TABLE 1. *Clinical features of patients*

Patient	A2	A3	A4	B1	C1	C2	D
Origin	Japan	Japan	Japan	Japan	Hong Kong	Hong Kong	Morocco
Age at onset	15	12	20	30	18	18	35
Disease duration	38	25	9	36	22	17	18
Sex	M	F	F	F	M	F	M
Resting tremor	+	+	+	−	−	−	+
Rigidity	+	+	+	NA	+	+	+
Bradykinesia	+	+	−	+	+	+	+
Postural instability	+	+	−	−	+	+	−
Frozen gait	−	+	−	+	−	−	+
Clinical response to levodopa	+	+	+	+	+	+	+
Wearing off	−	+	−	+	+	+	+
On off	−	+	−	+	+	+	+
Asymmetry at onset	+	+	+	−	−	−	+
Incontinence	−	−	−	−	−	−	−
Urinary urgency	−	−	−	NA	−	−	+
Levodopa-induced dyskinesia	+	+	−	+	NA	+	+
Sleep benefit	−	−	−	+	+	+	NA
Dystonia at onset	−	+	−	−	+	+	+
Hyperreflexia	−	−	−	−	+	+	−
Dementia	−	+	−	NA	−	−	−
Depression	−	−	−	−	−	−	+
Hallucination	+	+	−	+	−	−	−
UPDRS III (on/off)	20/NA	32/NA	NA	15/34	NA	NA	NA
Other psychosis	sch	sch	−	−	−	−	−
Special comment	−	−	−	−	−	−	RLS, RBD, facial dyskinesia with grimacing, severe dysarthria from onset

sch, schizophrenia; UPDRS, unified Parkinson's disease rating scale (motor score) in on and off condition; NA, not applicable or not available; RLS, restless legs syndrome; RBD, REM sleep behavior disorder; +, present; −, absent.

induced schizophrenia with hallucination. None of the patients in this cohort other than family A had schizophrenia. In addition, Patient B1 had hallucination and Patient D had depression.

DISCUSSION

In the present study, we set out to investigate whether Parkin and PINK1 could influence each other in patients with PD, based on the reports that Parkin and PINK1 share a common pathway using *Drosophila* models.[6,7] We identified digenic mutations of *parkin* and *PINK1* and found that *PINK1* mutation could modify the clinical course of *parkin* mutation-positive parkinsonism. Our results suggest that a single heterozygous mutation of *PINK1* might act not only as a susceptibility gene[3] but also as a modifier gene, in the pathogenesis of PD.

The relatively high frequency of *PINK1* heterozygous mutation identified in the present study (2.2% in PD vs. 0% in controls) is similar to that reported in a recent study (1.2% in PD vs. 0.4% in controls).[3] These results suggest that *PINK1* heterozygous mutation might also increase the risk of development of PD in patients who have mutations in other PD genes. Considering Patient D (Table 1), heterozygous *PINK1* p.E476K mutation was reported previously in three patients and two control subjects.[3,10] In addition, heterozygous p.P437L of *parkin* was found at the same frequency in patients and control subjects,[11] whereas none of Japanese 300 normal chromosomes harbored these mutations in the present study. This could represent differences based on ethnicity. Observation of patients carrying single nucleotide polymorphisms in both *parkin* and *PINK1* might be somewhat related to the position of mutated amino acids, the type of mutation, and one or more of the other gene mutations. On the other hand, the presence of asymptomatic carrier with the digenic mutations (family A-A1) also indicates the role of heterozygous mutation of *PINK1* in disease modification and suggests that other factors such as aging and environment are required for the development of the disease.

Based on recent reports, asymptomatic carriers of heterozygous *parkin* or *PINK1* mutations exhibit low ^{18}F-dopa uptake in the putamen on positron emission tomography.[12,13] These studies suggest that heterozygous mutation of *parkin* or *PINK1* gradually impairs the function of dopaminergic neurons. Interestingly, our patients of Family A, B, and D also developed

psychiatric disorders. Previous studies also reported that some *parkin* and *PINK1* mutations, even though heterozygous mutations, could be related to levodopa-responsive parkinsonism and psychiatric clinical pictures.[1,4,5] In this regard, our results might further indicate that *parkin* and *PINK1* mutations could be involved in psychiatric disorders not only singularly but also in combination. Furthermore, additional heterozygous *PINK1* mutation could hasten the age at onset of the disease. Combining the previous reports, our results emphasize that some heterozygous *PINK1* mutations might be related to the development of PD.[3,10] However, further genetic and functional analyses are required before one can make definite conclusions.

Intriguingly, digenic mutations of *PINK1-DJ-1* and *parkin-LRRK2* have recently been reported.[14,15] Screening for digenic or more mutations in responsible genes for familial PD could lead to the elucidation of the molecular pathway involved in nigral degeneration. In this regard, the mitochondrion is a good target for elucidating the pathogenesis of PD since Parkin, PINK1, and DJ-1 could be related to the mitochondrial function/dysfunction. Indeed, several studies highlighted the role of ARP gene products in maintaining mitochondrial function and in the pathogenesis of PD. Our results and these findings suggest that, multigenic mutation screening and analyses for interactions among related gene products could help enhance our understanding of the pathogenesis of PD.

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Screening *PARK* genes for mutations in early-onset Parkinson's disease patients from Queensland, Australia

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Abstract

A family history of Parkinson's disease (PD) is the most commonly reported risk factor after age, suggesting a genetic component to the disease in a sub-group of patients. Mutations in at least six genes have been identified that can lead to monogenic forms of PD. We screened a sample of 74 early-onset PD cases out of a cohort of 950 patients (onset <50 years) for genetic abnormalities in known familial Parkinsonism genes. A self-reported family history of PD existed for 30 patients (40.5%). Of these, 13 each had a first- or a second-degree relative with PD and four reported a more distant relative with PD.

The entire coding region of the *PRKN* (MIM 602544), *DJ-1* (MIM 602533) and *PINK1* (MIM 698309) genes, and exon 41 of the *LRRK2* gene (MIM 609007) were screened by direct sequencing. All exons of *PRKN* were examined for gene-dosage abnormalities.

Screening identified five patients with putative genetic disease: two patients carried *PRKN* mutations (p.G12R heterozygous and p.G430D homozygous), one patient carried a p.G411S heterozygous amino acid change in the *PINK1* gene and two individuals were heterozygous for the common p.G2019S mutation in *LRRK2*. No alpha-synuclein or *DJ-1* variants were observed.

Our data suggest that approximately 7% of early-onset PD cases seen in Queensland movement disorders clinics have mutations involving known *PARK* genes.

Keywords: Parkinson's disease; *PARK* genes; Mutations

1. Introduction

Parkinson's disease (PD) is a neurodegenerative condition with a typical onset in the seventh decade, however, about 4% of PD patients present with early-onset before the age of 50 years [1]. It is a complex, multifactorial disorder, comprising genetic and environmental components. The majority of cases appear to be sporadic or idiopathic, however, in the recent past a number of mutations in at least six genes (*PARK1, 2, 5, 6, 7,* and *8*) have been identified as being causative in the familial form of the condition, accounting for a small number of all PD cases. Mutations in these genes may lead to the disease phenotype and are often characterized by an earlier onset (under the age of 50 years) with or without Lewy body pathology. It is to be expected that more mutations causative for the disease in 'sporadic' PD will be identified in the future, adding to the number of distinct genetic forms of PD. The aetiology of the sporadic form of PD is still unclear but identification of molecular mechanisms and gene products underlying the disease in its monogenic form have shed some light on possible pathways involved in the non-hereditary form of the condition.

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As an early disease onset is frequent in familial PD, we undertook in this study to estimate the prevalence of known genetic forms of Parkinsonism in a typical Australian population (Queensland) by screening a subset of early-onset cases, derived from a large movement disorders clinic in Brisbane, Australia.

2. Methods

2.1. Sampling frame

Patients were derived from a case series of 950 patients with a diagnosis of PD seen in one specialist movement disorders practice in Brisbane, between 2000 and 2005. Informed consent was obtained from all participating patients.

2.2. Patient selection

Patients were included in the study if they (1) received a diagnosis of probable PD according to stringent clinical and neurological criteria; (2) exhibited onset of symptoms ≤50 years; and (3) had been seen at the clinic between 2001 and 2005.

