the molecule that compensates for the loss of parkin, since α-SN prevented apoptotic cell death induced by as-parkin. In this regard, Western blotting analysis showed that the dopaminergic SH-SY5Y cells did not express α-SN at significant levels, which is in marked contrast to the high abundance of dopaminergic neurons *in vivo*. Regardless of the compensating role of α-SN for the loss-of-function of parkin in Park2, α-SN is probably unable to cope with the accumulation of toxic molecules in the absence of parkin and thus apoptotic neuronal death perhaps occurs gradually, leading to degeneration of dopaminergic neurons. This is the first evidence for the anti-apoptotic role of α-SN and its involvement in the common pathway of parkin.

To date, several studies have demonstrated that α-SN could exert protective effect against various cellular stresses such as oxidative damage and related apoptosis of neurons. Considering the reason why mutation of α-SN causes familial PD, it is clear that this type of disease is due to the gain-of-toxic function of the mutant α-SN, differing from neuroprotective roles of the wild-type α-SN. In this context, it is noteworthy that α-SN is a major component of Lewy bodies, the pathological hallmark of PD, and at high concentrations, it oligomerizes to β-pleated sheets known as protofibrils (i.e., fibrillar polymers with amyloid-like characteristics). In addition, α-SN proteins with disease-causing mutations tend to generate protofibrils, suggesting that protein misfolding including α-SN plays a key role in the pathogenesis of PD. In addition to our finding, α-SN could play dual function such as neuroprotection and or neurotoxicity. Considering the presence of the patients with α-SN multiplication, overproduction of this molecule could cause for developing PD, and lower level expression would be also cytotoxic to the dopaminergic neurons. Indeed, α-SN knock out mice displayed the impairment of the dopamine release although neuronal loss has not been reported so far.

It remains unclear why dopaminergic neurons of the substantia nigra and locus coeruleus are selectively vulnerable to the loss of parkin in AR-JP patients. In the present study, we provided a clue for this enigmatic puzzle. Considering the specificity of the lesions in PD, it is possible that the high oxidative state associated with DA metabolism may cause deterioration of dopaminergic neurons. The mechanism underlying increased oxidative stress may involve DA itself, because oxidation of cytosolic DOPA/DA may be deleterious to neurons. Indeed, DA causes apoptotic cell death with morphological nuclear changes and DNA fragmentation. In this regard, we showed here that as-parkin directed loss of parkin leads to abnormality of DOPA/DA metabolism, which generated DOPA/DA-quinones in SHSY5Y cells. Thus, DA and its metabolites seem to exert cytotoxicity mainly by generating highly reactive quinones through auto-

oxidation. On the other hand, the toxicity of DOPA and DA is due to the generation of reactive oxygen species that could disrupt cellular integrity, causing cell death. However, the reason for the production of oxidative DOPA/DA-metabolites following loss of parkin is not clear at present.

The loss-of-function of parkin by full length antisense strategy could lead to the cell death of differentiated dopaminergic cells *in vitro*. In addition, the increasing of DOPA/DA-quinones was associated with the cell death, suggesting that quinines derived from dopamine metabolism are killer molecules in Park2 brains. This cell-based experiment enhances our understanding of the pathophysiology of PD and is potentially useful for drug screening in the future. Our results also showed that α-SN and parkin are involved in DA metabolism and its aberrant regulation is accompanied by accumulation of oxidative DOPA/DA metabolites.

Recently, parkin has been negatively regulated by S-nitrosylation modification and BAG5 (Kalia et al., 2004; Chung et al., 2004). Thus, loss-of-function through parkin mutation as in Park2, nitrosylation or binding with BAG5 results in nigral degeneration in not only Park2 brains but also sporadic PD. In the other word, such negative regulation system for parkin ubiquitin ligase suggests a possible mechanistic link between the familial and sporadic forms of PD. As s-nytrosylation for parkin has been reported in sporadic PD, DOPA/DA metabolites could be also involved in the pathogenesis for sporadic form of PD as well as Park2 brains. Finally, several gene products have been reported so far, the relationship among them is unclear at present. However, considering clinical similarities including neuropathologic findings between sporadic and FPD, most of the gene products may share a common pathway on the pathogenesis for PD.

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Progress in familial Parkinson's disease

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Summary. To date 11 forms of familial Parkinson's disease (PD) have been mapped to different chromosome loci, of which 6 genes have been identified as the causative genes, i.e., *alpha-synuclein* (*SNCA*), *parkin*, *UCH-L1*, *PINK1*, *DJ-1*, and *LRRK2*. For *UCH-L1*, additional families with this mutation are necessary before concluding that *UCH-L1* is the definite causative gene for PARK5, as only one family so far has been reported. *SNCA*, *UCH-L1*, and *LRRK2* mutations cause autosomal dominant PD and the remaining gene mutations autosomal recessive PD. Age of onset tends to be younger in familial PD compared with sporadic PD, particularly so in autosomal recessive PD. Generally familial cases respond to levodopa quite nicely and progression of the disease tends to be slower. It is an interesting question how familial PD-causing proteins are mutually related each other. In this article, we review recent progress in genetics and molecular biology of familial PD.

Introduction

To date 11 forms of familial Parkinson's disease (PD) have been mapped to different chromosome loci (Table 1). In this article we review recent progress in these familial forms of PD.

