TABLE 1. *Clinical features of three patients (A-1, A-2, and B-1) with ALS/PDC of the Kii peninsula*

	A-1	A-2	B-1
Suspected mode of inheritance	AD	AD	AD?
Age at onset (yr)	70	52	70
Duration of the illness (yr)	7	8	6
Sex	M	F	F
Clinical presentation	ALS with Dementia	PDC with ALS	PDC
Dementia	+	+	+
Psychosis	−	−	−
Resting tremor	−	−	+
Bradykinesia	−	+	+
Rigidity	−	+	+
Gait disturbance	+	+	+
Asymmetric sign at onset	−	+	+
Clinical response to levodopa	NA	+	−
Hoehn-Yahr stage (best on stage)	0	4.5	5
Hyperreflexia	−	+	+
Babinski's sign	−	+	+
Bulbar palsy	+	+	+
Respiratory failure	+	−	−
Amyotrophy	+	+	−
Fasciculation	+	+	−
Sensory disturbance	−	−	−
Orthostatic hypotension	−	−	−
Incontinence	−	+	+
Urinary urgency	−	−	−

ALS/PDC, amyotrophic lateral sclerosis/parkinsonism-dementia complex; AD, autosomal dominant; F, female; M, male; NA, not available; +, present; −, absent.

tex and brainstem, loss of anterior horn cells of the spinal cord, together with degeneration of pyramidal tract, and loss of Betz cells in the motor cortex. NFTs with neuronal loss were prominent in the medial temporal lobe without senile plaques.[4,17,18] Blood samples for genetic analysis and clinical information were collected after obtaining informed consent from the participants.

Genetic Analysis

Genomic DNA samples were isolated from peripheral blood using standard protocols. They were amplified by polymerase chain reaction (PCR) for each exon and sequenced for all exons and splice junctions of 19 genes (*SOD2*,[19] *SOD3*,[20] *ALS2/alsin*,[21,22] *SMN1*,[23] *PGRN*,[24,25] *ANG*,[26] *VEGF*,[27] *VCP*,[28] *VAPB*,[29] *DCTN1*,[30] *CHMP2B*,[31] *TDP-43*,[32] *GSK3β*,[33] *alpha-synuclein*,[34] *LRRK2*,[35] *parkin*,[36] *DJ-1*,[37] *PINK1*,[38] and *ATP13A2*[39]) using BigDye Terminator v1.1 Cycle Sequencing kit and 310 and 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA). Gene dosage analyses of exons 1, 9, 10, 13 of *MAPT*, exon 3 of *alpha-synuclein*, exon 3 of *TDP-43*, exon 5 of *GSK3β*, and all exons of *parkin* were performed by real-time PCR using TaqMan probes and ABI PRISM 7700 Sequence Detector (Applied Biosystems). We used β-actin or β-globin as an internal standard for each real-time PCR. We used the primers and probes prepared by "Custom TaqMan Genomic Assays" (Applied Biosystems). Sequences of the primers and probes, and conditions of PCR, sequencing, and real-time PCR are available upon request to the corresponding author or the first author.

RESULTS

Genetic Studies

Direct sequencing of all exons and splice junctions of the 19 genes (*SOD2*, *SOD3*, *ALS2/alsin*, *SMN1*, *PGRN*, *ANG*, *VEGF*, *VCP*, *VAPB*, *DCTN1*, *CHMP2B*, *TDP-43*, *GSK3β*, *alpha-synuclein*, *LRRK2*, *parkin*, *DJ-1*, *PINK1*, and *ATP13A2*) revealed no mutations that were shared by all three patients. A homozygous non-synonymous SNP (*ALS2/alsin* V368M: rs3219156) was detected in all three patients. This SNP showed a high allele frequency in the dbSNP database of normal Asian population (http://www.ncbi.nlm.nih.gov/SNP/) and all our 100 controls of healthy Japanese population had homozygous V368M. Gene dosage was normal in exons 1, 9, 10, 13 of MAPT, exon 3 of *alpha-synuclein*, exon 3 of *TDP-43*, exon 5 of *GSK3β*, and all exons of *parkin*.

DISCUSSION

In families with Kii ALS/PDC, many affected members in more than two generations have been described, with age at onset of 57-63 (mean 60.0) years for Kii ALS and 53-74 (mean 66.5) years for Kii PDC.[1] Anticipation has not been observed. Some unaffected siblings of parents with ALS/PDC were identified.[4] No marked gender differences in prevalence have been seen. These patterns suggest autosomal dominant inheritance with low penetrance rather than autosomal recessive one. Considering this genetic background and the clinicopathological features, previous studies on *MAPT* and *SOD1* mutations as well as *APOE* polymorphism of Alzheimer's disease (AD), CYP2D6B of Parkinson's disease (PD) and polymorphic dinucleotide repeats in *MAPT* intron of progressive supranuclear palsy were reported to be negative.[1,4] In this study, for further clarification of genetic factors, we extended candidate gene analyses for variants in coding regions

and exon–intron boundaries and gene dosages of neurodegenerative disease-related genes.

