2006; Tan et al. 2005). On the other hand, *LRRK2* G2385R variant has recently been found the most common genetic risk factor among Chinese and Japanese, but not Caucasians (Di Fonzo et al. 2006; Funayama et al. 2007; Tan et al. 2007; Farrer et al. 2007). Moreover, in a recent report (Wu et al. 2006), a heterozygous *LRRK2* p.P755L (c.2264c > *t*, rs34410987) mutation within *LRRK2* exon 19, corresponding to a predicted ankyrin-repeat-like domain of LRRK2, was found in 2% (12/598) of Chinese sporadic PD and 0% (0/765) of Chinese normal controls, suggesting its association with the disease. However, *LRRK2* P755L was reported as a polymorphism (3% of 92 normal controls) in the dbSNP database of Taiwanese. Thus, to determine the frequency and the role of *LRRK2* P755L in Asian PD, we screened for *LRRK2* exon 19 in Japanese sporadic PD patients.

Subjects and methods

The nucleotide sequences of *LRRK2* exon 19 were determined by direct sequencing in 501 sporadic Japanese PD patients and 583 controls of the Japanese general population (Table 1). All blood samples and clinical information were obtained by the attending neurologists after obtaining informed consent from their patients. The study was approved by the ethics review committees of Juntendo and Osaka Universities. Diagnosis of PD was made by the attending neurologists based on the presence of parkinsonism and good response to anti-PD treatment. Controls of the Japanese general population were evaluated by neurologists to ensure none of them had PD. DNA was prepared using standard methods. They were amplified by polymerase chain reaction (PCR) of exon 19 and sequenced using BigDye Terminator Chemistry and ABI310 and 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequences of the primers, conditions of PCR, and conditions of sequencing were based on a previous report (Zimprich et al. 2004).

Results

We found 6 patients (6/501 = 1.2%) and 8 controls of the Japanese general population (8/583 = 1.6%) with a heterozygous P755L variant ($P = 0.80$, odds ratio = 1.15, 95% CI: 0.40–3.32, $\chi^2 = 0.064$) in *LRRK2* exon 19 (Table 2). No other variants were found in exon 19.

Discussion

The purpose of the present study was to clarify the role of an ethnic-specific variant in the causative gene for PD. Although PD is considered a heterogeneous disease with genetic-environmental interaction, some cases certainly exhibit a Mendelian-inherited disease or are associated with strong genetic and ethnic background. Indeed, the reported frequency of *LRRK2* G2385R was higher in Asian sporadic PD patients than in controls (Di Fonzo et al. 2006; Funayama et al. 2007; Tan et al. 2007), although this is not the case in Caucasians. Moreover, Wu et al. (2006) in Nanjing, China, recently reported that a heterozygous *LRRK2* P755L mutation was found in 2% (12/598) of Chinese sporadic PD and 0% (0/765) of normal controls, whereas none (0/463) of the Caucasian PD patients had this mutation (Deng et al. 2007), suggesting ethnic differences, like *LRRK2* G2385R. However, our results of large case-controlled study in Japanese revealed that *LRRK2* P755L is a non-disease associated polymorphism. Consistent with our data, this variant was present at similar frequency in Taiwanese PD patients (7/578 = 0.99%) and Taiwanese normal controls (10/339 = 0.97%) (Di Fonzo et al. 2006). Furthermore, the latest report in the Chinese population in Singapore showed the absence of segregation and association of P755L with PD (case 4/204 = 2.0%, control 6/235 = 2.6%, $P = 0.76$) (Tan et al. 2008). These findings might be based on ethnic or native differences in human migration history or human genetics.

We reported previously that the most common *LRRK2* G2019S mutation in Mendelian-inherited and sporadic PD

Table 1 Profile of analyzed samples in this study

Parameter	Patients	Controls of general population
Total sample, *n* (%)	501 (100)	583 (100)
Male, *n* (%)	249 (49.7)	312 (53.5)
Female, *n* (%)	252 (50.3)	271 (46.5)
Age at sampling (years)[a]	65.0 ± 9.6 (28–92)	45.0 ± 17.0 (21–98)
Male[a]	64.3 ± 10.2 (28–92)	43.6 ± 15.0 (22–92)
Female[a]	65.4 ± 9.9 (28–92)	46.8 ± 19.0 (21–98)
Age at onset (years)[a]	58.0 ± 10.5 (20–88)	
Male[a]	57.7 ± 10.9 (20–88)	
Female[a]	58.3 ± 10.1 (25–82)	

[a] Data are mean ± SD (range)

Table 2 Allele frequency of *LRRK2* c. 2264C > T (p. P755L) in Japanese patients with Parkinson's disease and controls of general population

	Genotype, *n* (%)			Allele, *n* (%)			
	C/C	C/T	T/T	C	T	χ^{2a}	OR (95% CI)
Patients ($n = 501$)	495 (98.8)	6 (1.2)	0 (0)	996 (99.4)	6 (0.6)	0.06	1.15 (0.40–3.32)
Controls of general population ($n = 583$)	575 (98.6)	8 (1.4)	0 (0)	1,158 (99.3)	8 (0.7)		

[a] Compared with the control

OR odds ratio, *CI* confidence interval

was rare in Asians compared to North Africans or Caucasians (Tomiyama et al. 2006). *LRRK2* variants are reported to spread worldwide with some ethnic differences among each variant, such as R1441G, R1441C, R1441H (exon 31, ROC domain), G2019S, I2020T (exon 41, MAPKKK domain), and G2385R (exon 48, WD40 domain) (Mata et al. 2005). Since *LRRK2* consists of as many as 51 exons, it is important to decide which exon(s) of this gene should be screened first for efficient analysis of mutation in patients with various ethnic backgrounds. In this regard, *LRRK2* exon 41 and 31 are reasonable to be screened first; however, exon 19 is not likely a candidate exon for causative mutation screening in PD. In addition, although MAPKKK and ROC domain are reported to be associated with kinase activity of LRRK2 (Paisán-Ruíz et al. 2004; Zimprich et al. 2004; Smith et al. 2006), the existence and the role of the predicted ankyrin repeat-like domain in LRRK2 have not been established yet.