2.3. Patient ethnicity

Patients in this sample were in the majority (95%) of European extraction. Two patients reported Australian aboriginal ancestry, one case was of New Zealand Maori extraction and one patient reported Asian ancestry.

2.4. Screening methodology

DNA was extracted from peripheral blood according to the standard methods for use in gene-dosage studies. A whole genome amplification of the original DNA was performed prior to sequencing studies. The entire coding region of the *SNCA* (MIM 163890), *PRKN* (MIM 602544), *DJ-1* (MIM 602533) and *PINK1* (MIM 698309) genes, and exon 41 of the *LRRK2* gene (MIM 609007) were screened by direct sequencing using standard methods. All exons of *PRKN* were examined for gene-dosage abnormalities using TaqMan based methods. Details have been given elsewhere [2,3]. Primers and TaqMan probes used in the quantitative PCR amplification analysis are listed in Table 1. *UCHL1* (*PARK5*) was excluded from the study for reason of its extreme rareness; the importance of the gene in PD is still unclear. For similar reasons, no attempt was made to identify mutations in the *ATP13A2* (*PARK9*) gene, that has been identified as causative for rare cases of Kufor Rakeb disease.

3. Results

Seventy-four patients met the inclusion criteria. Demographic data are shown in Table 2. In this sample, males were slightly over-represented ($n = 44$, 59.5%). A self-reported family history of PD existed for 30 patients (40.5%). Of these, 13 each had a first- or a second-degree relative with PD and four patients reported a more distant relative affected by the condition. Among the first-degree relatives, there were six affected fathers and four affected mothers. More specific unambiguous inheritance patterns could not be ascertained for any of the patients. The average age of onset was 42.4 ± 5.7 years.

Screening identified mutations in five patients with putative genetic disease (Table 3 and Fig. 1). Two patients carried *PRKN* mutations (c.34G > C and c.1289G > A), the first leading to a heterozygous p.G12R amino acid change in exon 2, and the second to a homozygous p.G430D amino acid change in exon 12.

Table 1
Primers and TaqMan probes used in the *PARK2* analysis

Primer name	Forward (5′–3′)	Reverse (5′–3′)	Probe
Parkin Ex1(MGB)	CGCAGCCGCCACCTA	GGCGCAGAGAGGCTGTAC	Fam-CCCAGTGACCATGATAGGTA-MGB
Parkin Ex2	CACAGTCCAGTCATTCCTCAGC	GTTCAACTCCAGCCATGGTTTC	Fam-CCTTCCCTGCGAAAATCACACCGCAT-Tamra
Parkin Ex3	GAGGACTGAGCTGCTGAGG	AGAGCATTGTTCACATTGTGC	Fam-TCGTCGCCTCCAGTTGCATTCAT-Tamra
Parkin Ex4	TCTTCTCCAGCAGGTAGATCAATC	TGCTGACACTGCATTTCCTTAC	Fam-ATGTGTATTGCAAAGGCCCCTGTCAAT-Tamra
Parkin Ex5(MGB)	CCCAAAGGGTCCATCTTGCT	ACTAGTCCCAGGGCAGTGT	Fam-ACCACTCATCCGGTTTGG -MBG
Parkin Ex6	TAGAGGAAAAATGAGCAGCCG	CGTAATGCAAGTGATGTTCCGA	Fam-AGCACACCCCACCTCTGACAAGGAAAT-Tamra
Parkin Ex7	TGCCGATCATTGAGTCTTGTCA	CCAGTTGCCTTTCCACACTGA	Fam-AGTGGAAACAGTCTAAGCAAATCACGTGGCT -Tamra
Parkin Ex8	ATTCTTCTTTCCAACAGCTGGC	ATGACAGTCTGATGCAGCCTTT	Fam-CCCAACTCCTTGATTAAAGAGCTCCATCACT-Tamra
Parkin Ex9	TTTTGCAGTACAACCGGTACCA	AGCAAACAAGGACAGGAACACA	Fam-AGTATGGTGCAGAGGAGTGTGTCCTGCAT-Tamra
Parkin Ex10	CCAAATGCAACCTAATGTCCC	TGGAGGAATGAGTAGGGCATTC	Fam-AGTGCAGTGCCGTATTTGAAGCCTCAT-Tamra
Parkin Ex11	AGCTGAGATTAAACGCCTTTCC	TTTTTCCACTGGTACATGGCAG	Fam-CTTTGTTTCCCCAGGCCTACAGAGTCGGAT-Tamra
Parkin Ex12	GTTTTCCAGGTACTTGCTGCG	AAGGTAGACACTGGGTATGCTCC	Fam-ACCACACCTTTGTTTTCTGCCCCCT-Tamra

Table 2
Patient demographic data

Total number of patients, *n* (%)	74 (100.0)
Male, *n* (%)	44 (59.5)
Female, *n* (%)	30 (40.5)
Age at onset (years)	
All[a]	42.4 ± 5.7 (26–50)
Male[a]	42.4 ± 6.3 (26–50)
Female[a]	42.5 ± 4.9 (35–49)
Age at examination (years)	
All[a]	58.0 ± 8.1 (40–78)
Male[a]	58.9 ± 8.6 (40–77)
Female[a]	56.5 ± 7.2 (43–78)

[a] Data given as mean ± SD (range).

One patient possessed a heterozygous p.G411S mutation resulting from a c.1231G > A mutation in exon 6 of the *PINK1* gene.

Two individuals were found to be heterozygous for the common p.G2019S mutation in *LRRK2*.

No alpha-synuclein or *DJ-1* variants were observed. The results are summarized in Table 4.

The previously reported *PRKN* (p.S167N) and *PINK1* (p.Q115L) polymorphisms were also identified (data not shown).

Gene copy assays were performed for the *PRKN* gene only. The possibility that exonic or intronic rearrangements and deletions have occurred in the other *PARK* genes cannot be excluded.

4. Discussion

The importance of genetic factors for the aetiology of PD has been debated controversially for a long time. Longitudinal twin studies argued for a genetic element contributing to the condition whereas other cross-sectional studies could find no evidence for inheritance [4,5]. In the meantime, at least five genes have been identified that are implicated in the

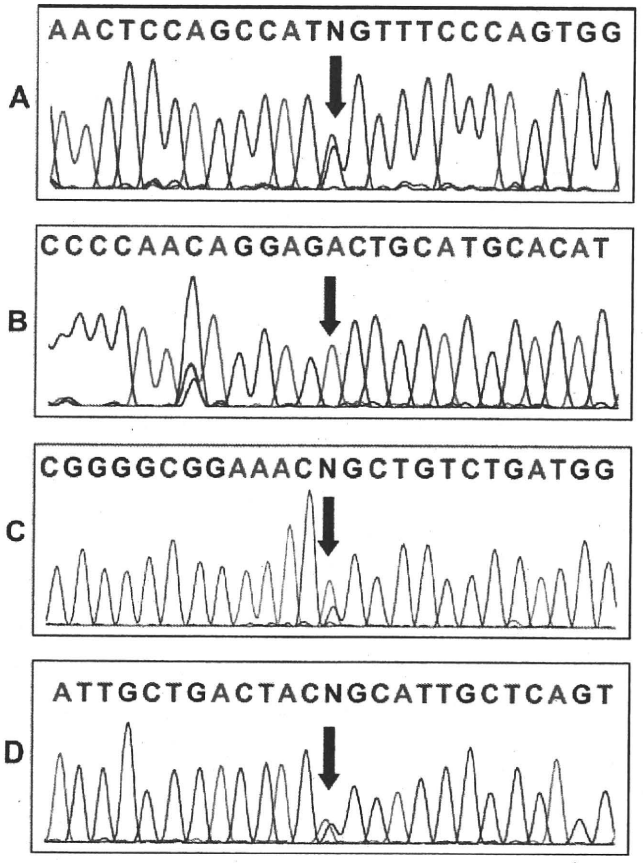

Fig. 1. Electropherogram of parts of the *PRKN* (A, B), *PINK1* (C) and *LRRK2* sequence, showing mutated sites, indicated by arrows. A. Heterozygous *PRKN* mutation p.G12R (c.34G > C, exon 2); B. Homozygous *PRKN* mutation p.G430D (c.1289G > A, exon 12); C. Heterozygous *PARK6* mutation p.G411S (c.1231G > A, exon 6); D. Heterozygous *PARK8* mutation p.G2019S (c.6055G > A, exon 41).

