1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

厚生科学研究費補助金（脳科学研究事業）

分担研究報告書

神経変性疾患でのミクログリアの毒性転換のメカニズムに関する研究

研究代表者：澤田　誠　名古屋大学環境医学研究所　教授

研究要旨　nef 導入により形質転換したミクログリアと、遺伝子変異を持つ不活性型 nef を導入したミクログリアにおいてディファレンシャル２次元タンパク質解析法により発現タンパク質の変化を調べ、発現するタンパク質に大きな差違があることを見いだした。

A．研究目的

HIV が中枢神経系に感染すると神経細胞死や神経機能障害を誘発し痴呆が生じることが知られている。このとき生じる生物反応はパーキンソン病などの神経変性疾患が発症する場合にも共通性がみられると考えられている。ミクログリアはこのときに中心的な役割を果たすと考えられているが、その詳細や実体は明らかでない。最近我々は、本来神経保護的に働くミクログリアに HIV 由来 nef 遺伝子を導入することによって神経障害性の活性酸素を産生するようになること（J Biol. Chem.277:42136-43, 2002 ）を見いだした。多くの神経変性疾患の場合、脳実質内で産生される活性酸素により神経障害が生じることから、nef 遺伝子導入によるミクログリアの形質転換が神経変性の引き金となっている可能性が強く考えられる．そこで、本研究では nef 遺伝子導入によるミクログリアの毒性転換の性質と nef 遺伝子産物の細胞内での作用点である細胞内因子を特定することを目的とし、nef 導入ミクログリアと非導入ミクログリアおよび変異型 nef 導入ミクログリに於いてそのタンパク質発現を解析した。

B．研究方法

nef 導入により形質転換したミクログリアと、遺伝子変異を持つ不活性型 nef を導入したミクログリアにおいてディファレンシャル２次元タンパク質解析法により発現タンパク質の変化を調べた。

（倫理面への配慮）本年度は細胞のタンパク質分析を実施したため倫理的な配慮に関しては該当する事項がない。

C．研究結果

- nef 遺伝子を導入したミクログリアは活性酸素産生が増大し、その発現料に伴って神経毒性を発揮した。
- N 末端に変異を持つためにミリストイル化がおこらない nef 変異体 G2A を導入した場合は活性酸素産生の増大や神経毒性の発現がみられなかった。
- nef 導入により形質転換したミクログリアと、遺伝子変異を持つ不活性型 nef を導入したミクログリアにおいてディファレンシャル２次元タンパク質解析法により発現タンパク質の変化を調べ、発現するタンパク質に大きな差違があること、活性型 nef 導入細胞で特

徴的な塩基性タンパクの集積が見られることを見いだした。
nef 抗体による免疫沈降分析から活性型 nef は活性酸素産生の主体である NADPH オキシダーゼサブユニットの一部に結合していることがわかった。

D. 考察

今回得られた nef 導入細胞に特徴的に見られるタンパク質はミクログリアの毒性転換に重要な役割を果たしていると考えられるため、今後質量分析法、Western blot 法などで分析して同定する必要がある。また、N 末端に変異を持つ不活性型 Nef ではミクログリアの形質転換作用が見られないことから、Nef の作用もしくは細胞内因子への調節作用（相互作用）にはそれ自身のミリストイル化が関与すると考えられる。また、ミクログリアの毒性転換において細胞膜、もしくは細胞内膜系への相互作用も考えられ、ミクログリアでの主要な活性酸素産生酵素である NADPH オキシダーゼと nef 遺伝子産物との直接結合および活性化には重要な意味があると考えられる.

E. 結論

ミクログリアに HIV 由来 nef を導入することによって細胞内のたんぱく質の性質が変化し、神経細胞に対する毒性を発現することがわかった。

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H．知的財産権の出願・登録状況

1．特許取得

・国際特許出願「脳移行活性を有するポリペプチド、およびその利用」平成 16 年 8 月 6 日提出、PCT 出願
・国内特許出願　「脳移行性骨髄前駆細胞」、平成１６年１０月１２日提出、特願２００４－２９８１７０

2．実用新案登録

なし

3．その他

なし

研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

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研究成果の刊行物・別刷り

CHAPTER

Do Familial Parkinson's Disease Genes Share a Common Pathway Involved in the Nigral Degeneration?

Nobutaka Hattori, Yasuko Hatano, Yuanzhe Li, Hiroyuki Tomiyama, Yutaka Machida, Kenichi Sato and Yoshikuni Mizuno

Introduction

Parkinson' s disease (PD) is the most common progressive movement disorders characterized by resting tremor, cogwheel rigidity, bradykineasi, and impaired postural reflexes with a prevalence of approximately 200/100,0000 among white populations[1] and 100/100,000 in Japanese.[2] Considering the age at onset, this disease affects 1 to 2 percent of persons older than 65 years of age.

The exact cause of this disease has been unclear, however, there has been growing evidence that mitochondrial dysfunction, oxidative stress, and genetic factors contribute the pathogenesis of PD. Moreover, progress in understanding the pathogenesis of this disease has been done after the identification of causative genes or loci for familial PD (FPD). Therefore, there is no question about the genetic influence on the development for PD. Studies on the frequency of PD among the first relatives of index patients with PD was reported as twice and three times than that in the control population.[3-5] Furthermore, the role of genetic factors in FPD is supported by the high concordance in twins using PET scans.[6-8] Thus, it is now clear that clinically defined PD represents a heterogeneous group of disorders that encompasses a small proportion of individuals with inherited disease and a larger population with seemingly sporadic disease.