So far, *LRRK2* P755L as well as G2385R variants have been found in only Chinese, Taiwanese, and Japanese (Asians) with similar frequencies in some Asians, but have not been found in Caucasians. Thus, these variants could occur independently in very ancient Asians with a single founder effect (Farrer et al. 2007). Although the HapMap project has been very successful, the presence of ethnic differences among *LRRK2* variants such as G2019S, R1441G, G2385R, and P755L suggest that further establishment of ethnic-specific or native-specific data is essential for more accurate SNP analyses and genome-wide association studies.

Conclusion

Our extended association study in Japanese with large sample size suggests that *LRRK2* P755L is a non-disease-associated polymorphism in PD patients.

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Progress in the pathogenesis and genetics of Parkinson's disease

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ABSTRACT

Recent progresses in the pathogenesis of sporadic Parkinson's disease (PD) and genetics of familial PD are reviewed. There are common molecular events between sporadic and familial PD, particularly between sporadic PD and *PARK1*-linked PD due to *α-synuclein* (*SNCA*) mutations. In sporadic form, interaction of genetic predisposition and environmental factors is probably a primary event inducing mitochondrial dysfunction and oxidative damage resulting in oligomer and aggregate formations of α-synuclein. In *PARK1*-linked PD, mutant α-synuclein proteins initiate the disease process as they have increased tendency for self-aggregation. As highly phosphorylated aggregated proteins are deposited in nigral neurons in PD, dysfunctions of proteolytic systems, i.e. the ubiquitin–proteasome system and autophagy–lysosomal pathway, seem to be contributing to the final neurodegenerative process. Studies on the molecular mechanisms of nigral neuronal death in familial forms of PD will contribute further on the understanding of the pathogenesis of sporadic PD.

TOP

1. INTRODUCTION

Clinical features of Parkinson's disease (PD) were first described by Parkinson (1817). He reported six patients in his monograph published in 1817 and described most of the typical clinical features such as bradykinesia, rest tremor, postural instability, stooped posture and micrographia. He did not describe rigidity, which was described by Charcot (1888); he proposed to call this disease as PD. In this review, we will focus on the recent progress in the pathogenesis of neuronal death in sporadic as well as familial PD.

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2. CLINICAL FEATURES OF PD

Clinical features of PD include bradykinesia, rest tremor, rigidity and postural instability. In addition to these four cardinal symptoms, loss of automatic movements such as loss of arm swing, loss of blinking, reptile stare, masked face and difficulty in two simultaneous motor acts comprise motor features of PD. Furthermore, many non-motor symptoms frequently appear in PD, such as cognitive impairment, hallucination, delusion, behavioural abnormalities, depression, disturbances of sleep and wakefulness, loss of smell, pain, and autonomic dysfunctions such as constipation, hypotension, urinary frequency, impotence and sweating. Definition of PD has been proposed in various ways. For the research purpose, British Brain Bank criteria (Hughes *et al.* 1992) are frequently used, which define PD as those patients who have bradykinesia and at least one of the remaining four cardinal symptoms. Also, other causes of Parkinsonism have to be excluded by appropriate tests and/or

clinical observation.

3. NEUROPATHOLOGY OF PD

The most characteristic features of neuropathology are loss of pigmented neurons in the substantia nigra (SN; Tretiakoff 1919) and the presence of eosinophilic cytoplasmic inclusion bodies (Lewy bodies); Lewy (1912) discovered these inclusions in the substantia innominata. Lewy bodies are usually absent in autosomal recessive young onset Parkinsonism due to *parkin* mutations (Takahashi *et al.* 1994; Mori *et al.* 1998). Also, in *PARK8*-linked PD, Lewy bodies may or may not be present (Wszolek *et al.* 2004).

Neuronal loss and Lewy body formation are seen not only in SN but also in locus coeruleus, pedunculopontine nucleus, raphe nucleus, dorsal motor nucleus of the vagal nerve, olfactory bulb, parasympathetic as well as sympathetic post-ganglionic neurons, Meynert nucleus, amygdaloid nucleus and cerebral cortices. These lesions are responsible for non-motor symptoms of PD. Braak *et al.* (2003) proposed a hypothesis that in PD and PD with dementia (PDD), Lewy bodies were first formed in the dorsal motor nucleus and the olfactory bulb and slowly involved higher structures along the brain stem, diencephalon and the cerebral cortex.