Table 3
Detected mutations

Gene and ID	Mutations	Family history of PD	Age at onset
PARK1 (alpha-synuclein)	None detected		
PARK2 (PRKN)			
10782	p.G12R (c.34G > C: exon 2) heterozygous	Negative	40
12238	p.G430D (c.1289G > A: exon 12) homozygous	Negative	30
PARK6 (PINK1)			
11280	p.G411S (c.1231G > A: exon 6) heterozygous	Positive: Uncle, grandmother, cousin	26
PARK7 (DJ-1)	None detected		
PARK8 (LRRK2)			
10002	p.G2019S (c. 6055G > A: exon 41) heterozygous	Positive: Aunt	49
12248	p.G2019S (c. 6055G > A: exon 41) heterozygous	Positive: Father, Mother	46

Table 4
Clinical characteristics of mutation carriers

10782	Female, aged 48 years at consultation. Initial symptoms: rigidity, loss of dexterity and dystonia of right foot. Good response to levodopa. No family history of PD
12238	Female, aged 35 years at consultation. Initial symptoms: right hand tremor and dystonia of right foot. No family history of PD. Good response to 100 mg levodopa b.i.d. suffers from depression, requiring treatment
11280	Male, aged 39 years at consultation. Initial symptoms were speech problems and loss of dexterity at age 26 years. Family history of Parkinson's disease: paternal grandmother, uncle and cousin
10002	Female, aged 58 years at consultation. Initial symptoms: unilateral tremor and gait disturbance at age 49 years. Family history: aunt. Currently, well responding to treatment with 100 mg levodopa t.d.s.
12248	Male, aged 58 years at consultation. Initial symptoms: cramping, "turning in" of right leg and gait disturbances. Family history: father and mother (father subsequently found to carry the *LRRK2* G2019S mutation). Currently well responding to treatment with 200/50 Madopar and 5 mg Artane t.d.s

development of a monogenic form of PD (*PARK1, 2, 6, 7,* and *8*). These monogenic forms of the condition may mimic clinically sporadic PD but generally (though not exclusively, with the probable exception of *LRRK2*) appear at an earlier age of onset. In this study, we therefore screened a cohort of 74 PD patients with age of onset earlier than 50 years, taken from a case series of 950 patients, for monogenic disease.

The first gene implicated in the development of PD was *SNCA*, coding for the alpha-synuclein protein (*PARK1*). Three point mutations and gene multiplications leading to familial Parkinsonism have been reported, although these mutations are considered rare and are estimated to contribute <1% of monogenic cases of PD. In our cohort, no mutations in this gene were detected, in agreement with statistical expectations with respect to the sample size.

Mutations in the *PRKN* gene (*PARK2*) were first found to be causative for autosomal recessive juvenile Parkinsonism in Japanese families [6]. The frequency of *PARK2* mutations has been estimated to be as high as 40–50% in early-onset disease [7–9] and 10–20% in sporadic cases [7,9–11]. In our study, two patients (2.7% of all subjects screened) possessed *PRKN* mutations (Table 3). Notably, no confirmed exon-dosage abnormalities were observed. This number is lower than that reported in several comparable studies, which report *PRKN* mutations to be present in between 10.4 and 18.0% of early-onset cases [9,10,12,13]. Our data is comparable to the reported 3.8% frequency of *PRKN* mutations in patients screened in a cohort of 313 North American PD cases [14]. The ability to detect *PRKN* mutations depends on factors such as sample size, ethnic extraction, inclusion criteria for cases and the methods used for mutation detection. Of the two identified *PRKN* mutations reported in the current study, one has been reported previously (p.G430D) [12]. To the best of our knowledge, there have been no previous reports of the p.G12R variant. The functional significance of these sequence variants remains to be established. This particular *PRKN* mutation (G12R) is predicted to be 'possibly damaging' (PolyPhen, http://coot.embl.de/PolyPhen/) and the amino acid in this position is highly conserved through *Bos taurus*, *Sus scrofa*, *Rattus norvegicus*, *Mus musculus*, *Gallus gallus* and *Danio rerio*. The mutation has not been found in a cohort of 170 European control subjects, comparable in ethnicity to subjects in our study [15]. *PRKN* mutations are generally presumed to be recessive, so the possibility that additional non-coding region sequence variants or additional factors contributing to the disease outcome in these individuals cannot be ruled out. There is a growing body of evidence that heterozygous *PRKN* mutations can be pathogenic and may be causative for disease [7,9,16]. In a recent report from Denmark, 10 out of 87 patients screened possessed putative disease-causing *PRKN* mutations; eight of these mutations were heterozygous in nature [13]. It has also been proposed that a heterozygous genotype may lead to a comparatively later age of onset. Our data are not necessarily consistent with this argument. Our mutation carriers developed symptoms well before the age of 45 years at 40 and 30 years of age, respectively (Table 4).

PARK6 (*PINK1*) mutations have been reported in 3–15% of early-onset recessive Parkinsonism cases while 5% of sporadic cases reportedly carry a single heterozygous *PINK1* mutation [17–19]. An estimated contribution of <1% for *PINK1* mutations to familial PD is probably more realistic [20,21]. Statistically, the one *PINK1* mutation carrier identified in our study accounts for 1.4% of the cohort. This mutation leading to a p.G411S amino acid substitution has been previously described in at least five PD patients [22–24]. Whether this particular mutation in its heterozygous form is causative for the disease phenotype is a matter of speculation. Interestingly, all five patients reported to date were heterozygous for this mutation, and no homozygous case has been described so far. Given that the amino acid change occurs within the kinase domain, in a sequence highly conserved in vertebrates, and that it has not been observed in normal subjects despite considerable investigation [22,23] it seems reasonable to assume that the mutation at least contributes significantly to the development of Parkinsonism. As no assays covering large genomic rearrangements for the *PINK1* gene were carried out in this study, the possibility that such rearrangements occurred in this particular patient cannot absolutely be ruled out.

No *PARK7* (*DJ-1*) mutations were detected in our study, consistent with previous studies that suggest that <2% of early-onset cases of PD carry coding region mutations in *PARK7* [25].

Two of our patients carried the common *LRRK2* p.G2019S mutation in exon 41 (2.7%, Table 3). Funayama et al. linked the *PARK8* locus to chromosome 12 in 2002 [26]. It has subsequently been found that *LRRK2* mutations constitute the most frequent form of monogenic PD. The p.G2019S mutation occurs in more than 2% of North American and English patients [27,28], and is found in >10% of North African, Ashkenazi Jewish and Portuguese populations [29–31]. The mutation falls within an activation segment of the MAPKKK domain, changing a highly conserved glycine at the start of the activation loop. Alternatively, it has been proposed that a reduction in kinase enzyme activity may be caused through changes in the magnesium-binding loop or by introduction of new phosphorylation sites. A recent Australian study, that did not include any of the subjects investigated in the current report, identified eight of 830 PD patients (1%) with this mutation.

4.1. Conclusions

Our data suggest that approximately 7% of early-onset PD cases seen in Queensland movement disorders clinics have mutations involving known *PARK* genes. However, whether these mutations were disease causing in all patients must remain open. The number of mutations found will increase as additional causal genes are identified from current gene-hunting strategies.