To identify susceptibility gene for PD, earlier efforts to identify a genetic defect in PD were mainly based on the candidate gene approach. Many polymorphisms have been screened by linkage analysis or association studies with PD, including those involved in mitochondrial respiratory chain, dopamine biosynthesis, neurotransmitter, and enzymes regulating the metabolism of neurotoxins or free radicals. Although controversies exist with regard to the results of genetic association studies so far, there may be genetic risk factors that increase the likelihood of developing PD, much in the same way that the ApoE4 allele increases the risk of developing AD. On the other hand, several genes for inherited forms of PD have been mapped (Table 1). The classification of FPD was divided as two groups based on the presence of Lewy bodies. At least, six causative genes have been identified such as *α-synuclein, parkin, UCH-L1, PINK1, DJ-1, and dardarin or LRRK2* for*SNCA and Park4, Park2, 5, and 6, 7, and 8,* respectively.[9-16] In addition, *NR4A2* has been identified as a causative gene of autosomal dominant form of FPD.[17] Furthermore, other loci in families with PD have been mapped to chromosomes 2p13[18] as Park3 and 1p36 as *Park9.*[19] In addition, the susceptibility gene of the late onset form of PD has been mapped to 1p32 locus as *Park10*[20] *and additional locus for* Park11 has been mapped to 2q36-37.[21] The presence of several causative genes and loci for FPD

Molecular Mechanisms in Parkinson's Disease, edited by Philipp Kahle and Christian Haass.

Table 1. Classification of familial Parkinson's disease

	Gene	Locus	Hereditary Form	Lewy Body
PARK 1	α-Synuclein	4q21-23	AD	+
PARK 2	Parkin	6q25.2-27	AR	-
PARK 3	?	2p13	AD	+
PARK 4	α-Synuclein triplication	4q13-22	AD	+
PARK 5	UCHL-1	4p14-15	AD	?
PARK 6	PINK1	1p35-36	AR	?
PARK 7	DJ-1	1p36	AR	?
PARK 8	Dardarin/LRRK2	12p11.2-q13.1	AD	-/+
PARK 9	?	1p36	AR	?
PARK10	?	1p32	AD	?
PARK11	?	2q36-37	AD	?
NR4A2	Nurr 1	2q22-23	AD	?

AD, Autosomal dominant form; AR, autosomal recessive form.

indicates the mechanisms of pathogenesis of sporadic PD are also complicated. However, considering the selective dopaminergic neuronal cell death, the gene products could share a common pathway including oxidative stress, mitochondrial dysfunction, and proteasome pathway. In this communication, we review recent progress in the molecular genetics of FPD.

SNCA (α-Synuclein) and Park4 (Triplication of α-Synuclein)

Golbe and colleague firstly reported the autosomal dominant form of FPD in the Contursi family.[22] The average age of onset was 45.6 years, and initial symptoms were variable including resting tremor, bradykinesia, or postural instability. The affected members of this family responded well to levodopa, however, the average duration of the illness was reported to be 9.2 ± 4.9 years, somewhat shorter than that of sporadic PD. Dementia was not uncommon in this family. Pathologically, Lewy bodies and cortical Lewy bodies were observed. The disease gene has been mapped to chromosome 4q21, and subsequently, mutations in the *α-synuclein*, located within the disease region were found to be associated with the autosomal dominant form of FPD, similar to the Contursi family.[9] Firstly, two separate point mutations such as A53T and A30P have been identified.[9,23] In addition, another mutation such as E46K has been reported.[24] Totally, only three different mutations have been identified so far. Although this form of FPD is very rare, this molecule has been found to be one major component of Lewy bodies that characterize the pathological hallmark of PD.[25]

In 1962, Spellman reported a family with autosomal dominant form of FPD in the United State.[26] Muenter et al made extensive studies for the clinical features of this family.[27] Clinical features of this family consisted of levodopa responsive parkinsonism and dementia. In addition, Walter and Miller reported another family with autosomal dominant FPD that has similar clinical features reported by Spellman and Muenter.[28] In autopusied brains from the patients from this family, many cortical Lewy bodies were observed and the pathological diagnosis suggested diffuse Lewy body disease. Later, the family by Walter and Miller tirned out to be blood-related to be the family reported by Spellman and Muenter. This family was mapped to the short arm of chromosome 4 that was assigned as Park4.[29] Very recently, assignment of this family to this region appears to be an error of the linkage analysis. Instead, triplication of *α-synuclein* in the affected members of this family; the 1.5 Mb region including several genes on both sides of *α-synuclein* was tripliacted in a tandem fashion.[30] Therefore, the protein level of *α-synuclein* is expected to be two-fold higher than that of normal individuals. Thus, it would

be possible that overproduction of this protein could cause developing to PD. Very recently, duplication of this gene has been reported in the autosomal dominant from of FPD.[31,32] Therefore, there are two different types of multiplication of this gene at least. In contrast, the patients with duplication of the *α-synuclein* had no dementia. Taken together with autosomal domonant FPD with multiplication of this gene, overproduction of *α-synuclein* from a single gene may relate to the phenotype of PD, PD with dementia (PDD), or dementia with LB (DLB). These findings provide us that genetic variations including single nucleotide polymorphisms promoter region of this gene.