PARK1

PARK1 is an autosomal dominant familial PD caused by mutations of *alpha-synuclein* (*SNCA*). Clinical features of PARK1 were first described by Golbe et al. (1990) on large autosomal dominant kindreds immigrated to USA from Contursi, a village in the hills of Salerno Province in southern Italy. Ancestors of this family are believed to have moved to Italy from Greece. Clinical features consist of L-dopa-responsive parkinsonism and variable degrees of cognitive impairment. The average age of onset of the original families reported by Golbe et al. (1990) was 46.5 ± 10.8 years (range, 28–68, $N = 33$).

Alpha-synuclein has been mapped to the long arm of chromosome 4 at 4q21-q23. To date, 3 missense mutations, i.e., A30P (Krüger et al., 1998), E46K (Zarranz et al., 2004), and A53T (Polymeropoulos et al., 1997) and triplication (Singleton et al., 2003) and duplication (Chartier-Harlin et al., 2004; Ibenez et al., 2004) of the entire *alpha-synuclein* are known (Fig. 1). Alpha-synuclein is a neuron-specific protein localized mainly in the presynaptic terminal membranes and synaptic vesicles. Although the function of alpha-synuclein is not well known, aggregated alpha-synuclein is accumulated in the nigral neurons in PD indicating that alpha-synuclein plays an important role in the pathogenesis of PD. Recently reported families with triplication and duplication of *alpha-synuclein* suggest that overexpression of normal alpha-synuclein per se is neurotoxic to nigral neurons.

Table 1. Inherited forms of Parkinson's disease

Name	Inheritance	Locus	Gene
PARK1	AD	4q21-23	*α-synuclein (SNCA)*
PARK2	AR	6q25.2-27	*parkin*
PARK3	AD	2p13	*unknown*
PARK4	AD	4q21-23	*α-synuclein*
PARK5	AD	4p14	*UCH-L1*
PARK6	AR	1p35-36	*PINK1*
PARK7	AR	1p36	*DJ-1*
PARK8	AD	12p11.2-q13.1	*LRRK2/ dardarin*
PARK9	AR	1p36	*unknown*
PARK10	AD/AR/SP	1p32	*unknown*
PARK11	AD	2q36-37	*unknown*

AD autosomal dominant, *AR* autosomal recessive, *SP* sporadic

Regarding the relationship between the types of mutation and clinical features, triplication and E46K mutations are associated with dementia in addition to parkinsonism and wide-spread neuropathologic changes with cortical Lewy bodies in addition to nigral neurodegeneration (Farrer et al., 1999; Zarranz et al., 2004). Actually neuropathological characteristics are consistent with those of diffuse Lewy body disease. On the other hand, duplication was associated with pure L-dopa-responsive parkinsonism without dementia. Ala53Thr mutation is associated with variable degrees of cognitive impairment. Ala30Pro is less likely to show cognitive impairment.

Functions of alpha-synuclein are not well known. Alpha-synuclein is a natively unfolded brain specific protein consisting of 140 amino acids without significant amount of secondary structure (Weinreb et al., 1996). From its localization in presynaptic terminals, it has been speculated that it may be related to neurotransmitter regulation. Alpha-synuclein has a tendency for self-aggregation and oligomer formation. Soluble oligomers ultimately form insoluble aggregates, which are the major component of Lewy bodies (Spillantini et al., 1998). Particularly, oligomers of alpha-synuclein are toxic to neurons inducing release of dopamine into the cytoplasm from synaptic vesicles (Volles and Lansbury, 2002), impairment of 26S proteasome (Snyder et al., 2003), and mitochondrial dysfunctions (Tanaka et al., 2001). Mitochondrial impairment results in reduced ATP synthesis. As 26S proteasome is an ATP-dependent protein degrading enzyme, mitochondrial impairment reduces its catalytic activity. Thus vicious cycles are formed within nigral neurons leading them to slowly progressing neuronal death. Mutated alpha-synuclein proteins show increased tendency for self-aggregation (El Agnuf et al., 1998).

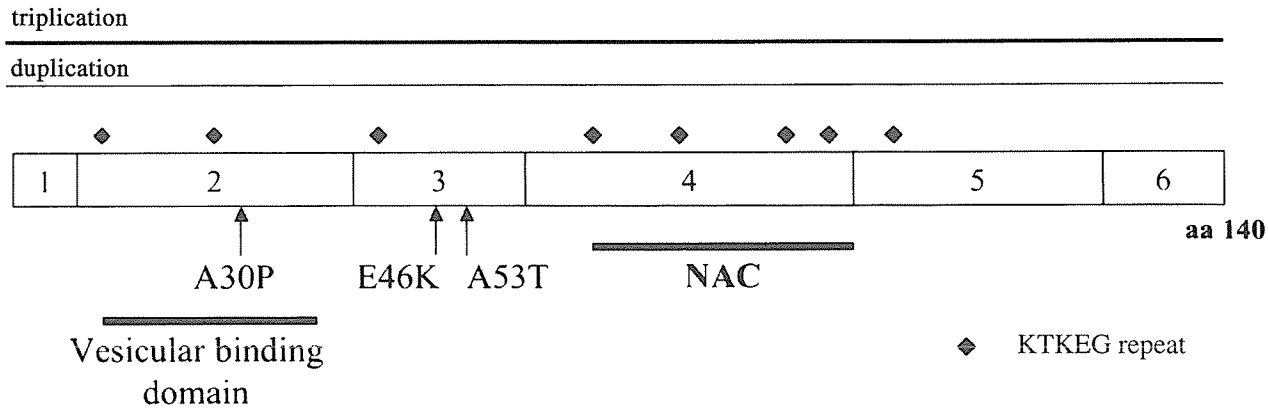

Fig. 1. Schematic presentation of the exons of *alpha-synuclein* and its mutations. Three missense mutations, duplication (thin line) and triplication (thick line) are known. Closed diamonds indicate approximate positions of the KTKEGV repeats. NAC represents non-amyloid component of the senile plaque

This is likely to be a reason for earlier onset of ages in familial PD due to alpha-synuclein mutations compared with sporadic PD. Furthermore, aggregated insoluble alpha-synuclein proteins are likely to impair transport of vital substances within nigral neurons. The insoluble aggregate of alpha-synuclein is highly phosphorylated at Ser-129 (Fujiwara et al., 2002).