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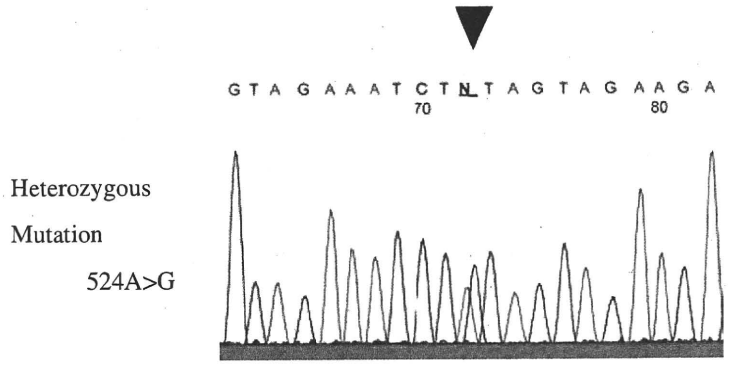
Letter to the Editor

A new mutation in the *GCH1* gene presents as early-onset Parkinsonism

The *GCH1* gene encodes GTP cyclohydrolase I (GCH1), which catalyzes the first step (GTP to 7, 8-dihydroneopterin-triphosphate) in the biosynthesis of tetrahydrobiopterin (BH4). This enzyme is rate-limiting and deficiency causes Segawa disease, characterized by hereditary progressive dystonia with marked diurnal fluctuation (HPD/DRD) and autosomal recessive GCH-deficient hyperphenylalanemia. Conventionally, the first symptoms of DRD develop before 10 years of age, and few cases of adult onset have been described [1,2]. To date, over one hundred kinds of the *GCH1* mutation have been reported to lower enzymatic activity, which has also been observed in mononuclear blood cells of juvenile Parkinsonism [3]. However, no reports have described only Parkinsonism occurring with a *GCH1* gene mutation.

Herein, we highlight a 33-year-old Chinese male computer engineer who developed resting tremor, and noted difficulty in skillful movement when manipulating objects during computer work. The patient showed a Myerson's sign, tremor in the left arm and leg at rest, and sluggish gait with anteversion posture. Neither autonomic nervous system dysfunction nor dystonic movement was apparent. Blood examination and magnetic resonance imaging (MRI) of the brain showed no abnormality. Based on the clinical presentation, the patient was diagnosed with early-onset Parkinsonism in Yahr stage 2 for which levodopa was started. Levodopa improved clinical symptoms, disease progression was slow and no dyskinesia appeared. At age 41 years (8 years after onset), medications were 250 mg of levodopa/carbidopa, 0.75 mg of cabergoline



Homo sapiens	A	R	I	V	E	I	**Y**	S	R	R	L
Mus musculus	A	R	I	V	E	I	**Y**	S	R	R	L
Rattus norvegicus	A	R	I	V	E	I	**Y**	S	R	R	L
G. gallus	A	R	I	V	E	I	**Y**	S	R	R	L
S. cerevisiae	A	R	L	A	E	M	**Y**	A	R	R	L
K. lactis	A	R	L	A	E	M	**Y**	A	R	R	L
E. gossypii	A	R	L	A	E	M	**Y**	A	R	R	L

Fig. 1. The arrowhead indicates point mutation c.524A > G (p.Y175C). This mutation was not detected in 192 chromosomes. The tyrosine position (Y) is preserved in various species.

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and 2.5 mg of selegiline hydrochloride. Daily life and job were well-managed. When medication effects disappeared, the patient displayed tremor in the left upper limb at rest, left-sided rigidity, frozen gait and disability in skillful movement of fingers. Notably, his father was reported to have exhibited resting tremor of the left upper limb.

Parkin gene analysis was negative; however, sequence analysis of the *GCH1* gene identified a heterozygous mutation (c.524 A > G; p.Y175C) in exon 4 (Fig. 1).

The father was subsequently found to carry the same mutation. This mutation was also not observed in 96 healthy control subjects and this tyrosine residue is conserved across species supporting the pathogenicity of p.Y175C (Fig. 1). The GCH1 enzyme activity was measured in the proband according to the methods previously reported by Ichinose et al. [3] at 1.80 pmol/h/mg protein (normal control, 4.17 pmol/h/mg protein). Next, concentrations of neopterin and biopterin in cerebrospinal fluid were measured. Both neopterin and biopterin are metabolic products of GTP. Concentrations were 13.3 pmol/ml for neopterin and 5.2 pmol/ml for biopterin (normal, around 22–32 pmol/l for neopterin and 21–26 pmol/l for biopterin) [4].

Neopterin concentrations in the cerebrospinal fluid of patients with dopa-responsive–dystonia (DRD) due to *GCH1* mutation are reportedly <10 pmol/ml. Therefore, in the present patient neopterin concentration was not as low as that in DRD, and was similar to that in patients with Parkinsonism. Differences among *GCH1* gene mutation positive patients may be influenced by the position of mutated amino acids, the type of mutation, and one or more of the other gene mutations. To elucidate genotype-phenotype correlations, examination of abnormalities in the *GCH1* gene in patients who present with only Parkinsonism is important and may contribute to better understand the pathogenesis of this syndrome.

Conflict of interest

The author has no conflicts of interest.

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Mutation analysis for *DJ-1* in sporadic and familial parkinsonism: Screening strategy in parkinsonism

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ABSTRACT

DJ-1 mutations cause autosomal recessive parkinsonism (ARP). Although some reports of *DJ-1* mutations have been published, there is lack of information on the prevalence of these mutations in large-scale studies of both familial and sporadic parkinsonism. In this genetic screening study, we analyzed the distribution and frequency of *DJ-1* mutations by direct nucleotide sequencing of coding exons and exon–intron boundaries of *DJ-1*, in 386 *parkin*-negative parkinsonism patients (371 index cases: 67 probands of autosomal recessive parkinsonism families, 90 probands of autosomal dominant parkinsonism families, 201 patients with sporadic parkinsonism, and 13 with unknown family histories) from 12 countries (Japan 283, China 27, Taiwan 22, Korea 22, Israel 16, Turkey 5, Philippines 2, Bulgaria 2, Greece 2, Tunisia 1, USA 2, Ukraine 1, unknown 1). None had causative mutation in *DJ-1*, suggesting *DJ-1* mutation is very rare among patients with familial and sporadic parkinsonism from Asian countries and those with other ethnic background. This is in contrast to the higher frequencies and worldwide distribution of *parkin*- and *PINK1*-related parkinsonism in ARP and sporadic parkinsonism. Thus, after obtaining clinical information, screening for mutations in (1) *parkin*, (2) *PINK1*, (3) *DJ-1*, (4) *ATP13A2* should be conducted in that order, in ARP and sporadic parkinsonism, based on their reported frequencies. In addition, haplotype analysis should be employed to check for homozygosity of 1p36, which harbors a cluster of causative genes for ARP such as *DJ-1*, *PINK1* and *ATP13A2* in ARP and sporadic parkinsonism, especially in parkinsonism with consanguinity.

To date, four genes, *parkin* (*PARK2*) [16], *PINK1* (*PARK6*) [30], *DJ-1* (*PARK7*) [4], and *ATP13A2* (*PARK9*) [25] have been identified as the causative genes for familial autosomal recessive parkinsonism (ARP). *Parkin*, *PINK1*, and *DJ-1* mutations cause levodopa-responsive parkinsonism with or without dystonia at onset and psychiatric problems [16,30,4,20,31,11,19,17]. On the other hand, *ATP13A2* mutations cause rare atypical parkinsonism with multisystemic neurodegeneration [25,23]. Mutations in *parkin* have been detected in approximately 50% of patients with ARP and 15% of patients with sporadic early-onset parkinsonism [20]. The reported frequency of *PINK1* mutations in ARP is approximately 4.5% [19]. Single heterozygous *PINK1* mutations were reported in 5% of patients with sporadic early-onset parkinsonism [31]. In some ethnic groups, the incidence of *DJ-1* mutations have been estimated to be not more than 1–2% [1,9,7,6,12,14], although further large studies are needed.

In patients with ARP, it is important to determine the best screening method that can detect mutations in these genes. In addition, the roles of single heterozygous mutations of *parkin*, *PINK1*, *DJ-1*, and *ATP13A2* in familial parkinsonism and sporadic Parkinson's disease (PD) remain unclear. However, to our knowledge, there is currently a lack of information about the frequency of mutations, including single heterozygous mutation, for *DJ-1* in both familial and sporadic parkinsonism, especially in large population samples. In this study, we report the results of mutation analysis for *DJ-1* in a large number of patients with familial and sporadic parkinsonism and discuss the best strategies for genetic screening.