4. NEUROTOXIN-BASED MODELS OF PD

The following substances have been used in producing animal models of PD, i.e. 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-tetrahydropyridine (MPTP), mitochondrial complex I-inhibitors such as rotenone, and α-synuclein overexpression. Systemic administration of MPTP produces selective degeneration of the dopaminergic neurons in SN in both humans and animals (Davis *et al.* 1979; Burns *et al.* 1983; Langston *et al.* 1983). We were interested in how MPTP killed nigral neurons. MPTP is oxidized by glial monoamine oxidase B to 1-methyl-4-phenylpyridinium ion (MPP^+; Chiba *et al.* 1984), which kills nigral neurons (Chiba *et al.* 1985; Javitch *et al.* 1985). We noted structural similarity between MPP^+ and NAD^+, which is an important cofactor of respiratory enzymes. We thought that MPP^+ might inhibit the activities of mitochondrial NAD^+-linked respiratory enzymes. Two groups reported inhibition of complex I by MPP^+ (Nicklas *et al.* 1985; Ramsay *et al.* 1986). We independently made the same observation (Mizuno *et al.* 1987*a*). Furthermore, we found inhibition of the α-ketoglutarate dehydrogenase complex of the mitochondrial tricarboxylic acid cycle by MPP^+ (Mizuno *et al.* 1987*b*). This enzyme synthesizes succinate from α-ketoglutarate; succinate is an electron donor for complex II of the electron transport chain. Thus dual inhibition of the activities of complexes I and II would impair deleteriously the electron transport and ATP synthesis. Inhibition of the electron transport induces oxidative damage by increasing the formation of reactive oxygen species.

5. AETIOLOGY AND PATHOGENESIS OF PD

We thought that mitochondrial function might be impaired in PD. Schapira *et al.* (1989) reported decreased activity of complex I in SN of PD. We found a decrease in complex I proteins by immunoblotting (Mizuno *et al.* 1989) and by immunohistochemistry (Hattori *et al.* 1991). We also found a decrease in the amount of α-ketoglutarate dehydrogenase complex in SN of PD by immunohistochemistry (Mizuno *et al.* 1994). Thus biochemical changes in PD were essentially similar to those of the MPTP-induced Parkinsonism.

Oxidative damage is also an important factor for nigral neuronal death; increase in iron (Youdim *et al.* 1989), increase in lipid peroxides (Dexter *et al.* 1989), decrease in glutathione (Sofic *et al.* 1992), increase in hydroxynonenal-modified proteins (Yoritaka *et al.* 1996) and increase in 8-hydroxy-deoxy guanine (Shimura-Miura *et al.* 1999) were reported in SN of PD. Reactive oxygen species impair mitochondrial proteins, further aggravating mitochondrial function. Ultimate outcomes are the dissipation of mitochondrial membrane potential and the release of cytochrome *c* into cytoplasm

activating the apoptotic cascade. Apoptotic nigral neuronal death has been postulated in PD (Mochizuki *et al.* 1996; Anglade *et al.* 1997). The interaction of genetic predisposition and environmental factors is believed to trigger mitochondrial dysfunction and oxidative damage in sporadic PD. Additional aetiological as well as pathogenetic factors are summarized in figure 1.

Figure 1
Aetiological and pathogenetic factors of PD. Aetiology refers to the cause of the disease and pathogenesis represents molecular events that lead nigral neurons to death.

6. DYSFUNCTION OF PROTEIN DEGRADATION IN PD

In recent years, dysfunction of protein degradation has emerged as an important contributor to nigral neuronal death in PD. Impaired protein degradation is likely to follow mitochondrial dysfunction and oxidative damage. In the presence of oxidative stress or mutated proteins, the folding process of proteins may be impaired resulting in an increase in misfolded proteins. Misfolded proteins are generally cytotoxic and have to be removed by protein degradating systems. In eukaryotic cells, the ubiquitin–proteasome system and the autophagy–lysosomal pathway are two major protein degradation systems. The ubiquitin system consists of three enzymes, i.e. the ubiquitin-activating enzyme (E1), the ubiquitin-conjugating enzyme (E2) and the ubiquitin-protein ligase; these enzymes work together to transfer ubiquitin molecules to target proteins that have to be metabolized by the 26S proteasome, which is an ATP-dependent proteolytic enzyme (Tanaka *et al.* 2004). Proteins with four or more than four ubiquitin molecules attached to lysine 48 residue of ubiquitin are recognized by the 26S proteasome. The 26S proteasome predominantly degrades short-lived nuclear and cytosolic proteins and misfolded proteins in the endoplasmic reticulum (Rubinsztein 2006).

The autophagy–lysosome pathway is able to degrade oligomers and aggregates of proteins as well as intracellular organelles (Yorimitsu & Klionsky 2005). In this pathway, double membrane-bound autophagosomes are generated by the elongation of small membranous structures, which encircle proteins to be digested. Then the autophagosome fuses with the lysosome to form the autophagosomes–lysosome. Then acidic hydrolases within the lysosome digest proteins that were incorporated into autophagosomes (Rubinsztein 2006).

What evidence do we have to suggest the dysfunction of protein degradation in PD? First of all, presence of Lewy bodies is strong evidence of impaired protein degradation. Lewy bodies consist of aggregated proteins and α-synuclein is the major component (Spillantini *et al.* 1997). Further interestingly, missense mutations of the *α-synuclein* gene (*SNCA*) cause autosomal dominant familial PD (Polymeropoulos *et al.* 1987; Krueger *et al.* 1998; Zarranz *et al.* 2004). α-Synuclein is a neuron-specific protein expressed predominantly in presynaptic membranes and the nucleus (Maroteaux *et al.* 1988). It is natively unfolded without a significant amount of secondary structure consisting of 140 amino acids (Weinreb *et al.* 1996). Thus the aggregation of α-synuclein emerged as one of the most important processes in nigral degeneration in PD. The mutant α-synuclein has increased tendency for self-aggregation (El Agnaf *et al.* 1998). α-Synuclein is mainly located in the lipid raft in membranes (Fortin *et al.* 2004; Kubo *et al.* 2005) and this localization appears to be important in the trafficking of α-synuclein and its final localization in presynaptic and synaptic vesicular membranes.