We selected 19 genes (*SOD2*, *SOD3*, *ALS2/alsin*, *SMN1*, *PGRN*, *ANG*, *VEGF*, *VCP*, *VAPB*, *DCTN1*, *CHMP2B*, *TDP-43*, *GSK3β*, *alpha-synuclein*, *LRRK2*, *parkin*, *DJ-1*, *PINK1*, and *ATP13A2*) as candidate genes for Kii ALS/PDC in this study. Among these ALS-, FTLD-, tauopathy-, and synucleinopathy-related genes, some of the genes might be linked in a common pathway leading to neurodegeneration. With regard to gene–gene and protein–protein interactions, there are many interactions between *GSK3β* and key components related to neuropathology of AD such as tau. *GSK3β* phosphorylates tau in the fly model and could modulate τ-induced neurodegeneration or at least widespread NFT formation.[40] Moreover, phosphorylation of human tau following overexpression of the *Drosophila* GSK3 homolog Shaggy resulted in the formation of neurofibrillary pathology, including paired helical filaments.[40] In addition, *LRRK2* was reported to link tauopathy and synucleinopathy.[35]

We also analyzed gene dosage abnormalities in exons 1, 9, 10, 13 of *MAPT*, exon 3 of *alpha-synuclein*, exon 3 of *TDP-43*, exon 5 of *GSK3β*, and all exons of *parkin*. Recently, further evidence for causative rearrangements has been presented in synucleinopathy and tauopathy. Indeed, multiplications of *alpha-synuclein* have been reported in PD/diffuse Lewy body disease with gene dosage effect. On the other hand, mouse models that expressed the shortest isoform of tau in a wild-type background acquired age-dependent pathology that was similar to FTDP-17 and Guam ALS/PDC: presence of insoluble, hyperphosphorylated tau and argyrophilic intraneuronal inclusions formed by tau immunoreactive filaments.[41] These were the first transgenic mice to recapitulate key features of human tauopathies associated with motor weakness observed in ALS/PDC.[41]

In this study, we showed the absence of causative mutation in all the major 19 related genes examined by direct sequencing and no gene dosage abnormalities in exons 1, 9, 10, 13 of *MAPT*, exon 3 of *alpha-synuclein*, exon 3 of *TDP-43*, exon 5 of *GSK3β*, and all exons of *parkin*. To clarify the regulatory system for abundant tau deposits, approaches to promoters or intronic regions, other genetic factors that could not be detected by direct sequencing, gene rearrangements, epigenetics, or gene–gene interaction, might also be needed. A recent study reported the deposition of TDP-43 in the Guam PDC[42] and Kii ALS/PDC (unpublished data by Kuzuhara et al.). Deposition of TDP-43 in addition to tau might be a major feature in ALS/PDC. More recently, mutations of *TDP-43* were identified to cause familial non-*SOD1* ALS and sporadic ALS.[43] Thus, further studies are needed to identify the roles of TDP-43, phosphorylated TDP-43, tau, and phosphorylated tau in ALS/PDC. Although we could not detect multiplication of *MAPT*, *GSK3β*, and *TDP-43*, overexpression of tau or other genes remains an important issue.

In this study, a homozygous nonsynonymous SNP (*ALS2/alsin* V368M: rs3219156) shared in all three patients is not likely to be a harmful polymorphism because of the high allele frequency in dbSNP database of normal Asian population and in our controls of healthy Japanese population. However, because the phenotype of ALS and PDC is heterogeneous even in the same family, some common genetic factors such as SNPs that are sensitive to certain agents in the early stages of life might be also underlying mechanisms solely or in combination. On the other hand, because rare diseases such as ALS/PDC might be caused by certain rare variants, further association studies including healthy controls in the Kii peninsula would be needed then. Furthermore, although the ethnic background is different, it is intriguing to investigate whether some shared SNPs among patients with the western Pacific ALS/PDC exist or not. Thus, these issues should be investigated thoroughly in western Pacific ALS/PDC.

To date, the etiology of ALS/PDC of the Kii peninsula and Guam remains unclear, and we could not identify any causative mutations of the known genes related to ALS/FTLD, parkinsonism and dementia in our Kii ALS/PDC patients. However, aggregation of the disease in some families and absence of any confirmed environment factors suggest the involvement of other genetic factors in the pathogenesis of ALS/PDC.

Acknowledgments: We thank the patients and their family members who participated in this study. We also thank Dr. S. Tsuji (the University of Tokyo) and Drs. R. Kuwano and K. Hara (Niigata University) for their collaboration on gene analysis; and Dr. K. Nishioka and Ms. H. Yoshino (Juntendo University School of Medicine) and Ms. H. Akatsuka (Mie University School of Medicine) for the excellent technical assistance.

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Parkinsonism and Related Disorders 15 (2009) 105–109

Parkinsonism & Related Disorders

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

George D. Mellick [a,*], Gerhard A. Siebert [a], Manabu Funayama [b], Daniel D. Buchanan [c], Yuanzhe Li [d], Yoko Imamichi [d], Hiroyo Yoshino [b], Peter A. Silburn [a,c], Nobutaka Hattori [b,d]

[a] *Eskitis Institute for Cellular and Molecular Therapies, Griffith University, Nathan, Qld 4111, Australia*
[b] *Research Institute for Diseases of Old Ages, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan*
[c] *School of Medicine, University of Queensland, Woolloongabba, Qld 4102 Australia*
[d] *Department of Neurology, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan*

Received 12 September 2007; received in revised form 8 November 2007; accepted 19 November 2007

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.

* Corresponding author. Eskitis Institute for Cellular and Molecular Therapies, Griffith University, 170 Kessels Road, Nathan Campus, Brisbane, Qld 4111, Australia. Tel.: +61 7 3735 5019.
E-mail address: G.Mellick@griffith.edu.au (G.D. Mellick).