Blood samples and clinical information on 386 patients with parkinsonism (371 index cases: autosomal recessive = 67, autosomal dominant = 90, sporadic = 201, and unknown = 13) from 12 countries (Japan, 283; China, 27; Taiwan, 22; Korea, 22; Israel, 16; Turkey, 5; Philippines, 2; Bulgaria, 2; Greece, 2; Tunisia, 1; USA, 2; Ukraine, 1; and unknown, 1) were obtained from each neurologist (Table 1). In this study, we classified the mode of inheritance as autosomal recessive (families with consanguineous marriages or at least two affected siblings in only one generation) and autosomal

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Table 1
Details of 386 analyzed patients with parkinsonism.

	Total number of analyzed patients	Age at onset* (years) (for analyzed patients)
Sporadic parkinsonism	201 (males 113, females 88)	35.3 ± 10.6 (7–80)
Familial parkinsonism	185 (170 probands + 15 relatives) (males 90, females 95)	46.2 ± 15.2 (9–80)
ARP	74 (67 probands)	49.6 ± 14.9
ADP	97 (90 probands)	44.3 ± 14.9
No clear hereditary information	14 (13 probands)	41.8 ± 16.4
Total	386 (371 index patients = 201 sporadic patients + 170 probands)	40.5 ± 14.0

ARP: autosomal recessive parkinsonism; ADP: autosomal dominant parkinsonism.
* Data are mean ± SD (range).

dominant (one or more affected members in each consecutive two generations). The diagnosis of parkinsonism was established by the participating neurologists. The majority of patients had typical parkinsonism with good clinical response to levodopa. The study was approved by the ethics review committee of Juntendo University And blood samples were collected after obtaining informed consent from all participants. DNA was prepared by using standard methods. None of the participants had *parkin* mutation in the coding regions and exon–intron boundaries and no gene dosage abnormality, as confirmed in our previous study (unpublished data).

For sequence analysis, six coding exons and exon–intron boundaries of *DJ-1* were amplified by PCR using published primers and conditions [4]. Dideoxy cycle sequencing was performed with Big Dye Terminater Chemistry (Applied Biosystems, Foster City, CA). This was followed by nucleotide sequencing on ABI377, 310, and 3130 automated DNA sequence analyzers (Applied Biosystems).

We found no heterozygous or homozygous missense/nonsense mutations in the coding exons and no causative mutations in exon–intron boundaries of *DJ-1*. We also found a non-synonymous p.R98Q variant, which had been reported to be a polymorphism [13].

This extended large study identified no pathogenic *DJ-1* mutations in 386 patients (mainly Asian) with familial or sporadic parkinsonism by direct sequencing. Our cohort was more selected than the overall PD population, since all *parkin*-positive patients were excluded. In this regard, the lack of *DJ-1* mutations, especially among the rest of approximately 50% of autosomal recessive cases without *parkin* mutation, is even more striking. Furthermore, our study identified the lack of a single heterozygous *DJ-1* missense or nonsense mutation in a large number of patients with sporadic and familial parkinsonism. However, the role of single heterozygous mutation of causative genes for ARP such as *DJ-1*, *parkin*, *PINK1*, and *ATP13A2*, remains intriguing because multiple rare variants might cause the disease. In the 386 patients, the conventional PCR method showed no homozygous *DJ-1* deletion in contrast to *parkin*. Accordingly, as a screening strategy in parkinsonism, gene dosage study for *DJ-1* appears less important than *parkin*, although multiplication or heterozygous exonic deletion cannot be ruled out. Although other mutations in the promoter region or intron of *DJ-1* could exist, our large study suggests that *DJ-1* mutation is rare in Asian or Japanese as well as in parkinsonism patients of other ethnic origin.

In several cohort studies, the frequency of *DJ-1* mutation has also been reported to be lower than that of *parkin* or *PINK1* mutation [30,4,20,31,11,19,1,9,7,6,12]. Because of the rare and characteristic phenotype of *PARK9*-linked parkinsonism, we did not screen for *ATP13A2* (which contains 29 exons) in this study [25,23]. Thus, we propose that, after checking the clinical features carefully, one should screen for mutations in the following order: (1) *parkin*, (2) *PINK1*, (3) *DJ-1*, and (4) *ATP13A2* in ARP in Asian and worldwide populations [20,19,17,12]. Combining our previous data with previous reports indicate that the frequencies of causative mutations in *parkin*, *PINK1*, and *DJ-1* are approximately 50%, 4.5%, 0% in our ARP cohort, respectively [20,19,17]. Conversely, our data further suggests the existence of more yet unknown causative genes for ARP.

The locus of *DJ-1* is 1p36 [32], which harbors a cluster of other causative genes for ARP such as *PINK1* and *ATP13A2* around the small chromosomal region. Therefore, checking the homozygosity of this locus by haplotype analysis is a fruitful strategy for genetic analysis in ARP and especially in parkinsonism with consanguinity. Although mutations in *DJ-1* have not yet been identified in sporadic parkinsonism patients, analyses of *DJ-1*, *PINK1*, and *ATP13A2* should be considered even in sporadic parkinsonism patients (especially in patients with consanguinity or homozygosity in locus 1p36). Indeed, although patients with *ATP13A2* mutation seem to be quite rare, we applied the above strategy and could identify the first Japanese patient with a novel homozygous *ATP13A2* mutation, who was the sole parkinsonism patient without other affected family members and with consanguinity [23].

Interestingly, the locus of TDP-43 (*TARDBP*), which is associated with TDP-43 proteinopathy and amyotrophic lateral sclerosis (ALS), is located in the same small chromosomal region 1p36 [22,27]. Surprisingly, *DJ-1* was also reported to cause parkinsonism-dementia-ALS complex [3]. Moreover, DJ-1 colocalizes with tau inclusions linking parkinsonism to dementia [26]. Subjects with the ALS/parkinsonism-dementia complex (ALS/PDC) of Guam or Kii have been reported to have tau and TDP-43 pathology [18,10], although no mutations were identified [29]. The roles of genes clustering around 1p36 in neurodegenerative disorders needs further investigation.

Furthermore, in parkinsonism, digenic or trigenic mutations (multigenic mutations) or functional interactions among the causative genes for ARP have been reported, such as *DJ-1*-*PINK1* and *parkin*-*PINK1* [28,24,5,8]. Thus, *DJ-1* could play some roles in certain common pathways of neurodegeneration via oxidative stress and mitochondrial dysfunction [15]. Although *DJ-1* was initially described as a ubiquitously expressed protein and an oncogene related to the pathogenesis of cancer [21], recent evidence of its neuroprotective roles in neurodegenerative disorders and stroke has been reported [2]. Accordingly, loss of *DJ-1* function could lead to various diseases.

In conclusion, despite the large cohort of both familial and sporadic parkinsonism, no causative *DJ-1* mutations were identified in our patients. These results could reflect the crucial importance of homoeostasis and ability to survive lethal events from the early stages of life. Therefore, further genetic analyses of *DJ-1* and studies of DJ-1 functions might provide important insights into human diseases and survival.

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各疾患領域における遺伝子解析

パーキンソン病関連遺伝子の全ゲノム関連解析

戸田達史*

要　旨

メンデル遺伝性パーキンソン病家系から6つの原因遺伝子が明らかにされ，それらを切り口にして孤発性パーキンソン病の病態解明が進んでいる．患者の大多数を占める孤発性パーキンソン病は多因子疾患であり，我々はαシヌクレインが孤発性パーキンソン病の確実な疾患感受性遺伝子として同定した．そのほか，まれな変異としてゴーシェ病遺伝子が重要である．現在パーキンソン病においてSNPチップを用いた全ゲノム関連解析が進行している．

はじめに

パーキンソン病は，臨床的には，振戦，筋固縮，寡動，姿勢反射障害を主徴とし，痴呆，自律神経障害などのさまざまな随伴症状を呈する神経変性疾患である．病理学的には，黒質緻密層のドーパミン細胞の脱落とレビー小体の出現を特徴とする．神経変性疾患ではアルツハイマー病に次いで頻度が高く，我が国には14万人以上の患者が存在するが，今後社会の高齢化に伴いさらなる患者数増加が予想されている．発症機序としてはMPTPやrotenoneといったドーパミン神経毒やパーキンソン病患者脳を用いた研究などから，ミトコンドリアの呼吸系酵素の障害，炎症反応，酸化ストレス障害によりアポトーシスが誘導され，神経細胞死に至ることが一因とされているが，それらが惹起される分子遺伝学的機序は明らかでない．