α-Synuclein is degraded by both autophagy and the proteasome; however, mutant forms of α-synuclein and oligomers are dependent on the autophagy–lysosome pathway for their clearance (Webb *et al.* 2003). Wild-type α-synuclein is translocated into lysosomes for degradation by the chaperone-mediated autophagy pathway; however, mutant A53T and A30P proteins can bind to the chaperon-mediated autophagy-pathway receptor on the lysosomal membrane, but act as uptake blockers inhibiting their own degradation and that of other proteins (Cuervo *et al.* 2004). In sporadic PD, dysfunctions of both ubiquitin–proteasome and autophagy–lysosome systems appear to be present. As 26S proteasome is an ATP-dependent enzyme, dysfunction of mitochondria will compromise its function. Furthermore, oxidative stress enhances oligomer formation of α-synuclein. Thus formed oligomers impair membrane structures (Volles & Lansbury 2002) such as synaptic vesicles and mitochondria, and further increase oxidative stress and mitochondrial dysfunction. In this way, vicious cycles will be formed within nigral neurons leading to neuronal death (figure 2). Decrease in the 26S proteasomal activity was reported in PD (McNaught *et al.* 2003).

Figure 2
Schematic presentation of nigral neuronal death in PD. In sporadic PD, interaction of genetic predisposition and environmental risk factors is believed to initiate the pathological cascade. In familial PD due to *SNCA* mutations, mutant α-synuclein (more ...)

We have only indirect evidence of lysosomal dysfunction in PD. Activation of lysosomal functions were reported by treating cell lines with Parkinsonism-inducing neurotoxins, such as overexpression of mutant α-synuclein (Stefanis *et al.* 2001), or proteasomal inhibitors (Ding *et al.* 2003; Rideout *et al.* 2004). Other indirect evidence came from studies on the association of Gaucher disease and PD. Gaucher disease is an autosomal recessive lysosomal lipid storage disease caused by mutations of a lysosomal enzyme, glucocerebroside β-glucosidase. Gaucher disease and its carrier state appear to be risk factors for PD (Tayebi *et al.* 2003; Ahron-Peretz *et al.* 2004; Goker-Alpan *et al.* 2004, 2006; Lwin *et al.* 2004; Sato *et al.* 2005; Kono *et al.* 2007). Furthermore, the recently identified *ATP13A2*, the disease gene for *PARK9*-linked PD (Kufor-Rakeb syndrome), encodes a lysosomal membrane protein (Ramirez *et al.* 2006). These two observations indicate the importance of lysosomal function for the maintenance of nigral neurons.

7. PROGRESS IN FAMILIAL PD

Thirteen chromosome loci have been identified to be linked to familial forms of PD (table 1). As *PARK1* and *PARK4* represent the same locus, the number of the familial forms is 12.

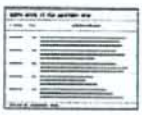

Table 1
Familial forms of PD. (AD, autosomal dominant; AR, autosomal recessive; LB, Lewy bodies; SP, sporadic.)

(a) *PARK1*- and *PARK4*-linked PD

PARK1- and *PARK4*-linked PD is an autosomal dominant one caused by mutations of the α-synuclein gene (*SNCA*); *PARK1* is caused by missense mutations and *PARK4* by multiplications of *SNCA*.

Three missense mutations, i.e. A53T (Polymeropoulos *et al.* 1997), A30P (Krüger *et al.* 1998) and E46K (Zarranz *et al.* 2004), duplications (Chartier-Harlin *et al.* 2004; Ibanez *et al.* 2004; Nishioka *et al.* 2006; Fuchs *et al.* 2007) and triplications (Singleton *et al.* 2003; Farrer *et al.* 2004) of *SNCA* are known. Missense mutations are very rare; A53T is limited to families with Greece origin; only one German family with A30P mutation and one Spanish family with E46K mutation are known. Multiplications of *SNCA* appear to be more common. Singleton *et al.* (2003) reported triplication of *SNCA* in a large kindred (Iowanian family). The triplication involved the 1.5 Mb region; exons of the adjacent genes on both side of *SNCA* were also triplicated. The amount of protein expressed would be doubled. Duplications of *SNCA* (Chartier-Harlin *et al.* 2004; Ibanez *et al.* 2004; Nishioka *et al.* 2006) were also reported. In the recently reported Swedish–American family (Fuchs *et al.* 2007), patients in the Swedish branch had duplication and those in the American branch (Farrer *et al.* 2004) had triplication. They suggested unequal recombination and unequal crossing over as the potential mechanisms for duplication and triplication, respectively (Fuchs *et al.* 2007).

There is a clinico-genetic correlation. E46K mutation and triplications are associated with Parkinsonism and dementia, and the age of onset is younger than the other mutations; neuropathological changes are those of diffuse Lewy body disease. A30P mutation is usually not associated with dementia. Duplication usually does not cause dementia but it can happen (Nishioka *et al.* 2006; Fuchs *et al.* 2007). A53T mutation may cause dementia and cortical Lewy bodies are reported (Golbe *et al.* 1990).

Regarding the pathogenesis of *PARK1*-linked PD, increased tendency for oligomer and aggregate formations of mutant α-synuclein is likely to be the cause (El Agnaf *et al.* 1998; Fredenburg *et al.*

2007). In duplication and triplication, increased amount of normal α-synuclein is probably predisposing nigral neurons for oligomer and aggregate formations. Recently, two groups independently reported that *SNCA* polymorphic mutations are significant risk factors for sporadic PD (Mueller *et al.* 2005; Mizuta *et al.* 2006); some of those polymorphic mutations were associated with increased α-synuclein expression. Thus the molecular mechanism of nigral degeneration is similar between *SNCA*-mutated and sporadic PDs.