PARK2

PARK2 is an autosomal recessive familial PD caused by mutations of *parkin*. Clinical features of PARK2 were first described by Yamamura et al. (1973). They reported 16 patients (13 familial patients in 5 unrelated families and 3 sporadic cases); clinical features of 11 patients from the initial 4 families were essentially identical. Ages of onset were between 17 and 28 years in 10 out of 11 patients and 42 in the remaining one. All the patients showed tremor, rigidity, bradykinesia, and postural instability. Atypical features included sleep benefit, temporary improvement of parkinsonism after a nap or sleep, and dystonic postures in the feet during walking (talipes equinovarus). Dementia was absent.

These patients show good response to L-dopa but they frequently develop motor fluctuations (wearing off and dyskinesia) sooner than late onset PD patients; usually two to three years after the initiation of L-dopa. Since the gene analysis became possible, many atypical features have been reported. For instance, age of onset can be as late as 72 (Lincolon et al., 2003). Other atypical features reported include dementia (Benbuman et al., 2004), psychosis and behavioral problem (Kahn et al., 2003), cerebellar ataxia (Kuroda et al., 2001), peripheral neuropathy (Tassin et al., 1998; Okuma et al., 2003; Kahn et al., 2003), hyperhidrosis (Yamamura et al., 1998), orthostatic hypotension (Kahn et al., 2003), urinary urgency and impotence (Kahn et al., 2003), and hemiparkinsonism-

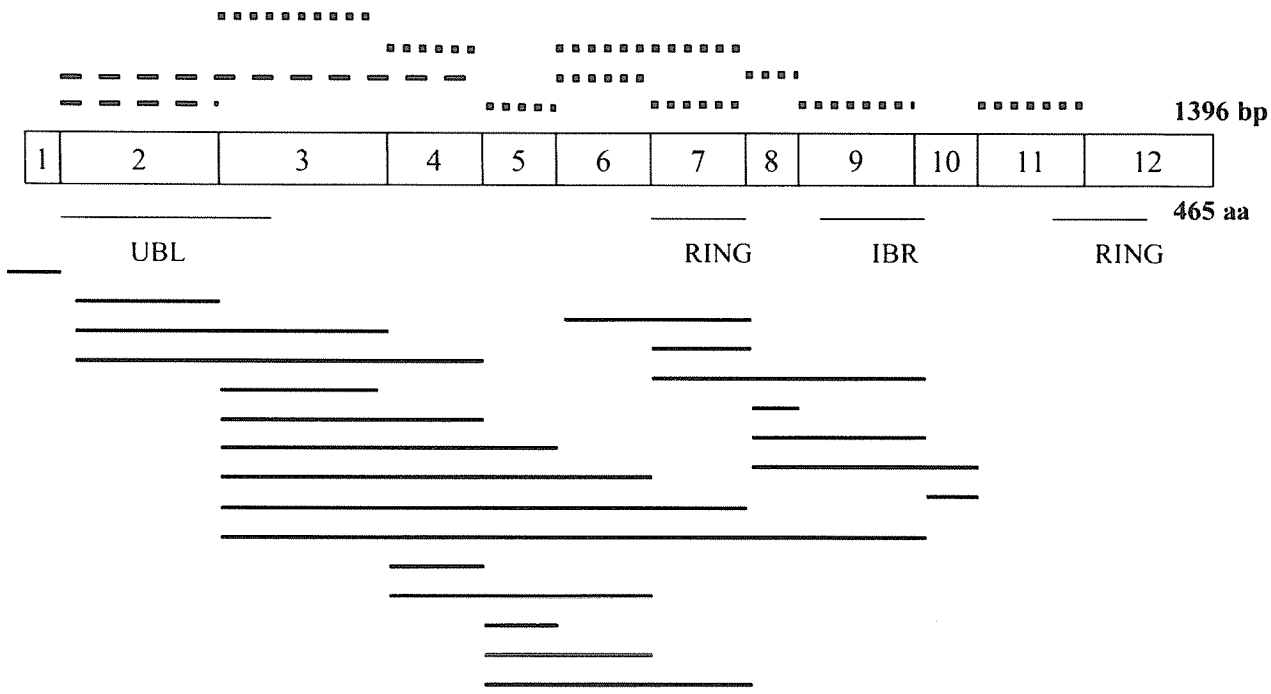

Fig. 2. Schematic presentation of exons of parkin and its exon rearrangements. summarized from the following literature, i.e., Hattori et al. (1998), Abbas et al. (1999), Lücking et al. (2000), Oliviera et al. (2003), and Hedrick et al. (2004). Broken lines indicate triplication, dotted lines duplication, and solid lines deletions of exons. *UBL* ubiquitin-like domain, *RING* RING domain, *IBR* in-between RINGs

hemiatrophy (Pramistaller et al., 2002) have been reported.