Recently, reduced mRNA expression of the G209A allele was reported in a Greek-American family.[33] Very recently, we reported that the mRNA expression of the mutant G88C and G209A alleles of the α-synuclein gene is significantly reduced relative to the wild-type allele in lymphoblastoid cell lines established from affected individuals, who had mutations either G88C and G209A alleles, with a severe clinical phenotype. In contrast, these mutant alleles are expressed at levels similar to the wild-type allele in lymphoblastoid cell lines established from less severely affected idividauals or asymptomatic carriers. This suggests that the ratio of expression levels of the wild type to mutant α-synuclein alleles may be able to be developed as a clinical marker of this type of FPD, particularly since α-synuclein is normally expressed in lymphocytes. Therefore, this haploinsufficiecy is a common mechanism for this form of FPD. Why the expression level in lymphoblastoid cells associated with the severity of clinical phenotypes remained to be determined. Furthermore, the expression level of wild type α-synuclein in the lymphocytes of PD may be also related to the progression of the disease. Considering the potential that overproduction of α-synuclein in brains may trigger the onset of PD, the expression of α-synuclein in even though the lymphocytes may be also useful to differentiate PD from PDD and DLB.

α-Synuclein is identical to NACP (nonamyloid component precursor);[34] NAC is deposited in the amyloid plaques of AD.[35] Furthermore, α-synuclein has been identified as a major component of the Lewy bodies in both familial and sporadic PD as well as in dementia with Lewy bodies (DLB).[24] Furthermore, α-synuclein is deposited in the cytoplasm and neuronal processes. In addition, α-synuclein aggregation occurs in the parkinsonian disorder of multiple system atrophy (MSA).[36] In this disease, there is abnormal oligodendroglial staining for α-synuclein, but no Lewy bodies. The identification of α-synuclein in pathological deposits in these neurodegenerative disorders such as PD, dementia with Lewy bodies, MSA, AD, and some prion diseases suggest that they may share common pathogenic mechanisms. Thus, the discovery of α-synuclein arose the concept that PD may be one part of a boarder group of "synucleinopathies", in which there is a fundamental defect in protein processing.

Why dopaminergic neurons in the substantia nigra are particular vulnerable to the gain of α-synuclein function including wild or mutant forms of this protein remains to be elucidated. Although α-synuclein is expressed ubiquitously, oxidative conjugation of dopamine to α-synuclein leads to the accumulation of the α-synuclein protofibril.[37] The conjugation of α-synuclein into oxidative form of dopamine, dopamine quinone provides an answer for the selective cell death of dopaminergic neurons. Thus, α-synuclein toxicity in dopaminergic neurons requires endogenous dopamine production and its toxicity could be related to reactive oxygen species. This adduct may form the complex proteins such as 54- to 83-kDa soluble proteins that contain α-synuclein and 14-3-3.[38] As α-synuclein contains no cystein residue, it would not be possible that dopamine quinone adduct does not modify the α-synuclein. The above-mentioned complex may be formed indirectly mediated by conjugation of α-synuclein into dopamine quinone. The question arises about the formation of cortical Lewy bodies in dementia with Lewy bodies (DLB). This difference between cortical and brain stem Lewy bodies on the structure and its distribution may be related to the neurotransmitters themselves on the location of Lewy bodies. Indeed, synphilin-1 as a marker of brain stem type Lewy bodies has been reported. In contrast, this immunoreactivity for cortiacal Lewybodies was less than brain stem type ones.[39] Further studies will be needed to elucidate the mechanism of the formation of Lewy bodies.

Ubiquitin (Ub) has also been identified as a major component of Lewy bodies, thus implicating abnormal protein degradation in the pathology of PD. The colocalization of both α-synuclein and Ub in Lewy bodies suggests that dysfunction of Ub-proteasome pathway may play a role in the pathogenesis of PD. Indeed, overexpression of α-synuclein is sufficient to induce inclusion formation and proteasome inhibition leads to an increase of α-synuclein accumulation.[40] Although it would be possible that α-synuclein is degraded by 26S proteasome, whether or not proteasomal pathway is involved in α-synuclein degradation has been controversial. However, recent works revealed that α-synuclein could be directly degraded in vitro assay, suggesting that an ubiquitin-independent mechanism of proteasomal degradation. The 26S proetasome requires the polyubiquitination chains, in contrast 20S particle that contains the protease active site does not require its multiubiqutination for degradation.[41] Thus, as α-synuclein belongs to the class of proteins known as natively unfolded, t is likely to that α-synuclein is directly degraded by 20S particle. Considering the colocalization of immunoreactivity for ubiquitin and α-synuclein within Lewy bodies, it would be possible that α-synuclein and ubiquitinated proteins incidentally accumulate during the process of Lewy body formation. Therefore further studies are warranted to investigate the mechanism of formation of Lewy bodies.