Regarding the toxicity of oligomers, Volles & Lansbury (2002) reported that protofibrillar Ala30Pro and Ala53Thr had greater permeabilizing activities per mole than the wild-type protein. The leakage of vesicular contents induced by protofibrillar α-synuclein exhibited a strong preference for low-molecular mass molecules like dopamine, suggesting a pore-like mechanism for permeabilization.

(b) *PARK2*-linked PD

PARK2-linked PD is an autosomal recessive young onset PD. Clinical features were first described by Yamamura *et al.* in 1973. The usual age of onset is between 20 and 40, but it can be before 10 years and above 60 years. When the age of onset is young, dystonic features and sleep benefits are characteristic symptoms; sleep benefit represents temporal improvement in Parkinsonism after a sleep or nap. They respond well to L-dopa; however, they will soon develop motor fluctuations. Pathologically, SN undergoes severe neuronal loss and gliosis; the locus coeruleus is much less severely involved. Usually no Lewy bodies are seen (Takahashi *et al.* 1994; Mori *et al.* 1998), although rare Lewy body positive cases were reported (Farrer *et al.* 2001).

We identified the disease gene as follows. While we were doing an association study between the genetic polymorphism of the manganese superoxide dismutase gene (*sod2*) and sporadic PD (Shimoda-Matsubayashi *et al.* 1996), we found a family that appeared to be linked to the *sod2* locus, which had been mapped to the telomeric region of the long arm of chromosome 6. We did linkage analysis on 13 similar families and mapped the disease locus to the long arm of chromosome 6 near the *sod2* locus (6q25.2–27; Matsumine *et al.* 1997).

While we were doing linkage analysis on additional families, we found a patient who showed deletion of one of the microsatellite markers (D6S305) that we were using in the linkage analysis (Matsumine *et al.* 1998). We thought that this microsatellite marker might be located within the disease gene. By screening the Keio BAC library (Asakawa *et al.* 1997) using D6S305, we cloned a cDNA consisting of 2960 base pairs, of which 1395 base pairs constituted the open reading frame (Kitada *et al.* 1998). As this was a novel gene, we named it *parkin*. The total size of *parkin* was 1.4 Mb, the second largest gene after *dystrophin*. The number of exons was 12. The gene product consisted of 462 amino acids. There were unique structures in parkin protein (figure 3). There was 30% homology to ubiquitin in the amino terminal domain and there were two RING-finger-like motifs in the carboxyl half of the protein. RING stands for rare interesting gene and RING-like structures have been found in proteins with ubiquitin-ligase activity (Lorick *et al.* 1999). By northern blot, parkin messengers were ubiquitously expressed including the systemic organs (Kitada *et al.* 1998).

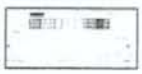

Figure 3

A schematic of the *parkin* gene. The coding region consists of 12 exons. In the amino terminal region, ubiquitin-like domain is indicated as 'Ubl', and in the carboxyl terminal side, two RING finger motives are indicated as 'RING1' (more ...)

Reported mutations in *parkin* now exceed 100 (Hattori *et al.* 1998; Abbas *et al.* 1999; Klein *et al.* 2000; Kann *et al.* 2002; Khan *et al.* 2003; Hedrich *et al.* 2004). Not only exonic deletions but also missense and nonsense mutations and multiplications of exons were reported.

We thought that parkin might be related to the ubiquitin–proteasome system (UPS); in fact the parkin protein had ubiquitin-protein ligase activity (Shimura *et al.* 2000). Since then many parkin-interacting proteins have been reported; the following are substrate candidates: CDCrel-1 (Zhang *et al.* 2000), which is believed to be negatively regulating transmitter release; glycosylated α-synuclein (Shimura *et al.* 2001); synphilin-1 (Chung *et al.* 2001), which is an α-synuclein-interacting protein; PAEL-receptor (Imai *et al.* 2001), which is an endoplasmic reticulum protein; p38 (Corti *et al.*

2003); polyglutamine (Tsai *et al.* 2003); α- and β-tubulins (Ren *et al.* 2003); cycline-E (Staropoli *et al.* 2003); SEPT5_v2 (Choi *et al.* 2003), which is also known as cell division-control protein-2; DJ-1 (Moore *et al.* 2005); RanBP2 (Um *et al.* 2006), which is a protein localized in the cytosolic filament of the nuclear core complex; and protein-1 (Ko *et al.* 2006). Also, parkin-regulatory proteins have been reported, i.e. CHIP (Imai *et al.* 2002), which is a chaperone; HSP-70 (Imai *et al.* 2002); Rpn10 subunit (Sakata *et al.* 2003); BAG5 (Kalia *et al.* 2004); Nrdp1/FLRF (Zhong *et al.* 2005); LRRK2 (Smith *et al.* 2005); and 14-3-3η (Sato *et al.* 2006*a*,*b*). In addition, non-lysine 48-related ubiquitylation substrates were reported. Lysine-63 polyubiquitylation is believed to be related to endocytosis, DNA repair, translation, iκB activation, DNA silencing, virus budding, protein sorting and protein trafficking (Tanaka *et al.* 2004). Recently, Lim *et al.* (2005) reported that synphilin-1 was polyubiquitylated at lysine 63 residue of ubiquitin. Other lysine sites are mono-ubiquitylated. Despite vast number of parkin-interacting proteins, there is no immunohistochemical proof of accumulation of above parkin-interacting proteins in autopsied patients.