PARK2 is caused by mutations of *parkin* (Kitada et al., 1998), which has been mapped to the long arm of chromosome 6 at 6q25.2-q27. To date more than 30 different exon rearrangements (deletion, duplication, and triplication) (Fig. 2), 30 missense mutations, and 8 nonsense mutations, and close to 20 small deletions or insertions (Fig. 3) have been reported (Hattori et al., 1998; Abbas et al., 1999; Lücking et al., 2000; Oliviera et al., 2003; Hedrick et al., 2004). These numbers are still increasing. Usually PARK2 patients harbor either homozygous mutations or compound heterozygous mutations of *parkin*. But at times single heterozygous mutations are seen. According to our hand, approximately 20% of patients with *parkin* mutations were single heterozygotes, in that only one allele of *parkin* showed a mutation and we could not find the second mutation. Question is how they could have got the disease. As PARK2 is an autosomal recessive disorder, it is expected that both of parkin alleles are mutated. Although exact mechanism is not known, numbers of possibilities can be considered. For instance, single normal parkin may not be suffice to its complete function (haploinsufficiency); mutated parkin protein in some way may interfere with the function of normal parkin protein (dominant-negative effect); single mutated parkin may predispose to late onset Lewy body-positive PD.

Parkin protein was found to be an ubiquitin-protein ligase (E3) of the ubiquitin system (Shimura et al., 2000). The ubiquitin-proteasome system (UPS) is an important intracellular proteolytic system responsible for wide variety of biologically important cellular processes, such as cell-cycle progression, signaling cascades, developmental programs, the protein quality control system, DNA repair, apoptosis, signal transduction, transcription,

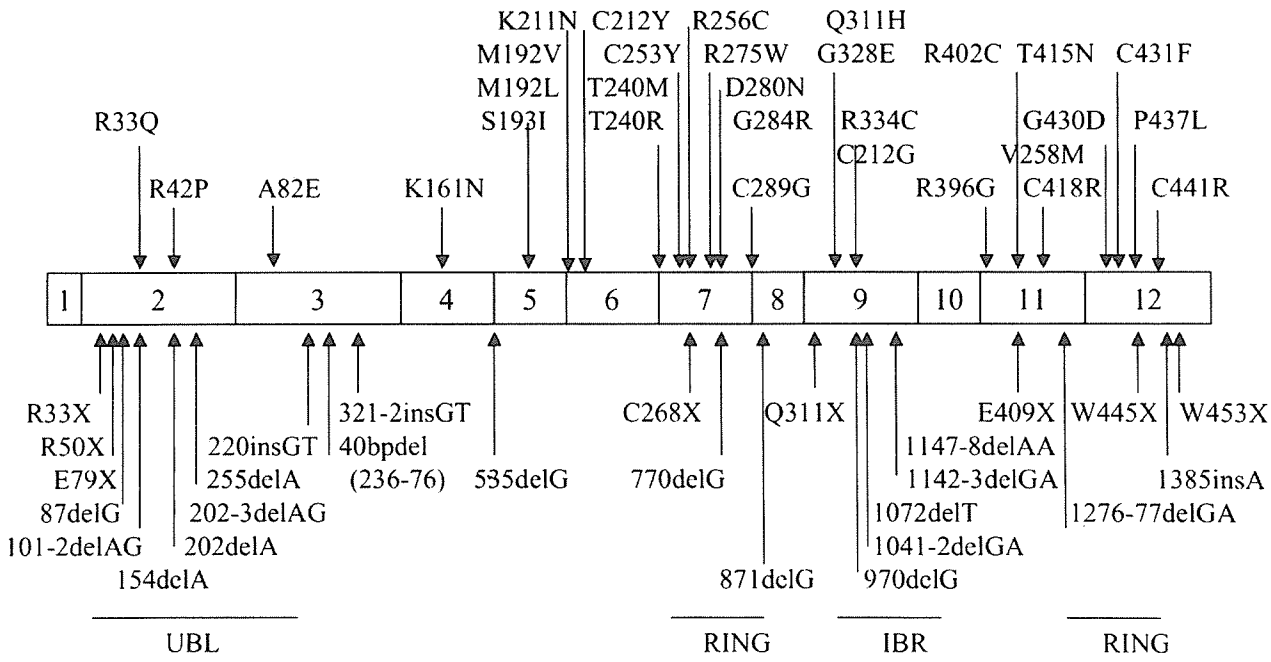

Fig. 3. Schematic presentation of exons of parkin and missense mutations, nonsense mutations, and small deletions summarized from the following literature, i.e., Hattori et al. (1998), Abbas et al. (1999), Lücking et al. (2000), Oliviera et al. (2003), and Hedrick et al. (2004). Missense mutations are shown above the exons and nonsense mutations and small deletions below the exons. *UBL* ubiquitin-like domain, *RING* RING domain, *IBR* in-between RINGs

metabolism, immunity, and neurodegeneration (Tanaka et al., 2004). The ubiquitin system consists of three enzymes, i.e., the ubiquitin activating enzyme (E1), the ubiquitin conjugating enzyme (E2), and the ubiquitin-protein ligase (E3). The E3 transfers ubiquitin molecules to target proteins forming a poly-ubiquitin chain which is recognized by 26S proteasome as the proteolytic signal. Therefore, in the presence of mutated parkin proteins, accumulation of parkin-substrate proteins is expected to be the major cause of nigral neuronal death. However, to date there is no clear immunohistochemical evidence to indicate accumulation of parkin-substrates in PARK2 patients, despite many parkin-interacting proteins have been reported such as CDCrel-1 (Zhang et al., 2000), glycosylated alpha-synuclein (Shimura et al., 2001), PAEL receptor (Imai et al., 2001), and synphilin-1 (Chung et al., 2001). We recently reported that parkin-knock down SH-SY5Y cells showed increased formation of dopamine/dopa-derived quinones and apoptotic cell death (Machida et al., 2005); these quinones appeared to be the mediator of cell death. Thus parkin appears to have a potent anti-oxidative property. As in the case of sporadic PD, oxidative damage may be an important mechanism of nigral neuronal death in PARK2.