Oxidative stress and protein modification may be a common event for neurodegenerative disorders.[42] Indeed, phospolylated α-synuclein deposited in human synucleinopathies as Lewy bodies and other hallmark lesions.[43] In addition, α-synuclein overexpressed in fly also undergo phosphorylation at the same site of this molecule, suggesting that a similar manner between fly model and human PD could be involved in formation of Lewy bodies.[44] This hyperphosphorylation could be also a common event in neurodegenrative disorders such as PD, AD, and various tauopathies. Thus, The α-synuclein studies including the mechanism of phosphorylation may help facilitate dissection of pathophysiologic mechanisms of various synucleinopathies and tauoopathies.

PARK2 (*Parkin*)

Park2 is characterized by early onset before 40 years (average onset, 26.1 years), mild dystonia, diurnal fluctuation, spontaneous improvement of movement of disability after sleep or nap, a good response to levodopa, and less frequent resting tremor compared with sporadic PD.[45] Gait disturbance was the initial symptom in 60.5% of patients. The pathological changes include selective degeneration of pigmented neurons in the SN and locus coeruleus, and generally lack of Lewy bodies.[46] *Parkin* mutations are the most frequent cause of autosomal recessive early-onset parkinsonism (AREP) including autosomal recessive juvenile parkinsonism (AR-JP); their frequency being estimated at 50% in AREP families with potentially autosomal recessive inheritance.[47,48] The clinical features of AREP with *parkin* mutations are highly variable compared with the AR-JP. Thus, AREP with *parkin* mutations are considered as parkin-related diseases that also include AR-JP. In this regard, autopsied cases of parkin-related diseases, with the exception of a single case, commonly lack Lewy bodies , suggesting that normal function of parkin is essential for Lewy body formation. In addition, the discovery that parkin is an ubiquitin ligase provides information suggesting that the ubiquitin-proteasome system may play an important role in maintaining dopaminergic neurons.[49] Furthermore, ubiquitin positive inclusions have reported in various neurodegenerative disorders such as Alzheimer disease (AD), multiple system atrophy (MSA), progressive supranuclear palsy (PSP), and poly-Q diseases. Thus, it is clear that ubiquitin-proteasome pathway may be a common cascade in the various neurodegenerative diseases. Therefore, the function of parkin provides a hint to elucidate the mechanisms of all the neurodegenerative disorders.

Mutations in *Parkin*

Parkin contains 12 exons spanning over 1.4 mega bases and encodes a protein of 465 amino acids, with moderate homology to ubiquitin at its amino-terminus (ubiquitin like domain, Ubl) and two RING finger motifs (RINGs) at the carboxy-terminus. In the preliminary study, *parkin* mutations are the most frequent in the young-onset PD. If the mode of the inheritance is

autosomal recessive, approximately half of such patients could have *parkin* mutations. To date, various *parkin* mutations have been identified such as exonic deletion, insertions, and several missense mutations in the patients with FPD originating from various races (Fig. 1).[50-56] Mutations in *parkin* gene have been distributed all over the world. Thus, this form is now considered to be one of the most frequent in FPD. In addition to homozygous mutations, compound heterozygous states that are different mutations in each allele are also frequent among the patients without affected members in the same family. It is difficult to detect the compound heterozygotes using conventional PCR due to its giant size of this gene. Thus, the gene dosage technique, that is quantitative analysis, is useful strategy to detect the compound heterozygotes. In the Orientals, the frequency of the point mutations is less than that of the white populations. In the Orientals, exonic deletions are high frequent compared to other types of mutations. The sites of the exonic deltions are located from exons 2 to 5. Thus, these regions are a hot spot for exonic deletions. In contrast, point mutations have been found from exons 6 to 12 of which involves two RING finger motifs and In-between RINGs. The clinical phenotype of this form is expanding with slowly progression, cerebellar ataxia. In addition, there are more than a few patients with *parkin* mutations who have psychiatric/behavioral symptoms. These sighs started prior to or after the onset of parkinsonism.[57] In this point, psychiatric problems are characterized symptoms for this form of parkinsonism. Therefore, it would be possible that some patients with *parkin* mutations have only the psychiatric/behavioral symptoms even though without parkinsonism.

Positron emission study (PET) using fluoro-dopa revealed that reduction of uptake was observed in even though carriers.[53] This finding indicates that carrier states potentially have a phenotype of PD. In addition, single heterozygous mutations also in exon 7 act as susceptible alleles for late-onset form of PD.[58] Furthermore, the recent association of haploinsufficiency of *parkin* with sporadic PD further implicates a role for parkin in the more common form of PD.[59] In this point, single heterozygous state could be also related to not the dominant negative effects, but the haploinsufficiency effects. In contrast, Lohmann et al[60] reported that some missense mutations might have a dominant negative effect as missense mutations in functional domains resulting in an earlier onset than mutations in other regions of this protein. Thus, we should examine whether or not all mutant parkins have no ligase activities according to the loss-of-function effects. We speculate that some mutant parkin may have ligase activities.

Parkin Function and Dopaminergic Cell Death

Ubiquitin (ub) is attached to covalently to target proteins. Protein ubiquitination is catalyzed by three enzymes, E1 (Ub-activating enzyme), E2 (Ub-conjugating enzyme), and E3 ubiquitin ligase. Mutations in the *parkin* gene result in a loss-of-fucntion of E3; subsequently, substrates for parkin could be accumulated within dopaminergic neurons and its accumulation may lead to young onset PD. Thus, it is important to identify the substrates for parkin. To date, ten candidate proteins have been reported to be degraded by parkin[61] (Table 2). Other types of proteins have been shown to interact with parkin such as E2s, multiprotein ubiquitin ligase complex such as cullin-1, CASK/Lin2 as a scaffolding protein containing postsynaptic density-95, disc large, zona occludens (PDZ) domain, actin filaments. In addition, CHIP and Hsp70 have been reported as biding partners.[62] Although it remains to be elucidated why parkin has two RING finger motifs, parkin may interact with various proteins including substrates due to two RING finger motifs.