Parkin-knockout (KO) mice do not show nigral neuronal loss or striatal dopamine deficiency (Goldberger *et al.* 2003; von Coelln *et al.* 2004; Perez & Palmiter 2005; Ko *et al.* 2005; Sato *et al.* 2006). What were reported are only subtle changes in dopaminergic functions. von Coelln *et al.* (2004) found some loss of neurons in the locus coeruleus and reduced startle response. Ko *et al.* (2005) found an increase in the amount of the aminoacyl-tRNA synthase cofactor p38 in the midbrain/hindbrain region of both young and old parkin-null mice. They postulated that p38 is a substrate of parkin as E3 ligase. They further showed that overexpression of p38 in the SN in mice lead to loss of dopaminergic neurons. They analysed the level of p38 in the cortical regions of the patients with parkin mutations by Western blotting; they found an increase in p38. We studied striatal dopamine receptors by *ex vivo* autoradiography. In *parkin*-KO mice, both striatal D1 and D2 receptor bindings were significantly increased when compared with wild mice. Midbrain dopamine content was increased in KO mice. Increase in D1 and D2 receptor bindings in the striatum would indicate reduction in dopamine release; increase in dopamine in nigral neurons would cause oxidative stress.

Ved *et al.* (2005) found increased sensitivity of mitochondria to complex I inhibitors such as rotenone, fenperoximate, pyridaben or stigmatellin in their *parkin*-KO *Caenorhabditis elegans*; they observed similar effects by overexpressing α-synuclein, or knocking down *DJ-1*. Further interestingly, *parkin*-KO *Drosophila* produced by Creene *et al.* (2003) exhibited reduced lifespan, locomotor defects and male sterility. Locomotor defects were due to muscle degeneration with mitochondrial damage consisting of disruption and disintegration of the cristae. There was no neuronal loss in the brain including dopaminergic neurons in these flies. Pesah *et al.* (2004) also reported similar findings. Then Clark *et al.* (2006*a*,*b*) and Park *et al.* (2006) made *PINK1/parkin* double KO *Drosophila*. Both groups reported that overexpression of parkin rescued PINK1-KO-induced muscle damage; but PINK1 overexpression could not rescue parkin-KO-induced damage. Furthermore, they showed interaction of parkin and PINK1. They concluded that parkin was functioning in the downstream of PINK1 in a common pathway to keep mitochondrial integrity. Their flies also showed dopaminergic neuronal degeneration.

At a cellular level, Machida *et al.* (2005) constructed a parkin-knockdown cell line using SH-SY5Y cells, which showed apoptotic cell death. Furthermore, they found increase in the auto-oxidized forms of L-dopa and dopamine (Dopa chrome+dopamine chrome), suggesting the presence of anti-oxidative property in parkin. We reported a profound accumulation of iron in the SN of a *parkin*-mutated patient (Takanashi *et al.* 2001). Taken together, oxidative stress appears to be a pathogenetic pathway common to *PARK2*-linked and sporadic PDs.

(c) *PARK6*-linked PD

PARK6-linked PD is another form of young onset autosomal recessive PD. The age of onset is slightly older than that of *PARK2*, i.e. from 32 to 48 years (Valente *et al.* 2001). Therefore, dystonic features and sleep benefit are uncommon. Affected patients show L-dopa-responsive Parkinsonism. The disease gene was identified as *PINK1 (PTEN-induced kinase 1*; Valente *et al.* 2004*a*,*b*). *PTEN* stands for *phosphatase with tensin homology*. *PINK1* has eight exons and cDNA spans 1.8 kb. It encodes a protein with 581 amino acids. The protein is ubiquitously expressed including brain and systemic organs. Interestingly, it is a mitochondrial protein located in the matrix and the

intermembrane space. It has a serine/threonine protein kinase domain. However, its function is not known (Valente *et al.* 2004).

Many missense and nonsense mutations have been reported (Hatano *et al.* 2004; Healy *et al.* 2004; Rohe *et al.* 2004; Valente *et al.* 2004; Li *et al.* 2005). In contrast to *parkin*, most of the *PINK1* mutations reported are either missense or nonsense mutations, although one family with a large deletion mutation is known (Li *et al.* 2005); this deletion involved exons 6–8 homozygously.

Regarding the molecular mechanisms of nigral neuronal death, as PINK1 has a protein kinase domain as LRRK2 has, dysfunction in some phosphorylation reactions may be important in the pathogenesis. PINK1 is inducible by PTEN and *PTEN* is an oncogene mutated in many neoplastic cells (Li *et al.* 1997). As oncogenesis and degeneration are the opposite sides of eukaryotic cell fate, elucidation of the function of PINK1 in relation to PTEN is an interesting topic.

(d) Do heterozygotes for *parkin* or *PINK1* mutations develop PD?

In autosomal recessive diseases, usually both alleles should have a mutation to show the disease. But in cases of *PINK1* (Valente *et al.* 2004; Bonifati *et al.* 2005; Fung *et al.* 2006; Criscuolo *et al.* 2006; Toft *et al.* 2007) as well as *parkin* mutations (Hedrich *et al.* 2002; Khan *et al.* 2002; West *et al.* 2002; Olivieira *et al.* 2003; Clark *et al.* 2006*a*,*b*; Schlitter *et al.* 2006), at times only one mutation can be found (heterozygote). The question is, how do they get the disease? There are several possibilities. First of all, the second mutation may be localized in a place that is difficult to find by the currently available methods. The second possibility is the haploinsufficiency (West *et al.* 2002); here the amount of normal gene product is not sufficient to keep nigral neurons alive. But usually in autosomal recessive diseases, only one normal gene is sufficient to prevent the disease; parents (usually carriers) of a patient are normal in most of the cases. The third possibility is the interaction at the protein level; the mutated protein might interfere with the functions of normal protein (dominant-negative effect); but this possibility has not been proved. Finally, single heterozygous state might be acting as a risk factor for sporadic PD (Schlitter *et al.* 2006). Another interesting observation in *parkin* heterozygotes was made by Olivieira *et al.* (2003) who reported that mutations in the first RING finger domain tended to be heterozygotic and associated with later age of onset.