Other mechanism that has been postulated is polyubiquitylation at the lysine-63 residue of the ubiquitin molecules. Polyubiquitin chains formed via the lysine-48 residue of the ubiquitin molecule mainly become a marker for proteolytic attack by the 26S proteasome. On the other hand, lysine 63-linked polyubiquitylation has many biological roles other than proteolysis, such as endocytosis, DNA repair, translation, IkB activation, DNA silencing, virus budding, protein sorting, and protein trafficking (Tanaka et al., 2004). Parkin promotes not only polyubiquitylation at lysine-48 but also at lysine-63. Recently, Lim et al. (2005) reported that parkin enhanced lysine-63 mediated polyubiquitylation of synphilin-1. Thus this is a novel aspect of the functions of parkin protein, however, exact molecular mechanism of nigral neurodegeneration in PARK2 is still open to question.

PARK3

PARK3 is an autosomal dominant familial PD linked to the short arm of chromosome 2 at 2p13 (Gasser et al., 1998). The disease gene has not been identified yet. Clinical features are essentially similar to those of sporadic late onset PD; the age of onset was 36 to 89. Interestingly, penetrance was 40% suggesting that some apparently sporadic PD patients may represent PARK3. Dementia developed in two out of six original families (Gasser et al., 1998). Autopsy findings from those families showed nigral neurodegeneration and neurofibirillary tangles in cortical neurons.

Recently, Strauss et al. (2005) reported a missense mutation (G399S) in *HtrA2/Omi*, which has been mapped to the same locus (2p13), in 4 sporadic PD patients; cells overexpressing S399 mutation was reported to be more susceptible to stress-induced cell death than wild type. But this mutation was negative in the original families of PARK3.

HtrA2 is a serine protease that has extensive homology to bacterial heat shock endoprotease (Faccio et al., 2000). Interestingly this is a mitochondrial protein localized in the intermembrane space and is released from mitochondria upon apoptotic stimuli initiating apoptosis cascade by activating caspase 3 (Suzuki et al., 2001). This is a proapoptotic protein; nonetheless, its mutation in its PDZ domain (carboxy-terminal side) was associated with familial PD. Further interestingly, a mutation in the protease domain caused motor neuron degeneration type 2 in mice (Jones et al., 2003). Knockout mice were reported to have shown striatal neuronal loss (Martins et al., 2004). This gene appears to be an interesting addition to the research on familial PD.

PARK4

PARK4 is an autosomal dominant familial PD caused by triplication of *alpha-synuclein* (Singleton et al., 2003). This mutation was found in the large kindred, which has been designated as Spellman–Muenter–Waters–Miller family or Iowanian family. Initial family was reported by Spellman (1962) who reported an autosomal dominant family with PD in the United States. Then Muenter et al. (1998) made extensive clinical studies on this family. Another autosomal dominant family later reported by Waters and Miller (1994) was found to be another branch of the kindred reported by Spellman and Muenter. Clinical features consist of L-dopa responsive parkinsonism and dementia, which are consistent with clinical diagnosis of diffuse Lewy body disease. In autopsied patients, many cortical Lewy bodies were found in addition to nigral neurodegeneration with Lewy body formation.

This family was reported to be linked to the short arm of chromosome 4 (Farrer et al., 1999) but in fact the causative gene of this family was found to be triplication of *alpha-synuclein* (Singleton et al., 2003); the 1.5 Mb region including introns on both sides of *alpha-synuclein* was triplicated in a tandem fashion. Therefore, PARK4 should be reclassified as a form of PARK1.

PARK5

PARK5 is an autosomal dominant familial PD linked to the short arm of chromosome 4 at 4p14-p15.1. To date only one family is reported (Leroy et al., 1998). Clinical features are essentially similar to those of late onset sporadic PD; the age of onset was 49 to 50.

Leroy et al. (1998) found I93M missense mutation in the ubiquitin carboxyterminal hydrolase-L1 gene (*UCH-L1*) (Fig. 4). UCH-L1 is a neuron specific enzyme that cleaves carboxyterminal peptide bond of polyubiquitin chains; UCH-L1 is an ubiquitin recycling enzyme. I93M-mutated UCH-L1 has half of the catalytic activity of the wild enzyme (Leroy et al., 1998). The supply of ubiquitin for proteins that have to be destroyed by 26S proteasome may be reduced with this mutation. Interestingly homozygous deletion of exon 7 and 8 in mouse UCH-L1 causes gracile axonal dystrophy (*gad*) mouse; this is an autosomal recessive condition characterized by axonal degeneration and formation of spheroid bodies in motor and sensory nerve terminals (Saigho et al., 1999).