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doi:10.1016/j.parkreldis.2007.11.016

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	GTTCAACTCCAGCCAATGGTTTC	Fam-CCTTCCCTGCGAAAATCACACCGCAT-Tamra
Parkin Ex3	GAGGACTGAGCTGCTGAGG	AGAGCATTGTTCACATTGTGC	Fam-TCGTCGCCTCCAGTTGCATTCAT-Tamra
Parkin Ex4	TCTTCTCCAGCAGGTAGATCAATC	TGCTGACACTGCATTTCCTTAC	Fam-ATGTGTATTGCAAAGGCCCTGTCAAT-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	ATTCTCTTTCCAACAGCTGGC	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

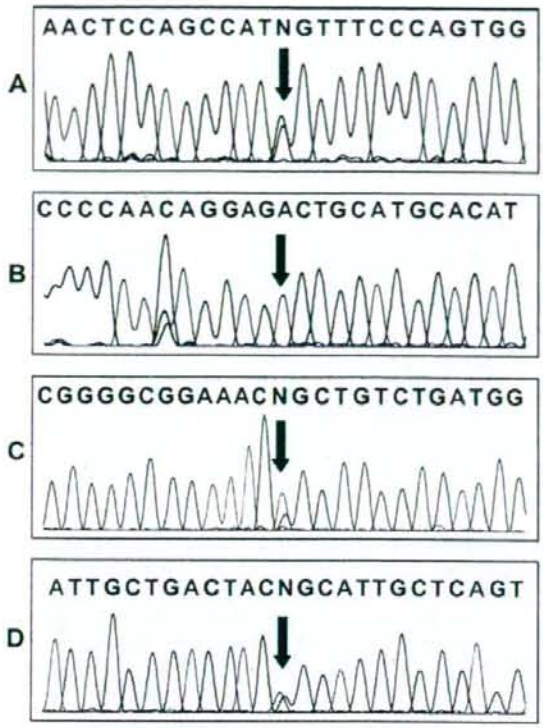

Fig. 1. Electropherogram of parts of the *PRKN* (A, B), *PINK1* (C) and *LRRK2* sequence, showing mutated sites, indicated by arrows. A. Heterozygous *PRKN* mutation p.G12R (c.34G > C, exon 2); B. Homozygous *PRKN* mutation p.G430D (c.1289G > A, exon 12); C. Heterozygous *PARK6* mutation p.G411S (c.1231G > 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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Parkinsonism and Related Disorders 15 (2009) 160–161

Parkinsonism & Related Disorders

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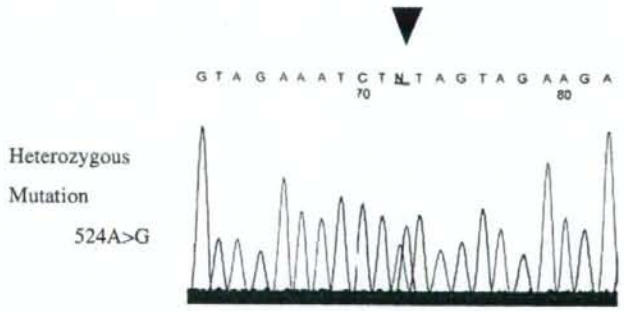
Letter to the Editor

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.

1353-8020/$ - see front matter
doi:10.1016/j.parkreldis.2007.12.010

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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Kazunari Momma
Department of Internal Medicine, Division of Neurology, National Defense Medical College, Saitama, Japan

Manabu Funayama
Research Institute for Diseases of Old Ages, Juntendo University School of Medicine, Tokyo, Japan

Yuanzhe Li
Department of Neurology, Juntendo University School of Medicine, Tokyo, Japan

Hiroshi Ichinose
Department of Bioscience and Biotechnology, Tokyo Institute of Technology, Tokyo, Japan

Kazuo Motoyoshi
Department of Internal Medicine, Division of Neurology, National Defense Medical College, Saitama, Japan

Nobutaka Hattori
Research Institute for Diseases of Old Ages, Juntendo University School of Medicine, Tokyo, Japan
Department of Neurology, Juntendo University School of Medicine, Tokyo, Japan

Yoshikuni Mizuno
Department of Neurology, Juntendo University School of Medicine, Tokyo, Japan

Keiko Kamakura*
Department of Internal Medicine, National Defense Medical College, Namiki 3-2, Tokorozawa, Saitama 359-8513, Japan.
*Corresponding author. Tel.: +81 4 2995 1617; fax: +81 4 2996 5202.
E-mail address: keiko-kamakura@umin.ac.jp

30 November 2007

Genomic Investigation of α-Synuclein Multiplication and Parkinsonism

Owen A. Ross, PhD,[1] Adam T. Braithwaite, BSc,[1] Lisa M. Skipper, PhD,[1] Jennifer Kachergus, BSc, MBA,[1] Mary M. Hulihan, MPH,[1] Frank A. Middleton, PhD,[2] Kenya Nishioka, MD,[3] Julia Fuchs, MD,[4] Thomas Gasser, MD,[4] Demetrius M. Maraganore, MD,[5] Charles H. Adler, MD,[6] Lydie Larvor, PhD,[7] Marie-Christine Chartier-Harlin, MD,[7] Christer Nilsson, MD,[8] J. William Langston, MD,[9] Katrina Gwinn, MD,[10] Nobutaka Hattori, MD,[3] and Matthew J. Farrer, PhD[1]

Objective: Copy number variation is a common polymorphic phenomenon within the human genome. Although the majority of these events are non-deleterious they can also be highly pathogenic. Herein we characterize five families with parkinsonism that have been identified to harbor multiplication of the chromosomal 4q21 locus containing the α-*synuclein* gene *(SNCA).*

Methods: A methodological approach using fluorescent in situ hybridization and Affymetrix (Santa Clara, CA) 250K SNP microarrays was used to characterize the multiplication in each family and to identify the genes encoded within the region. The telomeric and centromeric breakpoints of each family were further narrowed using semiquantitative polymerase chain reaction with microsatellite markers and then screened for transposable repeat elements.

Results: The severity of clinical presentation is correlated with *SNCA* dosage and does not appear to be overtly affected by the presence of other genes in the multiplicated region. With the exception of the Lister kindred, in each family the multiplication event appears de novo. The type and position of Alu/LINE repeats are also different at each breakpoint. Microsatellite analysis demonstrates two genomic mechanisms are responsible for chromosome 4q21 multiplications, including both *SNCA* duplication and recombination.