To elucidate the mechanism of parkin, the parkin knockout animal model is good strategy. Very recently, parkin null mice have been reported. Parkin null mice demonstrated that motor and cognitive deficits, inhibition of amphetamine-induced dopamine release and inhibition of glutamate neurotransmission.[63] In addition, the levels of dopamine were increased in the limbic brain areas and the metabolism of dopamine was shifted towards monoamine oxidase (MAO). The latter observation suggests the presence of oxidative stress in parkin related diseases. Indeed, iron accumulation in autopsied brains with AR-JP increased than that of controls and sporadic PD. Thus, oxidative stress is also a common cascade of pathogenic factors in both parkin related diseases and PD. However, why no

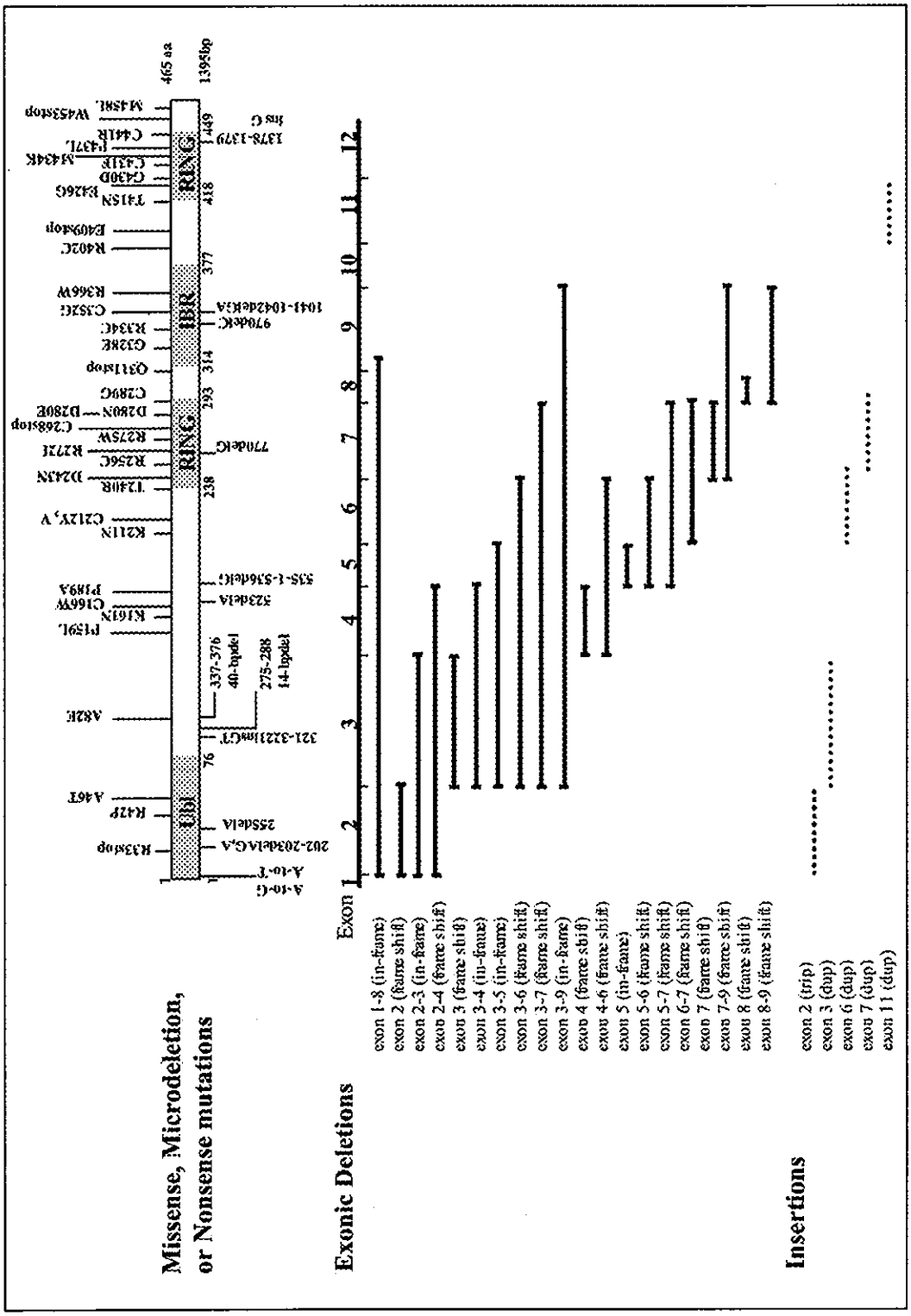

Figure 1. Sites of Parkin mutations.