PARK6

PARK6 is an autosomal recessive young onset familial PD caused by mutations of *PINK1* (*PTEN-induced kinase 1*) (Valente et al. (2001). Clinical features of PARK6 are essentially similar to those of PARK2; the age of onset of the original family studied by Valente et al. (2001) ranged from 32 to 48, somewhat older than those of PARK2. Reflecting this later age of onset, dystonia and sleep benefit which are common to young onset PARK2 are usually not seen in PARK6 unless the age of onset is young.

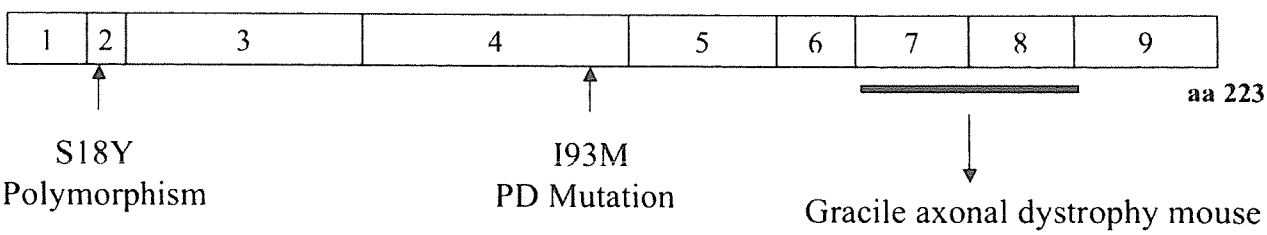

Fig. 4. Schematic presentation of exons of *UCH-L1* and its mutations. Only one mutation is known. I93M is associated with autosomal dominant PD. Interestingly homozygous exonic deletion involving exon 7 and 8 induces gracile axonal dystrophy (gad) mouse. S18Y polymorphism is said to confer neuroprotection for sporadic PD, but controversies exist

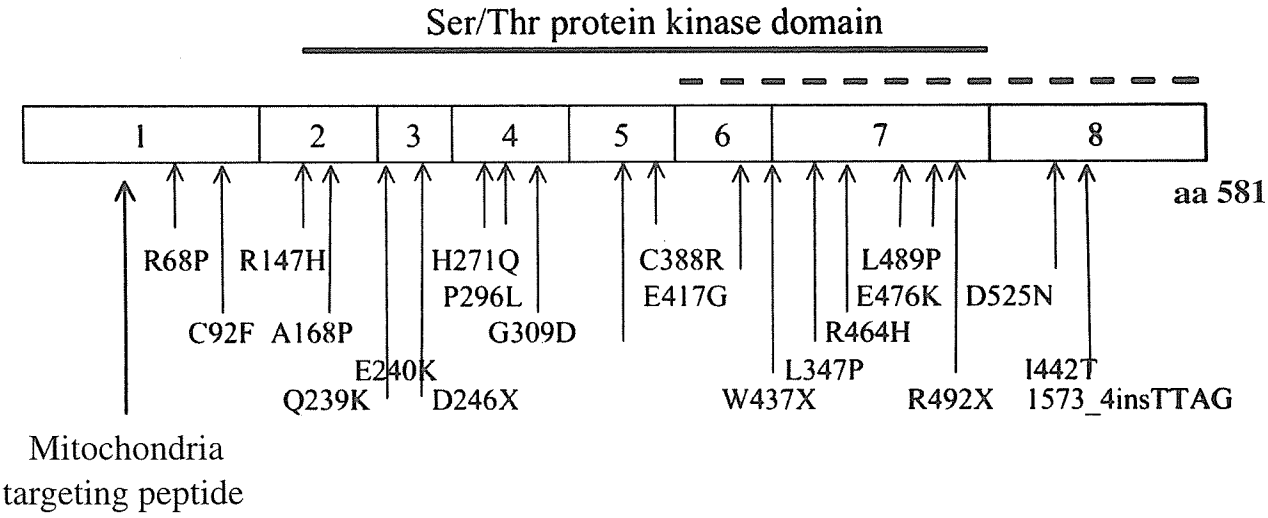

Fig. 5. Schematic presentation of exons of *PINK1* and its mutations summarized from the following literature, i.e., Valente et al. (2004), Hatano et al. (2004), Heary et al. (2004), Rohe et al. (2004), and Li et al. (2005). As PINK1 is a mitochondrial protein, it has a mitochondria-targeting sequence (exon 1). Two mutations in this targeting sequence are also known. Many missense and nonsense mutations are reported. Recently, we found an exonic deletion involving exon 6 to 8 indicated by the broken line. The solid line indicates the catalytic domain

PINK1 has been mapped to the short arm of chromosome 1 at 1p35-p36 (Valente et al., 2004). To date, 17 missense mutations, 3 nonsense mutations, one insertion, and one exon deletion are known (Valente et al., 2004; Hatano et al., 2004; Heary et al., 2004; Rohe et al., 2004; Li et al., 2005) (Fig. 5). We recently found a novel missense mutation (C388R) and an exonic deletion from exon 6 to 8; the latter was the first documented case with exonic deletion mutation in PARK6 (Li et al., 2005). PARK6 appears to be the second most common autosomal recessive PD after PARK2.

PINK1 is a mitochondrial matrix protein and has a protein kinase activity, however, its exact functions are not known. PINK1 stands for PTEN-induced kinase 1. PTEN stands for protein tyrosine phosphatase with homology to tensin: *PTEN* is a tumor suppressor gene on chromosome 10 mutated in many human tumors (Steck et al., 1997).