Interpretation: *SNCA* dosage is responsible for parkinsonism, autonomic dysfunction, and dementia observed within each family. We hypothesize dysregulated expression of wild-type α-synuclein results in parkinsonism and may explain the recent association of common *SNCA* variants in sporadic Parkinson's disease. *SNCA* genomic duplication results from intraallelic (segmental duplication) or interallelic recombination with unequal crossing over, whereas both mechanisms appear to be required for genomic *SNCA* triplication.

Ann Neurol 2008;63:743–750

The human genome displays a considerable level of interindividual variability from simple single nucleotide polymorphisms (SNPs) and short repeats to large-scale deletions, multiplications, and rearrangements. Recent studies have demonstrated that large gene copy number variations occur frequently in the general population with no determinable disadvantage to carriers. However, this phenomenon can also be pathogenic and result in severe disease phenotypes.[1–3]

In 2003, Singleton and colleagues[4] reported a triplication on one allele of the chromosomal locus (4q21) containing the α-*synuclein* gene *(SNCA)* in affected members of a family with parkinsonism known as the Iowan kindred. Although a relatively rare event, several families have since been described who carry multiplications of this region including both triplications and duplications that segregate with disease.[5–11] The severity of the clinical phenotype of *SNCA* duplication and

From the [1]Division of Neurogenetics, Department of Neuroscience, College of Medicine, Mayo Clinic, Jacksonville, FL; [2]Center for Neuropsychiatric Genetics, Microarray Core Facility, State University of New York Upstate Medical University, Syracuse, NY; [3]Department of Neurology, Juntendo University School of Medicine, Bunkyo, Tokyo, Japan; [4]Department of Neurodegenerative Diseases, Center for Neurology, Hertie-Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany; [5]Department of Neurology, Mayo Clinic College of Medicine, Rochester, MN; [6]Department of Neurology, Mayo Clinic College of Medicine, Scottsdale, AZ; [7]EA2683 ministère de l' éducation nationale de la recherche et de la technologie (MENRT), Diagnostic and Physiopathology of Parkinson's Disease, Institut de Recherches sur le Cancer de Lille (IRCL), Lille Cedex, France; [8]Department of Clinical Medicine, Division of Geriatric Psychiatry, Lund University, Lund, Sweden; [9]Parkinson's Institute and Clinical Center, Sunnyvale, CA; and [10]Neurogenetics, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD.

Received Nov 8, 2007, and in revised form Jan 29, 2008. Accepted for publication Feb 11, 2008.

This article includes supplementary materials available via the Internet at http://www.interscience.wiley.com/jpages/0364-5134/suppmat

Published online June 10, 2008, in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ana.21380

Address correspondence to Dr Farrer, Morris K. Udall Parkinson's Disease Research Center of Excellence, Mayo Clinic, Department of Neuroscience, 4500 San Pablo Road South, Jacksonville, FL 32224. E-mail: farrer.matthew@mayo.edu

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triplication families appears to be associated with gene dosage and messenger RNA (mRNA)/protein expression levels in brain.[6] The *SNCA* duplication families are generally reminiscent of typical, late-onset Parkinson's disease (PD),[5,7,9] whereas the two families (Iowan and Swedish-American) with monoallelic triplication of *SNCA* present with a severe form of early-onset parkinsonism with autonomic dysfunction and subsequent dementia.[6,12]

In the Iowan kindred, the region triplicated is reported to contain 17 gene transcripts (1.6–2.1Mb), whereas in both French and Japanese patients, much smaller genomic intervals are duplicated (approximately 0.5Mb).[4,5,7,9] Although *SNCA* multiplication appears necessary for parkinsonism, whether increased dosage of adjacent genes contributes to the phenotype is unclear. The mechanism underlying chromosome 4q21 genomic multiplication also has to be elucidated. The region appears to be evolutionarily fragile given the spontaneous deletion of *SNCA* within an inbred strain of C57BL/6J (OlaHsd) mice, albeit with no apparent deleterious effects.[13,14]

Herein we compare the phenotypes of *SNCA* multiplication families and present data on the genomic copy number, size, and breakpoints for each 4q21 multiplication mutation, using a combination of fluorescent in situ hybridization (FISH) and Affymetrix 250k SNP microarrays (CHIPS). Within each interval/family, we detail the genes with aberrant copy number and expression. We characterize the transposable repeat elements at each breakpoint and provide a mechanistic hypothesis for the genomic instability, rearrangement, and multiplication of this locus.

Subjects and Methods

Frequency, Clinical Manifestations, and Neuropathology

The frequency of *SNCA* multiplication is low and appears to be a relatively rare event.[15] Worldwide, seven families have been identified who harbor *SNCA* multiplication: one triplication (Iowa-US),[4,6,12] five duplication kindreds (two French, two Japanese, and one Italian),[5,7,9,11] and one kindred with individuals with either duplication or triplication mutations (Swedish-United States, now recognized as a branch of the "Lister family complex").[6,11] The clinical presentation and available pathological findings for five of these families are summarized in the Table. During the preparation of this article, Ahn and colleagues[16] reported the first *SNCA* duplication patients in Korea. Intriguingly, of the three PD patients identified, only one is described with a family history of parkinsonism. This familial *SNCA* duplication patient presented with symptoms at age 40 years and initially had a good response to L-dopa therapy; however, the disease course progressed rapidly with postural hypotension, personality changes, and dementia by the age of 46 years. The two sporadic patients presented with typical PD with ages at onset of 50 and 65 years. These alternate clinical presentations demonstrate the phenotypic range of *SNCA* multiplication symptoms.