(e) *PARK7*-linked PD

PARK7-linked PD is another young onset PD. Clinical features are very similar to those of *PARK2*-linked PD (Duijin *et al.* 2001); the age of onset is usually 20–40 years. Some atypical features such as psychiatric symptoms (anxiety attacks; Dekker *et al.* 2003), and short statue and brachydactyly (Dekker *et al.* 2004) have been reported. The disease gene was identified as *DJ-1* (Bonifati *et al.* 2003), which had been cloned by Nagakubo *et al.* (1997). The size of *DJ-1* is 24 kb with eight exons encoding a protein consisting of 189 amino acids. *PARK7*-linked PD is very rare (Bonifati *et al.* 2003; Hague *et al.* 2003; Hering *et al.* 2004).

The function of DJ-1 protein is not well known. The active form of DJ-1 is a dimer of monomeric DJ-1. DJ-1 is a cytoplasmic protein; however, it can translocate into the mitochondria. It has a strong anti-oxidative property (Nagakubo *et al.* 1997; Abou-Sleiman *et al.* 2003; Canet-Aviles *et al.* 2004; Moore *et al.* 2005) that depends on its cysteine residue at 106, which undergoes oxidation to form a disulphide bond (Canet-Aviles *et al.* 2004). Downregulation of endogenous DJ-1 protein of the neuronal cell line by siRNA was reported to enhance the cell death induced by oxidative stress, ER stress and proteasome inhibition, but not by pro-apoptotic stimulus (Yokota *et al.* 2003). The Leu166Pro mutant DJ-1 protein has a reduced anti-oxidative activity (Takahashi-Niki *et al.* 2004). DJ-1 protein expression is increased upon oxidative stress induced by paraquat (Mitsumoto *et al.* 2001). As nigral neurons are exposed to high oxidative stress owing to the presence of dopamine, DJ-1 may be acting as a strong anti-oxidative protein. As mutant DJ-1 was reported to interact with parkin (Moore *et al.* 2005), parkin might be acting as E3 ligase to remove mutated DJ-1.

(f) *PARK8*-linked PD

PARK8-linked PD is an autosomal dominant PD linked to the centromeric region of chromosome 12 (Funayama *et al.* 2002). Clinical features were described back in 1978 on a large Japanese family

(Nukada *et al.* 1978); clinical features are essentially similar to those of sporadic PD, except for slightly earlier onset of age. Dementia is not a common feature but it is known to occur (Wszolek *et al.* 1997).

The disease gene was identified as ***lrrk2*** (Paisan-Ruiz *et al.* 2004; Zimprich *et al.* 2004); ***lrrk2*** is a huge gene encompassing 144 kb, consisting of 7449 bp and encoding a protein consisting of 2517 amino acids, and has 51 exons. The carboxyl half of the LRRK2 contains several functional domains such as ANK (ankyrin-repeat domain), LRR (leucine-repeat-rich), ROC (Ras of complex proteins), COR (carboxy terminal of ROC), MAPKKK (mitogen activated protein kinase kinase kinase) and WD domain that is rich in tryptophan and aspartate repeats. Pathogenetic mutations are concentrated in these functional domains.

PARK8-linked PD is now believed to be the most common form of autosomal dominant familial PD and 20 missense or nonsense mutations have been reported (Paisan-Ruiz *et al.* 2004; Zimprich *et al.* 2004; Aasly *et al.* 2005; Di Fonzo *et al.* 2005; Funayama *et al.* 2005; Hernandez *et al.* 2005; Kachergus *et al.* 2005; Nichols *et al.* 2005; Paisan-Ruiz *et al.* 2005; Mata *et al.* 2006). ***lrrk2*** Mutations were also found in some of the apparently sporadic PD patients (Gilks *et al.* 2005). One of the polymorphic mutations, G2385R, is a genetic risk factor for sporadic PD in Asian populations (Di Fonzo *et al.* 2006; Funayama *et al.* 2007; Tan *et al.* 2007).

Four different neuropathologies were reported within the same family (Wszolek *et al.* 2004); one of their patients showed brain stem-type Lewy body disease, the second showed diffuse-type Lewy body disease, the third accumulation of tau in the remaining nigral neurons and the last simple nigral atrophy. This observation tells us the difficulty of defining a disease by neuronal inclusions.

Function of LRRK2 is not well known. The ROC domain is able to bind GTP but it does not have GTPase activity, but GTP binding is essential for the MAPKKK domain to exert kinase activity (Ito *et al.* 2007); some of the mutant LRRK2 have increased kinase activity (Gloeckner *et al.* 2006). Other functional domains are believed to be important in protein–protein interactions (Zimprich *et al.* 2004). LRRK2 also interacts with other familial PD proteins; Smith *et al.* (2005) reported interaction of LRRK2 with parkin through the ROC domain; however, the interaction with parkin did not enhance polyubiquitylation of LRRK2.