症例の90%以上は孤発性発症であるが，5～10%は家族性（その一部はメンデル遺伝性）に発症する．メンデル遺伝性パーキンソン病家系の連鎖解析などから，6つのメンデル遺伝性パーキンソン病原因遺伝子が明らかにされ，ミトコンドリア障害，酸化ストレス障害の病態への関与に加え，新たにユビキチン・プロテアソーム系の機能低下，つまりタンパク質分解異常からドーパミン細胞死に至る経路の重要性が示された．孤発性パーキンソン病，メンデル遺伝性パーキンソン病とも，一部共通の発症メカニズムが存在していると考えられ，それらを切り口にして孤発性パーキンソン病の病態解明が進んでいる．

一方で，パーキンソン病においては，患者

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表1　メンデル遺伝性パーキンソン病（PARK 遺伝子）

遺伝子座	遺伝形式*	遺伝子座	遺伝子	臨床的特徴	レビー小体
PARK1	AD	4q21	*α-synuclein*	早発性，急速に進行	＋
PARK2	AR	6q25.2-27	*parkin*	若年発症，ジストニー	－
PARK3	AD	2p13	未知	晩発性パーキンソン病	＋
PARK4	AD	4q21	*α-synuclein*	早発性，認知症，姿勢振戦	＋
PARK5	AD	4p14	*UCH-L1*	典型的パーキンソン病	？
PARK6	AR	1p35-36	*PINK1*	早・晩発性，緩徐に進行	？
PARK7	AR	1p36	*DJ-1*	早発性	？
PARK8	AD	12p12	*LRRK2*	典型的パーキンソン病	－～＋
PARK9	AR	1p36	*ATP13A2*	若年発症，核上性上方注視麻痺	？
PARK10	SP	1p32	未知		
PARK11	SP	2q36-37	未知		
PARK12	SP	Xq21-25	未知		
PARK13	SP	2p12	*HTRA2*		
GBA	SP	1q21	*glucocerebrosidase*		
SCA2	AD	12q24	*ataxin-2*		

* AD：常染色体優性遺伝，AR：常染色体劣性遺伝，SP：孤発性

の大多数を占める孤発性パーキンソン病は多因子疾患であると考えられ，孤発性パーキンソン病の疾患感受性遺伝子の発見を目指した研究が行われている．本稿では，パーキンソン病関連遺伝子について，家族性よりむしろ孤発性パーキンソン病の疾患感受性遺伝子に目を向け，その全ゲノム関連解析（GWAS）の現状とともに概説する．

メンデル遺伝性パーキンソン病と病態（表1）

一部のメンデル遺伝形式をとる家族性パーキンソニズムについては，α シヌクレイン遺伝子[1]やパーキン遺伝子[2]の変異が発見された．さらに，孤発性パーキンソン病のレビー小体中に α シヌクレインが存在すること，PARK4 が野生型 α シヌクレインを含む 1.6～2.0Mb の三重重複であったことが報告され，変異型のみならず野生型でも発現量が増加すれば発症に至ることが示された[3]．

パーキンタンパク質の機能については，ユビキチン・プロテアソーム系タンパク質分解において基質にユビキチンを結合させる E3 ユビキチンリガーゼであることが明らかにされた．ユビキチンリガーゼはプロテアソームによって分解を受けるべきタンパク質にユビキチンという目印をつける酵素であり，ユビキチン・プロテアソーム系の破綻によって，本来分解されるタンパク質（すなわちパーキンの基質）が蓄積することで神経変性を引き起こしていると考えられている．このパーキンの基質はさまざまな報告があるが，真の基質はどれであるかいまだ結論が出ていない．近年，パーキンと PARK6/PINK1 が同じ経路に位置していることが報告された[4]．

PARK7 の原因は DJ-1 タンパク質であり，抗酸化作用やプロテアソーム機能を持ち，ドーパミン細胞死との関係が注目される．また，PARK8 は常染色体優性遺伝形式を呈するものの中で最多であり，原因タンパク質は LRRK2 であり，シヌクレインに対するキナーゼ活性が注目されている[5]．PARK9 は近年

発見され，進行が早く，核上性上方注視麻痺を呈するなど非典型的である．原因遺伝子はlysosomal type 5 P-type ATPaseと呼ばれ，機能不明．リソソームに存在し，パーキンソン病黒質ドーパミン神経でRNA発現が上昇しているが，意義は不明である[6]．

メンデル遺伝性パーキンソン病の原因遺伝子は次々と同定され，これらのユビキチン・プロテアソーム系への関与や，従来から孤発性パーキンソン病の患者脳や神経毒を用いた動物モデルで報告されているミトコンドリア障害，酸化ストレス障害との関係が示されている．タンパク質の産生・分解・蓄積の異常といった他の神経変性疾患にも共通する機構が考えられ，興味深い[5]．

孤発性パーキンソン病は多因子遺伝性疾患である

症例的には大多数（90% 以上）の孤発性パーキンソン病の原因は現時点では不明であるが，環境因子と遺伝因子により発症する多因子疾患であると考えられている．

遺伝因子の存在に関しては，① 遺伝形式の推定可能な大家族の存在，② 約 10% のパーキンソン病患者の1親等内にパーキンソン病患者が存在すること，③ PBT にて潜在的異常を示す症例を含んだ PET study の結果では，1卵性双生児の疾患一致率が約 60% であり2卵性双生児の約3倍であること，などからも多因子遺伝性疾患であることが示唆されている．2000 年にはアイスランド国民を対象とした大規模な疫学的調査の結果が発表され，同胞再発危険率 λs は 6.7 で，パーキンソン病発症には遺伝因子が影響していることが示された[7]．

このようにパーキンソン病患者のほとんどは多因子遺伝と推定され，外的毒素の解毒を行う酵素のシトクロム P450 遺伝子，ドーパミン受容体やトランスポーターの遺伝子，カテコールアミンの代謝系の酵素遺伝子，酸化的ストレス関連遺伝子などが関連解析にて検討されたが，候補遺伝子アプローチは多数の関連解析の報告があるが，サンプル数も 200～300，P 値も 0.01～0.001 程度で，追試で反対の結果が出たりするなど，アルツハイマー病における ApoE4 多型のような確実に発症リスクを高める遺伝因子はなかなか確認されていなかった．

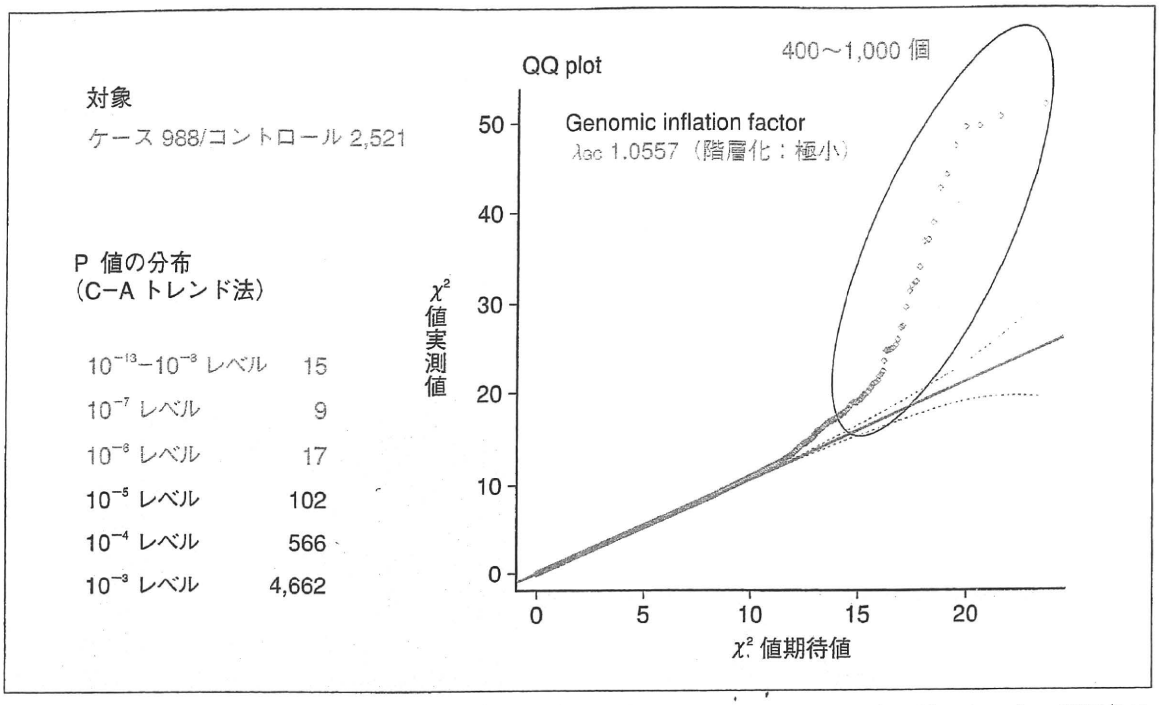
孤発性パーキンソン病感受性遺伝子 α シヌクレインの同定

そのような状況下で我々は，孤発性パーキンソン病の疾患感受性遺伝子同定による，病態解析と創薬，抗パーキンソン病薬の効果・副作用と SNP との関係に基づいたテーラーメイド医療の確立を目標にして，① ゲノムワイド関連解析，② 多数の候補遺伝子による関連解析を行っている．