The clinical phenotype in the *SNCA* triplication families is rapid, progressive parkinsonism with onset in the third and fourth decades of life. Movement disorder (resting tremor, bradykinesia, and rigidity) occurs early in the course with autonomic dysfunction (including hyposmia and orthostatic hypotension) and neuropsychological impairments (hallucinations, anxiety, paranoia, and depression), with subsequent cognitive decline and dementia. The neuropathology of *SNCA* triplication patients is reminiscent of diffuse Lewy body disease with numerous α-synuclein–positive Lewy bodies, Lewy neurites, and glial cytoplasmic inclusions, with neuronal cell loss in the *substantia nigra* and *locus ceruleus*. Extensive neuronal loss is also observed in the hippocampus CA2/3 region and is a feature of both missense and multiplication *SNCA* mutations.[6,17]

In contrast, most patients in *SNCA* duplication families present with signs and symptoms that closely resemble idiopathic PD. Onset of motor symptoms is in the fifth to sixth decades of life, neither cognitive decline nor dementia is prominent, and generally disease progression is slow with a sustained response to L-dopa therapy.[5,7,9] However, with each report, clinical variability within and among *SNCA* duplication families becomes more extensive. For example, Japanese A and B families are noted for reduced penetrance among carriers; patients may have a long duration of disease, may exhibit signs of cognitive decline and dementia, and have either a mild or excellent response to L-dopa therapy.[9] In contrast, affected carriers within Branch J of the Swedish Lister family initially present with dysautonomia (orthostatic hypotension and syncope) rather than motor problems but quickly develop rapidly progressive parkinsonism that is poorly responsive to L-dopa.[11] Neuropathological examination of *SNCA* duplication highlights α-synuclein pathology reminiscent of diffuse Lewy body disease comparable with that observed in *SNCA* triplication patients.[18] It is evident that disease onset in *SNCA* duplication carriers is several decades later than in *SNCA* triplication families.[11] Although *SNCA* dosage appears responsible and sufficient for disease, clinical variability may reflect the size of the duplicated segment and the aberrant expression of the additional genes.[9,11]

Genetic Analysis: Fluorescent In Situ Hybridization and CHIPS

FISH was performed on Epstein–Barr virus–immortalized lymphocytes from one affected member of each family, as described previously, with *SNCA* PAC 27M07 (146 kb; AF163864) labeled using fluorescein isothiocyanate, and *SNCA* promoter and intron four fragments (13 and 21kb) labeled with rhodamine.[4] Samples were considered duplicated/triplicated if they had 3/4 FISH probe signals in greater than 20% of interphase cells scored, from 100 interphase nuclei examined. To exclude the possibility of an artifact of Epstein–Barr virus immortalization, we performed semiquantitative polymerase chain reaction on genomic DNA extracted from blood and confirmed a multiplication of the region of chromosome 4 containing *SNCA* in all families. Affymetrix 250k SNP microarrays (CHIPS) genotyping and SNP dosage analysis was then performed on 250ng total

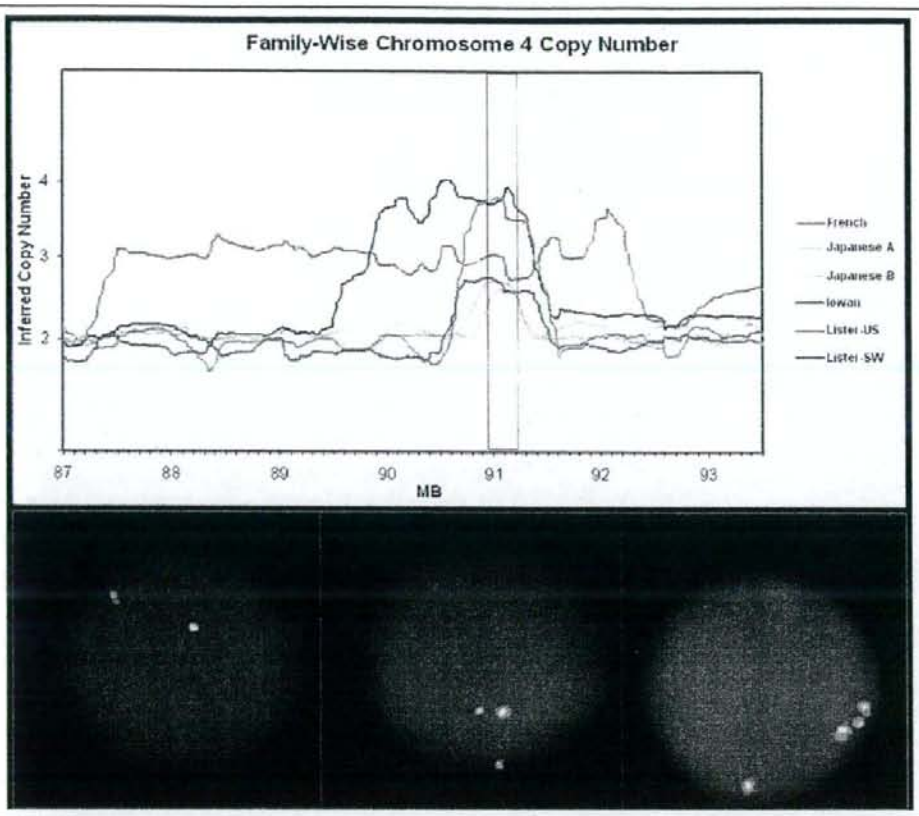

Fig 1. A representation of fluorescent in situ hybridization (FISH) and Affymetrix 250k SNP microarrays (CHIPS) that were used to examine the region of multiplication in the proband of each family. (A) Relative copy number estimates were plotted against physical genomic position on chromosome 4. Raw data are shown that have not been normalized with respect to integers. (B) FISH was performed on interphase cells with three labeled SNCA *probes directed at the entire locus (PAC 27M07 in green), with promoter and intron 4 fragments (visualized in red).* SNCA *multiplication was confirmed in all samples using both methodologies. SW = Swedish-American.*