***Table 2. Candidate substrates for* parkin**

Substrates	Function
CDCrel-1	Exocytosis (Dopamine storage?)
CDCrel-2	Exocytosis (Dopamine storage?)
Pael-receptor	ER stress (Unfolded protein response)
O-glucosylated a-synuclein	Lewy body formation
Synphilin-1	Lewy body formation
Cyclin E	Apoptosis (Kainate excitoxication)
á/âTubulin	Microtubules (assembly dysfunction)
p38 subunit (Amynoacyl tRNA synthase?	Protein biosynthesis or apoptosis
Synaptotagmine IX	Exocytosis
Expanded poly-Q	?

Lewy bodies formation is commonly observed in parkin related diseases is unclear. At least, parkin could be involved in the formation of Lewy body.

The presence of Ubl domain in parkin is an important clue to investigate the function of this protein. Very recently, the three-dimensional structure of this Ubl domain has been determined by NMR.[64] This study revealed that the parkin Ubl domain binds the Rpn10 subunit of 26S proteasome via the region of parkin that includes amino acid position 42. Rpn 10, so called S5a, can bind polyubiquitin conjugates in vitro, and could possibly function as a polyubiquitin-binding subunit. This site, position Arg 42, has been reported as a pathogenic mutation, in which Arg is substituted with Pro in one patient. According to the structure of parkin using NMR, the Arg 42 mutation induces a conformation change in the Rpn 10-binding site of Ubl, resulting in impaired proteasomal binding of parkin. Indeed, mutnt parkin carrying the Arg-to-Pro mutation was extremely difficult to dissolve at a submillimolar concentration for NMR analysis; this insolubility might be associated with loss of the correct functional conformation in the mutant form of parkin. It suggests that this hampers the formation of an efficient assembly line for protein degradation, and thereby causes the accumulation of parkin substrates regardless of the degree of ubiquitin ligase activity.

Pathologic findings of brains with *parkin* mutations revealed severe neuronal loss with gliosis in the substantia nigra (SN) and mild neuronal loss in the locus coeruleus (LC), suggesting that pathology of the mutated brains is mainly in the SN, in which the ventrolateral group is more severely affected than in sporadic PD, whereas the LC is less severely affected.[46] In addition, several atypical findings have been reported in the brains of this form. One of them is the accumulation of tau protein: neurofibrillary tangles (NFTs) in the SN, LC, red nucleus, and posterior hypothalamus, and NFTs and thorn-shaped astrocytes in the frontal, temporal, and parietal cortices.[46] In addition, accumulation of tau protein in the form of tufted astrocytes, but not NFTs, was reported in a patient with compound heterozygous mutations.[65] In this respect, a part of the pathology of parkin related diseases' brains is very similar to that of progressive supranuclear palsy. Therefore, parkin-related diseases are also considered as one of tauopathies, although which either isoforms of 3 or 4 repeat tau increased in this form of FPD remains to be determined.

To investigate the toxicity of the substrates for parkin, the fruit fly is a good model to elucidate the mechanism of dopaminergic neuronal loss. Yang et al used a transgenic fly to the expression of human Pael receptor (Pael-R), one of candidate substrates, under conditions of altered parkin activity.[66] This fly revealed the age-dependent degeneration of dopaminergic neuronal loss in spite of the same expression levels in all neurons. This Pael-R mediated neurotoxicity in the dopaminergic neurons was attenuated by the coexpression of human parkin and exacerbated by blocking the activity of endogenous parkin in the fly by RNA interference

(RNAi). In addition, overexpression of parkin can suppress α-synuclein-induced toxicity. However, there is no evidence that parkin directly interacts with α-synuclein. These findings suggest that parkin plays a central role in maintaining dopaminergic neurons. Put another way, parkin is an essential factor for the survival of dopaminergic neurons.

PARK5 (*UCH-L1*)

Only one family with autosomal dominant FPD caused by mutation of UCH-L1 has so far been reported.[12] Thus, UCH-L1 is one of candidate gene responsibel for FPD. Furthermore, no autopsy data are available at present; therefore, it is unclear whether or not the formation of Lewy bodies is observed in this form of FPD. However, considering the function of this protein, UCH-L1 could play an important role for FPD. In only one family with an UCH-L1 mutation, the affected member had a missense mutation (Ile93Met) in UCH-L1 and the mutation was segregated with the disease phenotype.[12] As no additional families have been identified so far, whether this mutation is responsible for familial PD remains to be determined and further studies are necessary to describe further cases. On the other hand, a common polymorphism (Ser18Tyr) has been frequently observed in various races. The Ser18Tyr is associated with decreased risk of PD and that the protective effect is dose-dependent manner.[67]

UCH-L1 hydrolyzes terminal small adducts of ubiquitin and generates free monomeric ubiquitin.[68] Mutation of UCH-L1 causes partial loss of its catalytic activity. In addition, immunoreactivity for UCH-L1 is present in Lewy bodies.[69] Thus, abnormalities of this enzyme may result in accumulation of structurally altered proteins that may interfere with normal cellular function.

Recently, UCH-L1 is also shown to exhibit a second, dimerization-dependent, ubiquitin ligase activity.[70] This ubiquitin ligase activity may be dependent on the K63-linked polyubiquitin chain on α-synuclein in a dimerization form. The Ser18Tyr polymorphism has reduced ligase activity but comparable hydrolase activity as well as wild-type UCH-L1. Thus, UCH-L1 posses both opposing enzyme activities such as a beneficial effect of hydrolase activity and dimerization-dependent ligase activity that is at least partly pathogenic. In a brief, the UCH-L1 gene encodes two opposing enzymatic activities that affect the degradation of α-synuclein.