うち候補遺伝子アプローチにて，数百個の候補遺伝子上 SNP を用いた患者 882 人，対照 938 人の関連解析を行った結果，α シヌクレイン遺伝子のイントロン4上に存在する SNP0070 に $P=5.0\times10^{-10}$ という極めて強い関連を見いだした．また SNP0070 を含めて，高い r^2 値（>0.85）をとる SNP がイントロン4，3'UTR，3'-flanking region に計6個あり，すべてパーキンソン病と強い関連（$P=2.0\times10^{-9}$～1.7×10^{-11}）を示した[8]．

α シヌクレインタンパク質はパーキンソン病の病理学的特徴であるレビー小体の主要成分であり，α シヌクレインの発現量が孤発性パーキンソン病発症にも影響すると考えられている．剖検脳前頭葉にて，パーキンソン病感受性アレルの数に応じて α シヌクレイン遺伝子発現が増加していた．以上の結果から，α シヌクレインは孤発性パーキンソン病の初めての確実な感受性遺伝子であると結論づけられ，またP値はさほど低くはないが，同様

図1　GWAS の結果



患者 988 検体，対照 2,521 検体で関連解析を行った結果．インフレーションファクターは 1.055 と，階層化は非常に小さいと考えられた．候補遺伝子アプローチから同定されたαシヌクレイン[8]も含めて，10^{-7}～10^{-13}レベルの SNP が 24 個，10^{-6}レベルが 17 個存在．

の結果はドイツ人，ノルウェー人からも報告された．

パーキンソン病の GWAS

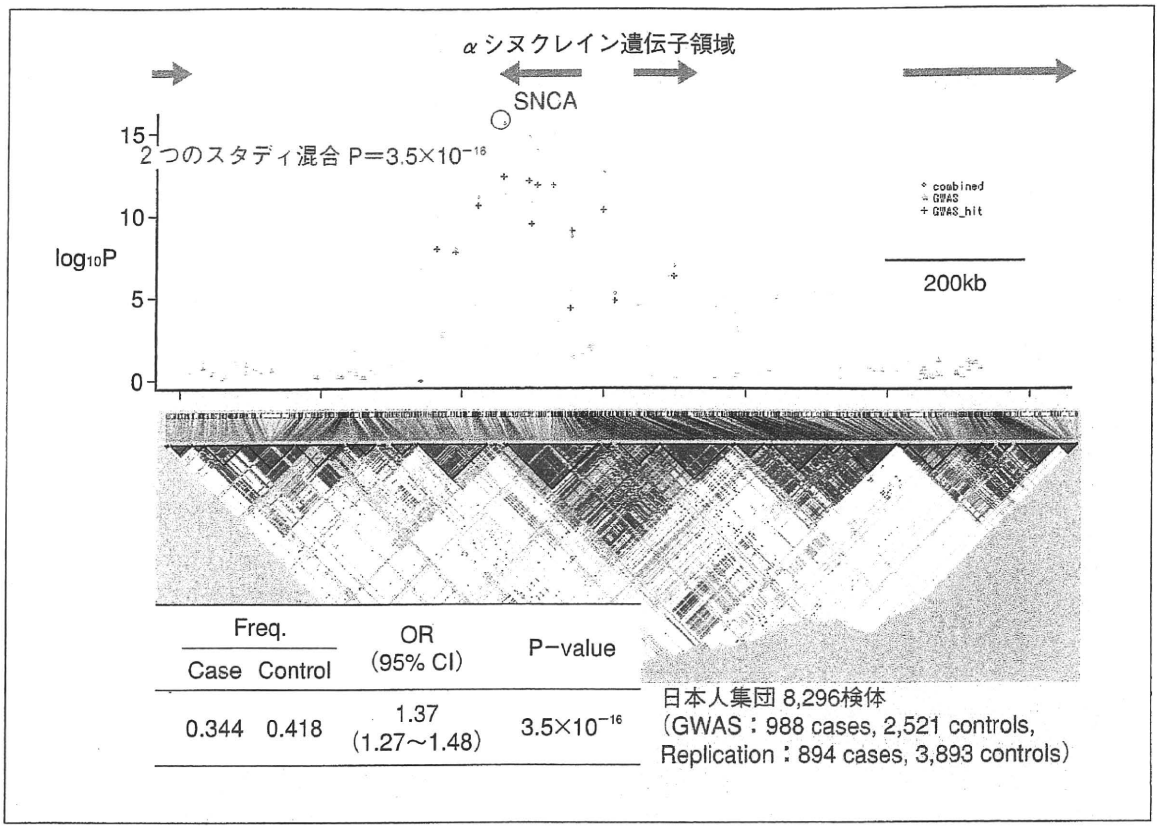
パーキンソン病に関しても SNP チップの GWAS が論文報告されているが，少人数の解析であり，確実なことは言えないのが現状である．

Maraganore らは，Perlegen 社の 198,345 個の SNP チップを用いて 443 人の不一致兄弟ペア（片方は発症，もう片方は非発症）で解析し，P＜0.01 で残った有意な 1,793 SNP について 332 人ずつの症例・対照関連解析を行った[9]．その結果最も低い P 値を示すものとして，神経発生に関与するセマフォリン 5A 遺伝子（$P=7.62\times10^{-6}$）を見いだしたが，その後の他グループ他検体によるレプリケーション解析，メタ解析でことごとく否定された．不一致兄弟ペアの解析にて，遺伝的負荷を持つ非発症者も近い将来発症したかもしれず，バイアスが増したものと考えられる．

また Singleton のグループは，Illumina 社の 408,000 個の SNP チップを用いて 267 人ずつの症例・対照解析を行い，後シナプスで働くキナーゼの 1 つ DLG2（$P=7.3\times10^{-6}$）など，有意な遺伝子リストを報告しているが[10]，Maraganore らのデータと共通するものはわずか 3 SNP しかない[11]．この程度の人数では偽陽性のことのほうが多く，大人数での検証を待たねばならない．

最近 Foroud と Myers のグループは，857 人の家族性パーキンソン病症例，867 人の対照について，Illumina 社の 370K SNP チップを用いて解析したところ，ゲノムワイド解析の P 値基準を満たす SNP はなかった．以前の Singleton グループの解析と併せたメタ解析で，GAK と DGKQ という 2 つのキナーゼ遺伝子領域で $P=10^{-7}$，シヌクレ

図2 GWAS＋レプリケーションの結果



患者 1,882 検体，対照 6,414 検体，計 8,296 検体の結果．最も有意な遺伝子はαシヌクレイン領域でさらに有意になり，$P=3.5\times10^{-16}$ を示した．
略語：巻末の「今月の略語」参照