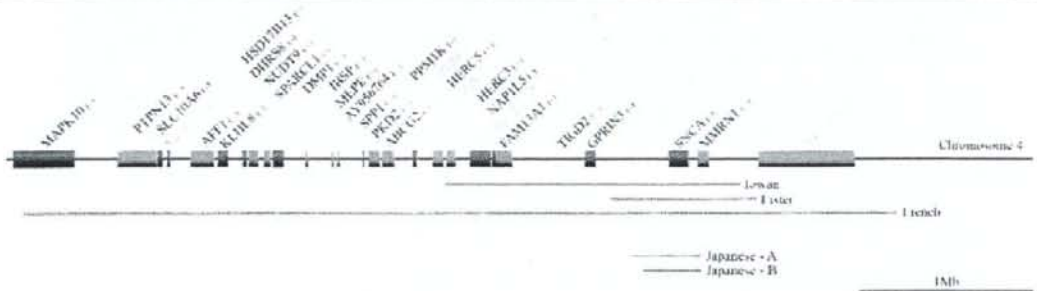

Fig 2. Representation of the genes in the multiplication region in each family. Displays the genes that are present in each of the multiplied regions of the families. Figure is drawn approximately to scale. The coding genomic DNA strand of each gene is indicated by (+) or (−). Genes are colored to represent their relative expression in brain according to the GNF Expression Atlas 2 (http://genome.ucsc.edu/), with red, black, and green representing high, medium, and low expression, respectively. Gene symbols in gray text *indicate hypothetical genes.* Gray bars *below the chromosome diagram show the regions of multiplication in each family.*

Table. **Clinical and Neuropathological Characteristics of the *SNCA* Multiplication Families**

Kindred	Iowan	Lister-US	Lister-Swedish	French	Japanese A	Japanese B
SNCA Multiplication	Triplication	Triplication	Duplication	Duplication	Duplication	Duplication
Number of patients with clinical data	12	3	5	5	3	1
Average age at onset (yr)	34 (20–48)	(31 to early 40s)	59 (40–71)	48 (35–65)	43 (38–77)	47
Rigidity	Yes	Severe, generalized	Yes	Yes	Yes	Yes
Bradykinesia	Yes	Yes	Yes	Mild to severe	Yes	Yes
Rest tremor	Some subjects: none to pronounced	Yes	Some subjects: mild to intermittent	Some subjects: none to pronounced	No	No
Postural tremor	Yes, not segregating with triplication	Yes	No	3/5	No	No
Postural instability	Yes, with falls	Mild to moderate	Pronounced, with falls	3/5	2/3	Yes
Myoclonus	Late	NA	Late, in distal upper extremities	NA	NA	NA
Response to L-dopa	Yes	Dramatic effect initially	Slight	Yes	None to good	Slight
Orthostatic hypotension	Yes, some subjects, sometimes requiring drug treatment	Moderate to severe, early in illness, partially requiring drug treatment	Early, symptomatic, required drug treatment	No	No	No
Other dysautonomia	Erectile, cardiac, and gastrointestinal dysfunctions	Urinary incontinence late in illness	Moderate urinary incontinence, dysphagia	No	No	No
Dementia/cognitive dysfunction	Memory loss, visuospatial dysfunction, decline of executive functions: may present with these features (LBD phenotype) or may be late in course (PD phenotype)	Early, severe	Not prominent (late)	Not prominent	No	Yes
Paranoia, anxiety	Not prominent	Early, pronounced	Yes	NA	NA	NA
Depression	Yes, may precede parkinsonism by a decade or more	History of depression between age 13 and 19, suicidal later in illness	Yes	2/5	Yes	NA

Table. Continued

Kindred	Iowan	Lister-US	Lister-Swedish	French	Japanese A	Japanese B
Hallucinations	Partial: some have prominent visual when phenotype is LBD	Pronounced, visual, auditory and olfactory	Visual, olfactory and auditory	No	No	Yes
Other remarks	Weight loss may be seen; rapidly progressive	NA	Rapidly progressive disease	NA	Psychosis in 1 of 2	NA
Neuropathology	Neuronal loss in SN and LC, extensive, pleomorphic and atypical LBs, GCIs, neuritic dystrophy, neuronal loss in HC (CA 2/3)	Neuronal loss in SN and LC (few LBs), NBM, CTX (widespread LBs), and HC (CA2/3)	NA	NA	NA	Neuronal loss in SN, LC, and HC (CA 2/3); Lewy neurites in the CA2; only a few LBs in SN and LC

NA = no data available; LBD = Lewy body disease; PD = Parkinson's disease; LB = Lewy body; SN = substantia nigra; LC = locus ceruleus; GCI = glial cytoplasmic inclusion; HC = hippocampus; NBM = nucleus basalis of Meynert; CTX = cortex.

genomic DNA samples for the probands of each family as described previously.[11] Copy number was estimated using dChipSNP software with GTYPE exported genotype calls and signal intensities (.cel files).[19–21] This algorithm uses a rigorous "within and between array" normalization method to compute estimates of the normal signal values for genotype calls observed with a set of arrays. Deviations from the normal signal values seen for any particular genotype in the set of abnormal DNA samples were compared with values observed for a set of 10 samples with normal 2N copy numbers throughout chromosome 4. Copy number changes in the probands and families were then inferred by median smoothing with a Hidden Markov Model applied. In our study, we present results based on a sliding window approach to average the inferred copy numbers across a continuous 250kb stretch centered on each SNP, and for simplicity, only a single proband is shown for each nuclear family.

The centromeric and telomeric ends of the breakpoints were confirmed and further refined using polymorphic microsatellite markers. Internal control peak height of heterozygous individuals were calculated and compared among patient samples, diploid, *SNCA* duplication, and triplication samples to give copy numbers. These analyses confirmed the Affymetrix 250K SNP microarray results showing a different length of the multiplicated region in each family (see Supplementary Table 1).