PARK6 (*PINK1*)

Recently, mutations of PINK1 have been identified as the causative gene for PARK6. Several mutations of this gene have been reported so far (Fig. 2).[13,71,72] Therefore, the PINK1 mutations may be more frequent next to the *parkin* mutations. Hatano et al reported that six families of 39 families with AREP had PINK1 mutations. Thus, the PINK1 mutations have been detected in approximately 15% of cases without parkin mutations.[71] In addition, PINK1-positive AREP are not limited to Europeans but also in Asians. Furthermore, different point mutations seem to be more frequently responsible for the disease phenotype than are deletions. Of course, it would be possible that deletion mutations may take place in this gene as nonsense mutations have been reported.

It is difficult to distinguish PINK-1-positive AREP from the PINK-1 negative one. The clinical features of Park6 included slow progression and commonly lack of dystonia at onset of the disease. Thus, the presence or absence of the dystonia provides us good information to differentiate the PINK1- positive from PINK1-negative one. In addition, the identification of a higher frequent ratio in patients with PD than that of normal controls carrying a single heterozygous mutation supports the hypothesis that haploinsufficiency of this gene as well as *parkin* and *DJ-1* may represent a susceptibility factor for developing to parkinsonism. Alternatively, some mutation types may have the dominant negative effect for this disease.

Although PINK1 function is unclear, it originally was reported to be upregulated by the tumor suppressor gene, *PTEN*, in cancer cells.[73] Preliminary results revealed that the loss-of-function effect of this gene might be associated with mitochondrial function that was known as one of causative factors for sporadic form of PD. In addition, this gene product, PINK1, has the kinase domain. Thus, the loss-of-function effect of PINK1 may be related the

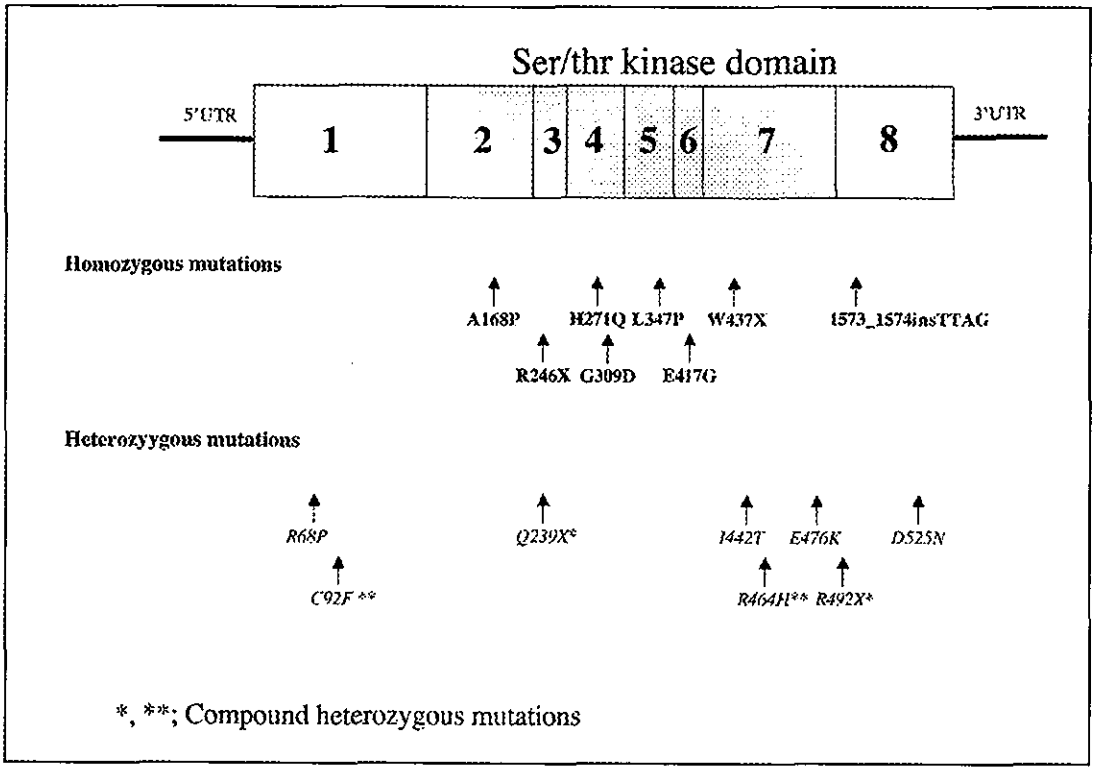

Figure 2. Sites of PINK1 mutations.

phosphorylation mechanisms. In this point, it is clear that the approach to pathogenesis of monogenically form of PD could be a useful strategy for elucidating that of common form of sporadic PD in which phosphorylated α-synuclein is accumulated.