インや MAPT で $P=10^{-5}$ 程度を示したことを報告している[12]．

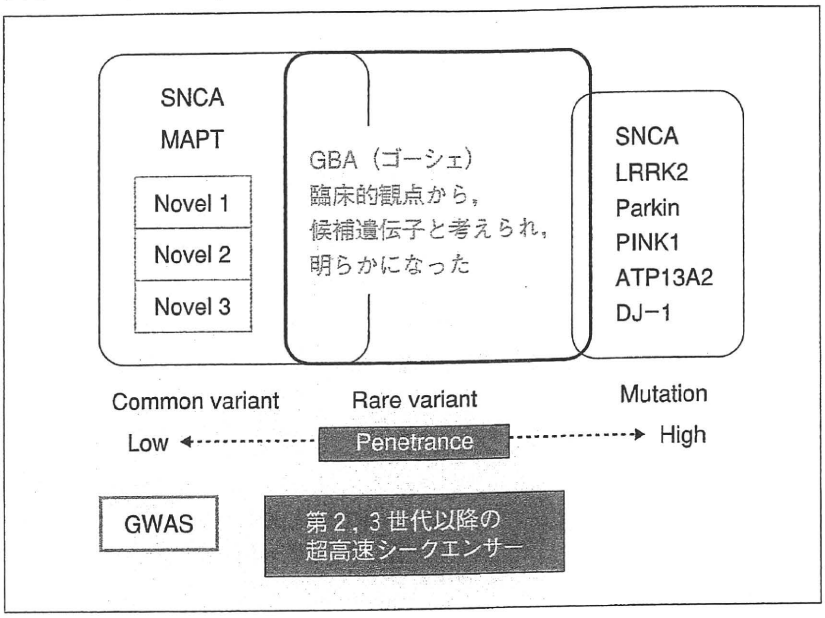
日本人パーキンソン病の GWAS

我々は 55 万個の SNP を搭載した Illumina Hap550 アレイを用いて，患者約 1,000 人規模の GWAS の実験を行った．

まずパーキンソン病患者 1,012 検体，理化学研究所の対照 2,573 検体の SNP 型を判定した（東京大学医科学研究所 中村祐輔教授との共同研究）．IBS 検定，Multidimensional scaling により，血縁者や非アジア人検体を除外し，患者 988 検体，対照 2,521 検体で関連解析を行った結果，QQ プロットから約 400～1,000 個が外れていた．インフレーションファクターは 1.055 と，階層化は非常に小さいと考えられた．最も有意であったのは，α シヌクレイン領域の SNP［$P=6.17\times10^{-13}$ (trend model)］であった．これは先の候補遺伝子アプローチからも同定されており，本実験の確からしさを物語っている．先の α シヌクレインも含めて，10^{-7}～10^{-13} レベルの SNP が 24 個，10^{-6} レベルが 17 個，10^{-5} レベルが 102 個存在し，新規のパーキンソン病感受性遺伝子を捕捉していると思われた（図1）．

さらに，関連を認めた上位 384 SNP について，別の患者 894 検体，対照 3,893 検体（計 8,296 検体：患者 1,882 検体，対照 6,414 検体）で，VeraCode を用いた GoldenGate 法でレプリケーション実験を行った．最も有意な遺伝子は α シヌクレイン領域で，さら

図3 パーキンソン病の遺伝子



メンデル遺伝性変異以外に，common variant として α シヌクレイン（SNCA），rare variant としてゴーシェ遺伝子（GBA）が重要．

に有意になり $P=3.5\times10^{-16}$ を示したが（図2），それ以外にも幾つかの有意に関連した領域を得ている．これらは従来からは全く新規な遺伝子であり，従来説にとらわれず，新規なものを同定できるところに GWAS の強みがある．

同規模の検体数のプロジェクトが米国，ドイツ，シンガポールで進行中であり，関連を認めた SNP に関しては，ドイツ人，米国人，シンガポール人で再現性を検討し，人種を越えて共通するパーキンソン病感受性 SNP，人種特異的なパーキンソン病関連 SNP を同定する．

Multiple rare variants とゴーシェ病

SNP をマーカーにする GWAS は，common disease – common variants 仮説に基づくが，頻度が非常に低くしかも多数の変異が疾患と関連する場合，そのような遺伝因子は多型との連鎖不平衡による検出が難しく，GWAS ではつかまえきれない．このような考えを common disease – multiple rare variants 仮説と呼ぶ．

リピドーシスの1つ，常染色体劣性遺伝のユダヤ人ゴーシェ病家系内にパーキンソン病患者が多いことから，パーキンソン病ではグルコセレブロシダーゼ（GBA，1q21）変異のヘテロ保因者が有意に多いことが報告された[13]．その後も各国から同様の報告が続き，東京大学神経内科 辻教授らと我々との共同研究で，GBA 遺伝子変異をヘテロで持つ保因者はパーキンソン病患者 534 人中 50 人（9.4%），対照 544 人中 2 人（0.37%）であり，パーキンソン病と GBA 変異は強く関連している（オッズ比 28.0）ことを見いだしている[14]．GBA 変異は確実なパーキンソン病危険因子であり，common disease – multiple rare variants 仮説によるものである（図3）．

おわりに

多因子神経変性疾患の疾患感受性遺伝子としては，アルツハイマー病における ApoE4 とパーキンソン病の α シヌクレイン，GBA など以外に確立されたものは少ないのが現状

であり，今後の GWAS からさらなる研究の発展が期待される．

しかし現在の SNP チップによる GWAS では，アレル頻度の高いものしか同定不可能であり，GBA などの rare variant は見すごされてしまう．高速の次世代シークエンサーによるリシークエンスが行われだしているが，現在のところ，候補遺伝子に限られよう．また最近，自閉症，統合失調症[15]などがゲノム中にたくさん存在するコピー数多型（CNV）と関連するとする報告があり，パーキンソン病も同様のことがないという保証はないと思われる．

Foroud らの研究のようにある程度の人数を行っても，さほど有意なものは同定できていない．それぞれの SNP が影響力が小さすぎるのか，検体が階層化していて均一でないことなどが理由として考えられる．同様の問題はアルツハイマー病，統合失調症[16]でも生じており（統合失調症など1万人規模の患者でも $P=10^{-7}$ 程度である），これらの精神神経疾患には，SNP 以外にある程度の割合で，multiple rare variant，CNV が関連している可能性も考えられる．

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病因・病態の解明

孤発性パーキンソン病のリスク遺伝子

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要　旨

患者の 95% を占める孤発性パーキンソン病は多因子疾患である．孤発性パーキンソン病のリスク遺伝子を同定するため，ゲノムワイド関連解析を行い，パーキンソン病発症にかかわる2つの新しい遺伝子座 *PARK16*, *BST1* を同定した．また，常染色体優性遺伝性パーキンソン病の原因遺伝子 *SNCA*, *LRRK2* の孤発性パーキンソン病への関与を証明した．一方ゴーシェ病変異も，頻度は低いが発症への effect size が大きい rare variant として重要である．さらなる遺伝子の解明が期待される．

はじめに

パーキンソン病症例の 95% は孤発性発症であるが，5% は家族性（その一部はメンデル遺伝性）に発症する．家系の連鎖解析などから6つのメンデル遺伝性パーキンソン病原因遺伝子が明らかにされ，ミトコンドリア障害，酸化ストレス障害の病態への関与に加え，新たにユビキチン-プロテアソーム系の機能低下，つまりタンパク質分解異常からドパミン細胞死に至る経路の重要性が示された．

一方で，ヒトゲノム解析の進展を受けて，生活習慣病を含めた多因子疾患の疾患感受性遺伝子の探索が実現可能となった．パーキンソン病においては，患者の大多数を占める孤発性パーキンソン病は多因子疾患であると考えられ，孤発性パーキンソン病の疾患感受性遺伝子の発見を目指した研究が行われている．メンデル遺伝性パーキンソン病原因遺伝子については他稿で詳述されているので，本稿では遺伝性よりむしろ孤発性パーキンソン病の疾患感受性遺伝子に目を向けて概説する．

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多因子遺伝性疾患としての孤発性パーキンソン病

症例的には大多数（95%）の孤発性パーキンソン病の原因は現時点では不明であるが，環境因子と1つ1つは影響力の弱い遺伝因子（おそらく数十個）によってなり，その総和がある閾値を超えたときに発症するという多因子疾患であると考えられている．アイスランド国民を対象とした大規模な疫学的調査の結果が発表され，同胞再発危険率は 6.7 で，パーキンソン病発症には遺伝因子が影響していることが示された[1]．

パーキンソン病の感受性遺伝子を発見するため，ここ 10 年間，多くの研究がなされて