Results

In each proband, all three *SNCA* FISH probes gave results that were consistent with Affymetrix dosage and microsatellite genotype analyses. Affymetrix CHIP dosage data were obtained from between 62 and 363 SNPs, within the chromosome 4q21 region of multiplication in each family. Illustrative results are shown for both *SNCA* duplication and triplication cell lines (FISH) and the proband of each family (CHIPS) (Fig 1). The longest region (4.93–4.97Mb) is present in the French duplication family (also reported as FPD-131) and encompasses 31 transcripts, including genes associated with epileptic encephalopathy *(MAPK10),* type II dentinogenesis imperfecta (*DMP1* and *SPP1*), and polycystic kidney disease II *(PKD2)* (http://www.ncbi.nlm.nih.gov) (Fig 2). Five transcripts including *SNCA* are expressed at high levels in the brain (microarray expression data retrieved from UCSC Web site: http://genome.ucsc.edu/). In contrast, the shortest region (0.4Mb) was observed in the Japanese B family with duplication of only *SNCA* and the 5′ region of the *MMRN1* gene.

Microsatellite genotype analysis demonstrated *SNCA* genomic duplication results from intraallelic (segmental duplication) or interallelic recombination with unequal crossing over, whereas both mechanisms are required for genomic *SNCA* triplication (Fig 3). The reason for genomic instability and chromosome 4q21 rearrangement remains unclear. Thus, VISTA software[22] was used for comparison of the DNA sequences, short and long interspersed repeats (SINE/LINE) at the centromeric and telomeric ends of the multiplicated region in

each family. It is reported that Alu repeats constitute approximately 10% of the human genome,[23,24] and mobile elements make up more than 45% of the human genome.[23] Although our analysis identified a number of transposable repeat elements with more than 70% conservation, the proportion of sequence occupied by SINE or LINE repeats in the breakpoint regions was not greater than observed within flanking sequence. Rather than one specific repeat, there was a variety of Alu subtypes at the 5′ and 3′ ends of the *SNCA* multiplication regions (see Supplementary Table 2). Nevertheless, the presence of these genetically mobile elements can lead to genomic instability, unequal recombination, and rearrangements that result in copy number variations including multiplication or deletion.[23,25]

Discussion

Multiplication of the *SNCA* locus is now reported to account for a greater number of families with autosomal dominant parkinsonism than the known pathogenic α-synuclein missense substitutions (A30P, E46K, and A53T).[26] However, this is still a small number of familial patients, given that approximately 10 to 15% of patients with PD report a family history of disease.

Only multiplication of *α-synuclein (SNCA)* appears necessary for parkinsonism because Japanese kindred B has only full-length *SNCA* and the 5′ end of *multimerin1 (MMRN1).* Deficiency of *MMRN1,* a specific platelet factor V/Va binding protein, is associated with an inherited bleeding disorder, factor V Quebec, although haploinsufficiency does not appear to be associated with any phenotype.[27] *MMRN1* is increased in copy number in all other *SNCA* multiplication kindreds. It may be noteworthy that *γ-synuclein (SNCG)* and *multimerin 2 (MMRN2)* lie in the same orientation to each other on human chromosome 10 (murine chromosome 14), suggesting that these paralogs arose because of an evolutionary duplication event. γ-Synuclein, also known as breast cancer-specific protein 1, is increased in cancer and may play a role in disease.[28,29] Given the instability of the *SNCA-MMRN1* region, *SNCG-MMRN2* multiplications/deletions may yet be identified.

Limited expression and functional data are available on other genes within regions of chromosome 4q21 multiplication. Nevertheless, only *SNCA* dosage appears to specifically contribute to the variability in clinical observations among families. Genetic and genealogical studies recently identified a Swedish family with *SNCA* duplication and a US family of Swedish descent with a *SNCA* triplication as branches of the "Lister kindred."[11,30,31] Within the families examined, this was the only example of copy number changes from one generation to another. Earlier onset, faster progression, and more fulminant disease are associated with increasing *SNCA* copy number, suggestive of "genetic anticipation," a clinical phenomenon usually confined to small simple repeats such as in spinocerebellar ataxias.[32]

It is remarkable that both segmental intraallelic duplication and interallelic recombination with unequal crossing over appear to be responsible for *SNCA* multiplications. Microsatellite genotyping clearly demonstrates both mechanisms operate; duplication does not necessarily precede unequal crossing over and the opposite may occur. Although our study identified a number of large repeat elements at either ends of the multiplicated regions, no single repeat was consistently identified at the breakpoints of all multiplications (see Supplementary Table 2). Thus, a variety of transposable repeat elements including Alu and LINE repeats may promote instability causing irregular gene duplication and recombination events. Cloning the exact multiplication breakpoints across repeat elements may yet be insightful. Rovelet-Lecrux and colleagues[33] have reported similar multiplication events on chromosome 21 that involve the amyloid precursor protein gene and result in Alzheimer's disease. The regions duplicated in five of these families ranged from 0.58 to 6.37Mb and differed in their haplotypic structure, suggesting these multiplication events are also independent.

Ahn and colleagues[16] recently reported two sporadic patients with *SNCA* duplication suggestive of age-related penetrance, as observed for other mutations causing parkinsonism. The frequency and direct relevance of *SNCA* multiplication to most PD patients remains to be determined. The hypothesis that α-synuclein overexpression contributes to disease susceptibility predates the discovery of *SNCA* multiplications. A number of classic association studies have examined the Rep1 microsatellite (D4S3481) in the *SNCA* promoter, a region implicated in transcript expression by *in vitro* luciferase assays.[34–37] Combined, pooled analysis by the Genetic Epidemiology of Parkinson's Disease Consortium observed a significant association with increasing Rep1 allele size, 259<261<263 base pairs.[38] Online meta-analysis of all published studies also highlight a SNP (rs356165) in the 3′ untranslated mRNA (www.pdgene.org). In contrast, genome-wide SNP association studies of PD have not highlighted common variants in *SNCA,* suggesting their power to identify susceptibility genes is limited.[39,40] A reanalysis of genome-wide data highlighted copy number variants in *PARKIN.*[41] The identification of heterozygous carriers and one homozygous early-onset patient demonstrates the method can detect both multiplications and deletions.