PARK7 (*DJ-1*)

Very recently, Bonifati et al identified the causative gene for Park7.[14] The causative gene was named as DJ-1. DJ-1 was first cloned independently by Ariga and his colleagues.[74] This gene product is a candidate of the oncogene product that interacts with *c-myc* and increases cell transformation in the presence of *myc* or *h-ras*. In addition, DJ-1 was also found to be an infertility-associated protein that was reduced in rat sperm treated with toxicants that cause infertility in rats.[75] This gene product, DJ-1, has been identified as a causative gene for Park7. The mutations appear to be a rare cause of FPD, accounting for 1-2 % of all early-onset cases with parkinsonism and PD. Several mutations such as exonic deletion, truncations, and, homozygous point mutations have been reported in this gene. In addition, heterozygous missense mutations such as A104T and D149A have been identified as a cause for developing to young-onset parkinsonism. This finding for this heterozygous mutations indicates the gene mutations have dominant negative effects or haploinsufficiency as well as *parkin* mutations. The clinical phenotypes of this form are very similar those of Park2 and Park6. Based on the clinical examination and imaging study indicates nigral neuronal loss although pathological findings are not yet reported.[76]

Although the biological function of the DJ-1 remains obscure, several possible functions have been proposed. Firstly, DJ-1 may function as an anti-oxidant protein as DJ-1 was identified as a hydorperoxide-responsive protein that becomes a more acidic isoform following oxidative stress.[77,78] Secondly, DJ-1 is sumoylated through binding the SUMO-1 ligase PIAS.[79] SUMO-1 is a small ubiquitin-related modifier. Although the homology between ubiquitin and SUMO is only 18%, the three-dimensional structure is very similar each other. SUMO-1 is covalently attached to other proteins as well as ubiquitin in a similar multistep process to ubiquitination.[80]

Sumoylation does not participate the degradation of proteins like uniqitin-proteasome system.[81] In addition, the modification of SUMO-1 is reversible unlike ubiquitination. Although the function of SUMO-1 is unknown, sumoylation might act as a modifier to alter the conformation of the sumoylated proteins. Furthermore, sumoylation competes ubiquitin for specific lysines in target proteins, suggesting that DJ-1 may be related to the regulation of protein degradation and its stability.[82] Indeed, the L166P mutant protein is impaired in its ability to form homo-dimers and markedly reduced protein stability. Moreover, there is evidence that sumoylation is actively involved in the nuclear import of substrates. Indeed, a number of transcription factors are sumoylated.[83,84] Considering the modification of DJ-1 by SUMO-1, DJ-1 may be also linked to U-P pathway like parkin. Although several possibilities of DJ-1 function have been proposed, how DJ-1 induces the dopaminergic neuronal death in PD remains to be determined. To address this question, further studies will be needed.

Park 8(Dardarin/*LRRK2*)

Very recently, two independent groups have identified the causative gene for Park8-linked PD. The gene product was named as dardarin and leucine-rich repeat kinase 2 (LRRK2) by each group, respectively.[15,16] In this review, we used the gene as *Park8* gene and used the gene product as Park8 product.

Most of Park8-linked families have a clinical phenotype of typical PD. In contrast, the pathological findings range from pure nigral degeneration in the absence of Lewy bodies as reported in the Sagamihara kindred that has been noted as an index family for Park8[85] to typical Lewy bodies formation in the Western Nebraska kindred.[86] Several missense mutations segregating with Park8-linkied families have been reported so far. The Park8-linked families distributed in the world-wide populations based on the haplotype analysis and mutation screening. Thus, this form may be high frequent compared with the frequency of α-synuclein mutations.

The clinical features of Park8-linked families revealed the typical PD, diffuse Lewy body disease, PD with dementia (PDD), and parkinsonism with amyotrophy or PDD with amyotrophy. In addition, pathologic findings also exhibited variable changes representing aspects of several of the major neurodegenerative disorders such as synucleinopathies and tauopathies. Thus, Park8 product may be central to the pathogenesis of several major neurodegenerative disorders associated with parkinsonism.

The Park 8 product remained to be determined. Considering the domain structure, this gene structure consisted of five functional domains such as leucine-rich repeat (LRR), a Roc (Ras in complex proteins) domain, a COR domain (C-terminal of Roc),a tyrosine kinase catalytic domain (TyrKc), and a WD40 domain. As the Park8 product may have the kinase activity, this protein potentially may be responsible for the phosphorylation of both α-synuclein and tau. Therefore, the kinase activity of Park8 product could be a key event in the accumulation and aggregation of these unfolded proteins within disease neurons.

Conclusions

The recent explosion of genetic information has indicated that PD is not a single entity but is rather a highly heterogeneous disorder. Indeed, there are several genetically, clinically, and pathologically distinct forms of FPD that can be caused by mutations of *α-synuclein, parkin, UCH-L1, PINK1, DJ-1, and dardarin or LRRK2* as well as yet unknown causative genes. Although mutations underlie a minority of the larger PD population, they nevertheless represent a cascade of events that culminates in the death of nigral neurons. Indeed, the causative gene products for FPD share a common biochemical pathway such as ubiquitin-proteasome pathway, mitochondrial function, oxidative stress, and phosphorylation for proteins. For examples, o-glycosylated α-synuclein is one of candidate substrates[59] and DJ-1 mutants specifically but differentially associated with parkin.[87] The experimental results suggest that FPD gene products may link each other in a common pathway that may have important implications for

understanding the pathogenesis of FPD and sporadic PD. Moreover, identification of the candidate genes will enhance our understanding of the mechanisms of nigral degeneration of PD as well as for developing methods to prevent nigral neuronal death.

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