Recently, Hatano *et al.* (2007) made a detailed observation on the intracellular distribution of LRRK2. It was found to be present in Golgi apparatus, plasma membrane, synaptic vesicles and particularly in the lipid rafts; presence in the lipid rafts suggests that LRRK2 is probably involved in signal transduction, membrane trafficking and cytoskeletal organization (Brawn & London 1998). Biskup *et al.* (2006) also reported the presence of LRRK2 in membrane structures, such as lysosomes, endosomes, transport vesicles and mitochondria. In this regard it is interesting to note that α-synuclein is also expressed in the presynaptic membranes and lipid rafts (Fortin *et al.* 2004; Kubo *et al.* 2005).

(g) *PARK9*-linked PD

PARK9-linked PD is an autosomal recessive disorder characterized by L-dopa-responsive Parkinsonism, supranuclear gaze palsy, pyramidal sign and dementia; it is also called as Kufor-Rakeb syndrome; the name of the initial Jordanian family with this disorder (Najim Al-Din *et al.* 1994). Age of onset was very early, between 11 and 16 years. MRI showed significant atrophy of the globus pallidus and the pyramids, as well as generalized brain atrophy in later stages. Some of them developed facial–faucial–finger mini-myoclonus, visual hallucinations and oculogyric dystonic spasm (Williams *et al.* 2005).

Hamprhire *et al.* (2001) performed linkage analysis on this Kufor-Rakeb family and mapped the disease locus to the short arm of chromosome 1 at 1p36 with a maximum LOD score of 3.6, the hot spot for autosomal recessive familial PD. The disease gene was identified as *ATP13A2* (Ramirez *et al.* 2006), which is a lysosomal membrane protein with an ATPase domain; exact function is still unknown. It is interesting to note that mutations of a lysosomal membrane protein can induce nigral degeneration, suggesting the importance of lysosomes for the maintenance of the integrity of nigral neurons.

(h) Other forms of familial PD

PARK3-linked PD is an autosomal dominant PD. Clinical features are essentially similar to those of sporadic PD with the age of onset between 36 and 89 (Gasser *et al.* 1998). Patients from two out of six families reported in that literature developed dementia. Autopsy findings in two families showed nigral degeneration and neurofibirillary tangles in cortical neurons.

PARK5-linked PD is an autosomal dominant PD. Only one family is reported (Leroy *et al.* 1998). Clinical features are similar to those of sporadic PD with the age of onset from 49 to 50. The disease gene was reported as ubiquitin carboxyl-terminal hydrolase-L1 (*UCH-L1*; Leroy *et al.* 1998). Ile93Met missense mutation was found in the affected members. Deletion of exons 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).

UCH-L1 is an enzyme that cleaves carboxy-terminal peptide bond of polyubiquitine chains. Thus UCH-L1 is an ubiquitin-recycling enzyme. UCH-L1 is a neuron-specific enzyme. Catalytic activity of Ile93Met-mutated UCH-L1 was reported to be half of the wild enzyme (Leroy *et al.* 1998). Thus the supply of ubiquitin for 26S proteasome may be reduced with this mutation.

PARK10 was found by genome-wide scanning. Hicks *et al.* (2002) studied 51 Icelandic families with more than one PD patient; they analysed 117 patients and 168 of their unaffected relatives using 781 microsatellite markers. Allele-sharing, model-independent analysis of their results showed linkage to a region on chromosome 1p32 with a LOD score of 4.9. They designate this region *PARK10*. The disease gene has not been identified yet. Clinical features are essentially similar to those of sporadic PD and the mean age of onset was 65.8 years.

PARK11 was also found by genome-wide scanning; Pankratz *et al.* (2003*a*,*b*) screened 85 families with a very strong family history of PD and found an evidence of linkage to the long arm of chromosome 2 (LOD=4.9). Clinical features are essentially similar to those of sporadic PD with the mean age of onset at 58 years.

PARK12 was also found by genome-wide scanning on sporadic PD; Pankratz *et al.* (2003*a*,*b*) screened 277 families without a strong family history of PD and detected linkage to the long arm of chromosome X (LOD=3.2).

PARK13-linked PD was reported to have a mutation (G399S) in *Omi/HtrA2* (Strauss *et al.* 2005); they found G399S mutation in four German sporadic PD patients. They also identified a novel A141S polymorphism that was associated with PD ($p<0.05$). Both mutations resulted in defective activation of the protease activity of Omi/HtrA2. Further studies are necessary before concluding that this is a new familial PD-inducing protein. A cDNA of HtrA2 was first isolated by Faccio *et al.* (2000); it encoded a protein (Omi) consisting of 458 amino acids and had homology to bacterial HtrA endoprotease and had a PDZ domain. Omi mRNA was expressed ubiquitously, and the gene was localized on human chromosome 2p12 near the *PARK3* locus; however, *PARK3*-linked PD patients did not have this mutation. Omi/HtrA2 is a nuclearly encoded mitochondrial protein localized in the intermembrane space. Further interestingly, it has a serine protease domain and it binds to apoptosis-inhibiting protein upon release into the cytoplasm (Suzuki *et al.* 2001). G399S mutant Omi/HtrA2 induces mitochondrial dysfunction associated with altered mitochondrial morphology and cells overexpressing G399S mutant Omi/HtrA2 are more susceptible to stress-induced cell death than wild-type (Strauss *et al.* 2005).

Finally, there are many families in which linkage analysis failed to show linkage to any one of the known loci that are associated with familial PD. By elucidating the functions of familial PD proteins, pathogenesis of sporadic PD will be better understood.

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FOOTNOTES

One contribution of 17 to a Theme Issue 'Japan: its tradition and hot topics in biological sciences'.

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