In vivo findings with respect to *SNCA* mRNA expression are inconsistent.[42] We find a decrease in *SNCA* transcript levels in specific brain regions such as the surviving neurons of the *substantia nigra,* as well as the putamen and frontal cortex in subjects with PD

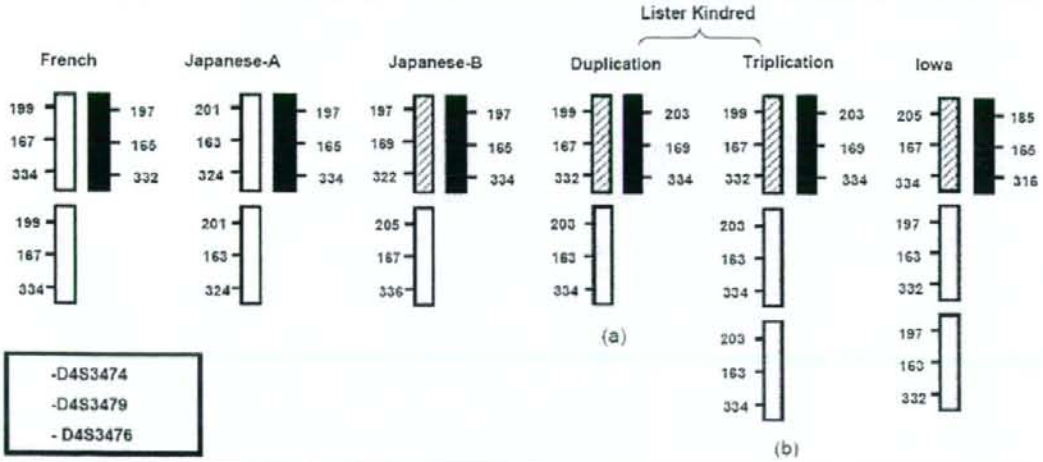

Fig 3. The allele sizes and dosage for the chromosome 4 markers D4S3474, D4S3479, and D4S3476 are shown for each family. These data demonstrate an intraallelic, segmental duplication in the French and Japanese A families. However, interallelic recombination occurred initially in Japanese B and the in Lister kindred duplication, indicated by the presence of three different alleles at marker D4S3479 (163, 167, and 169) (a). *A further segmental duplication is apparent in the Lister kindred branch with* SNCA *triplication* (b). *It is not possible to ascertain the sequence of events for the* SNCA *triplication in the Iowa kindred, but the presence of three allele sizes at all markers demonstrates a recombination event must have occurred.*

(unpublished data).[43] Changes in mRNA expression in end-stage disease may compensate for the accumulation of α-synuclein protein, but mRNA and protein expression levels have yet to be correlated within the same samples. Whether alternately spliced *SNCA* mRNA, predicted to lead to smaller isoforms (α-synuclein 98, 112, and 126), may also contribute to disease has yet to be determined.

The discovery of *SNCA* multiplication demonstrates aberrant α-synuclein expression is sufficient for parkinsonism and highlights a direct, dose response with age of onset, progression, and symptom severity. Whether *SNCA* multiplication is a distinct entity or a more aggressive form of typical PD, both are part of a spectrum of Lewy body disorders. The challenge is now to functionally translate genetic insights focused on *SNCA* into patient therapy.

This work is supported by the American Parkinson Disease Association (O.A.R.), Morris K. Udall Parkinson's Disease Research Center of Excellence (National Institute of Neurological Disorders and Stroke P50 #NS40256), the NIH (National Institute of Aging, P01 #AG17216), German National Genome Network (NGFN; 01GS0116), PHRC 2005/1914 the Universite de Lille 2 and Institut National de la Sante et de la Recherche Medicale, and the Draper Family Foundation. DNA and cell line samples and phenotypic data on the triplication subjects are available via the National Institute of Neurological Disorders and Stroke Repository (http://ccr.coriell.org/ninds; catalogue number ND00139), as are other affected and unaffected individual subject samples. The repository is supported by the NIH (National Institute of Neurological Disorders and Stroke contract #N01-NS-2-2349).

We acknowledge the Swedish Parkinson Foundation. We thank all those who have contributed to our research, including Drs A. Brice and S. Lesage for helpful discussion.

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Philos Trans R Soc Lond B Biol Sci. 2008 June 27; 363(1500): 2215–2227.
Published online 2008 April 15. doi: 10.1098/rstb.2008.2273.
PMCID: PMC2394571

Progress in the pathogenesis and genetics of Parkinson's disease

Yoshikuni Mizuno,* Nobutaka Hattori, Shin-ichiro Kubo, Shigeto Sato, Kenya Nishioka, Taku Hatano, Hiroyuki Tomiyama, Manabu Funayama, Yutaka Machida, and Hideki Mochizuki

Department of Neurology, Juntendo University School of Medicine, 2-1-1 Hongo Bunkyo, Tokyo 113, Japan

*Author for correspondence (Email: y_mizuno@med.juntendo.ac.jp)

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.

TOP
ABSTRACT
1. INTRODUCTION
▮ 2. CLINICAL FEATURES OF PD
3. NEUROPATHOLOGY OF PD
4. NEUROTOXIN-BASED MODELS OF PD
5. AETIOLOGY AND PATHOGENESIS OF PD

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