PARK7

PARK7 is an autosomal recessive familial PD caused by mutations of *DJ-1* (Bonifati et al., 2003). Clinical features are essentially similar to those of PARK2 including the age of onset, which is younger than that of PARK6. Affected patients show L-dopa-responsive parkinsonism of varying severity and drug-induced motor fluctuation and dyskinesia. Interestingly, three out of four patients in the original family showed psychiatric disturbances (anxiety attacks) (Dekker et al., 2003). Atypical clinical features include short statue and brachydactyly, which were found in Dutch kindred (Dekker et al., 2004).

DJ-1 has been mapped to the short arm of chromosome 1 at 1p36 and was identified as a novel oncogene that transformed mouse NIH3T3 cells in cooperation with activated Ras (Nagakubo et al., 1997). To date, 6 missense mutations, 1 intronic mutation, 1 small deletion, and 2 exonic deletions (exon 1 to 5 and exon 5 to 7) are known (Bonifati et al., 2003; Abou-Sleiman et al., 2003; Hague et al., 2003; Hering et al., 2004) (Fig. 6). *DJ-1* mutations are rare compared with *parkin* and *PINK1* mutations. We could not find *DJ-1* mutations among Japanese PD families studied.

DJ-1 is a potent anti-oxidative protein and this character depends on its 106-cysteine residue (Taira et al., 2004). DJ-1 is a cytoplas-

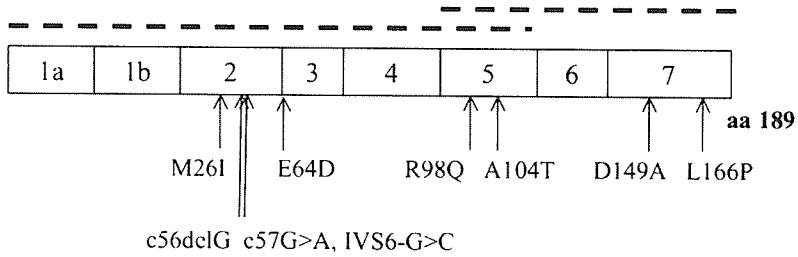

Fig. 6. Schematic presentation of exons of *DJ-1* and its mutations summarized from the literature, i.e., Bonifati et al. (2003), Abou-Sleiman et al. (2003), Hague et al. (2003), and Hering et al. (2004). Exon 1 and 2 are spliced out in the mature protein. Broken lines indicate exonic deletions

mic protein (Bonifati et al., 2003); however, oxidized DJ-1 is relocated to mitochondria (Canet-Aviles et al., 2004). DJ-1 undergoes dimmer formation to become active (Honbou et al., 2003; Tao and Tong, 2003). One of the PD-inducing missense mutations, L166P, interferes with dimmer formation (Wilson et al., 2003) and is degraded more rapidly than wild DJ-1 by ubiquitin-proteasome-system (Macedo et al., 2003; Miller et al., 2003) or by autoproteolysis (Gorner et al., 2004). This mutant DJ-1 is also mislocalized to mitochondria. Further interestingly, parkin interacts with mutated DJ-1 (L166P) but not with wild one (Moore et al., 2005), suggesting that parkin might be acting as a quality control protein for DJ-1. Thus molecular mechanism of nigral neuronal death in PARK7 appears to be at least in part related to dysfunction of anti-oxidative property of DJ-1.

PARK8

PARK8 is an autosomal dominant PD caused by mutations of *LRRK2/dardarin*. Clinical features were first described in large Japanese kindred (Nukada et al., 1978). They reported 36 patients in 5 generations. The age of onset ranged from 38 to 68 (mean = 53). Later the mean age of onset was reported as 51 ± 6 as the number of affected members increased (Funayama et al., 2002). Initial symptom was either gait disturbance or rest tremor. All of them showed L-dopa-responsive parkinsonism. Motor fluctuations and psychiatric side effects from L-dopa treatment can be seen. Clinical features are essentially similar to those of late onset sporadic PD except for slightly younger age of onset. Post-mortem examination in four patients from the original family showed pure nigral degeneration without Lewy body formation (Funayama et al., 2002). But later on another patient who came to autopsy from the same family showed nigral degeneration with Lewy bodies (Personal communication with Dr. K. Hasegawa).

The Western Nebraska family (Family D) reported by Wszolek et al. (1995), which inluded 18 patients in 5 generations, turned out to be PARK8. The age of onset was 48 to 78 (mean 63). Neuropathological features of this family are very interesting in that among the four patients who came to autopsy, one patient showed brain stem type Lewy body pathology; the second patient showed diffuse Lewy body disease pathology; the third patient showed nigral neuronal loss and gliosis with neurofibrillary tangles in the remaining nigral neurons without Lewy body formation; the fourth patient showed marked neuronal loss and gliosis in the nigra and locus coeruleus without any inclusions or tau-positive accumulations (Wszolek et al., 2004). Four different pathological findings in the same family would indicate the difficulty of defining a disease entity by neuronal inclusions. Family A reported by Denson and Wszolek (1995) was also turned out to be PARK8.

PARK8 has been mapped to the centromeric region of chromosome 12 (Funayama

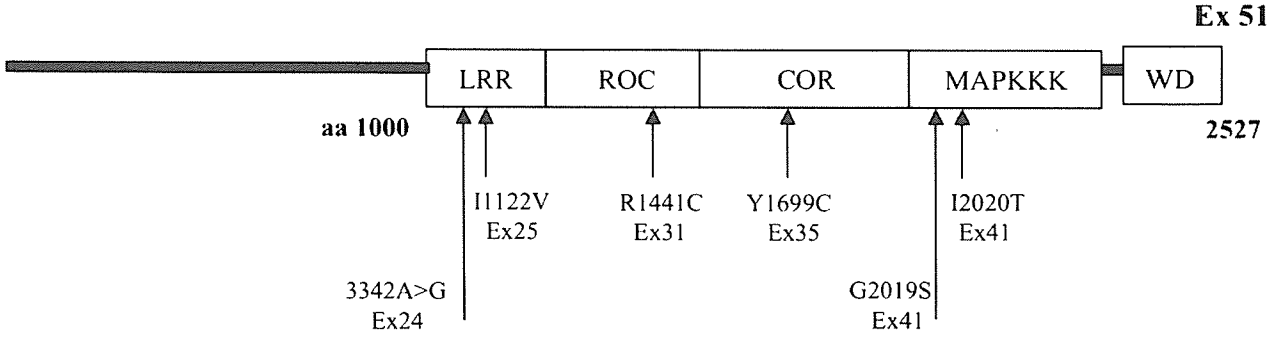

Fig. 7. Schematic presentation of *LRRK2* and its mutations summarized from the literature, i.e., Zimprich et al. (2004), Paisan-Ruiz et al. (2004), and Kachergus et al. (2005). LRRK2 protein belongs to ROCO protein family, which is characterized by the presence of ROC domain and COR domain. Many of the ROCO proteins also have LRR, MAPKKK, and WD domains. See the text for the explanations of these domains. To date 6 missense mutations have been reported in the homology region. Exon 41 appears to be a mutational hot spot

et al., 2002). The causative gene was identified as *LRRK2/dardarin* (Zimprich et al., 2004; Paisan-Ruiz et al., 2004). LRRK2 stands for leucine-rich repeat kinase 2 and dardar means tremor in the Bask language where families of PARK8 are found. *LRRK2* is a huge gene encompassing 144 kb and the open reading frame consists of 1449 base pairs in 51 exons. LRRK2 protein consists of 2527 amino acids and it is ubiquitously expressed in the cytoplasm of many organs. To date 6 missense mutations have been reported (Zimpricht et al., 2004; Paisan-Ruiz et al., 2004; Nichols et al., 2005) (Fig. 7).

LRRK2 protein belongs to the ROCO protein family. ROCO proteins are a group of proteins which has ROC and COR domain (Bosgraaf and Haastert, 2003). ROC stands for Ras in complex proteins belonging to the Ras/GTPase superfamily, and COR stands for carboxy terminal of ROC. In addition, many ROCO proteins have a LRR (leucine-rich repeat) domain, which has 3 to 16 leucine-rich repeats, a MAPKKK (mitogen-induced protein kinase kinase kinase) domain, and a WD domain, which is rich in tryptophan and aspartate repeats. The function of LRRK2 is still unknown but as it has protein kinase domain, it is likely that its role is phosphorylation of proteins that are important for the survival of nigral neurons. It is interesting to note that alpha-synuclein aggregates in PD are highly phosphorylated in Ser-129 (Fujiwara et al., 2002); therefore, it is an interesting question whether or not LRRK2 is in some way related to phosphorylation of alpha-synuclein.

PARK9

PARK9 is an autosomal recessive familial PD linked to the short arm of chromosome 1 at 1p36 (Hampshire et al., 2001). The causative gene has not been identified. Clinical features consist of L-dopa-responsive parkinsonism, supranuclear gaze palsy, pyramidal sign, and dementia, called Kufor-Rakeb syndrome. The age of onset is 10 to 20. Neuropathologically not only the substantia nigra but also the pyramidal tract, putamen, and the pallidum show neurodegeneration. PARK9 appears to be a form of multiple system atrophy.

PARK10

The PARK10 is linked to the short arm of chromosome 1 at 1p32. This locus was found by genome wide scanning on familial as well as sporadic cases of PD in Iceland (Hicks et al., 2002); they studied 117 PD patients and 168 of their unaffected relatives within 51 families using 781 microsatellite markers. The mean age of onset was 65.8. They showed linkage to chromosome 1p32 with a lod score of 4.9. The disease gene has not been identi-

fied yet. As expected from the source of the clinical subjects, clinical features are essentially similar to those of sporadic PD.

PARK11

PARK11 is an autosomal dominant familial PD linked to the long arm of chromosome 2 at 2q36 to q37 (Pankratz et al., 2003). The causative gene has not been identified yet. Clinical features are essentially similar to those of sporadic PD with the mean age of onset at 58. Neuropathological findings are not known.

Other forms of familial PD

There are many families in which linkage analysis failed to show linkage to any one of the known loci that are associated with familial forms of PD. Such reports are increasing every year. According to our hands, we have analyzed 347 families for known PD-causing genes including non-Japanese families with either autosomal dominant or recessive inheritance. We found 116 families with *parkin* mutations, 8 families with *PINK1* mutations, no *DJ-1* mutation, 10 families with *LRRK2* mutations, and 2 families with *alpha-synuclein* duplication. Overall mutation rate was 136 positive families out of 347 (39.2%). In another word, approximately 60% of familial patients with PD did not have known mutations. Mutual relationship among the familial PD causing proteins is an interesting and important subject to study. Identifying new genes for familial PD would give us important information on this topic. Such information would also give us important clues to investigate pathogenesis of sporadic PD.

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