general population and the prevalence of the disease, it is likely that the frequency of use of amantadine will increase steadily in the future. Neurologists and physicians in general should pay attention to amantadine, when they encounter sudden visual deterioration in patients with PD.

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PARK9-LINKED PARKINSONISM IN EASTERN ASIA: MUTATION DETECTION IN *ATP13A2* AND CLINICAL PHENOTYPE

PARK9, a form of autosomal recessive parkinsonism, or Kufor-Rakeb syndrome (KRS), is characterized by subacute or slowly progressive, juvenile-onset, levodopa-responsive parkinsonism, pyramidal signs, dementia, and supranuclear gaze palsy.[1-5] Recently, *ATP13A2* was identified as the causative gene for *PARK9* in Chilean and Jordanian families.[4] This gene contains 29 exons encoding a lysosomal type 5 P-type ATPase. Six mutations have been reported in only five probands so far.[4,5] Here, we describe a Japanese patient with KRS with a novel mutation who developed early onset parkinsonism, dementia, and other features. We also describe PET findings of *PARK9*-linked parkinsonism.

Methods. Haplotype analysis was conducted in 117 (mainly Japanese) patients with early onset (≤50, 26.8 ± 11.7 years, mean ± SD) parkinsonism. Among them, 14 patients had dementia. Patients who exhibited homozygosity on *PARK9* locus by haplotype analysis underwent direct sequencing for all 29 exons (e-Methods on the *Neurology*® Web site at www.neurology.org); the remaining patients underwent direct sequencing for exons 13, 16, and 26, in which mutations have been identified.[4] The methods of direct sequencing, sequences of the primers, and PCR conditions are available (table e-1). The study was approved by the ethics committee of Juntendo University and all subjects gave informed consent.

Results. Twenty-eight of 117 patients exhibited homozygosity on *PARK9* locus. Among them, we found a Japanese proband (Family A) with a novel homozygous F182L (c.546C>A) mutation (figure e-1A). The consanguineous parents and the other two unaffected siblings had heterozygous F182L mutation. This mutation was not detected by direct sequencing of exon 6 in 300 chromosomes of normal controls.

Haplotype analysis showed homozygosity spanning the *PARK7* and *PARK9* regions (figure e-1B) in the proband and heterozygosity in her parents and the other two unaffected siblings. No causative mutation was detected in *DJ-1* and *PINK1* in all patients.

The clinical features of the proband, a 43-year-old woman, are described in the table, the e-Case report, the video, and figure e-2. Neuroimaging showed several interesting findings: MRI showed diffuse brain and spinal cord atrophy, and ^{18}F-dopa PET study revealed reduced uptake in the striatum bilaterally (figure e-2).

Discussion. The cardinal features and diffuse brain atrophy of the proband closely resembled previously reported ones.[1-5] Therefore, it was possible that this patient was given a diagnosis of KRS clinically. Genetically, phenylalanine-182 is highly conserved throughout most species (figure e-1C). It has been reported that missense mutations in the loop between the transmembrane segment of the membrane protein (including *ATP13A2*) could affect disease phenotype significantly.[5] These findings and absence of F182L in normal controls support that the homozygous F182L mutation causes KRS.

Our findings of *ATP13A2* mutation in a Japanese family together with the reported Jordanian, Chilean, Brazilian, and Italian cases suggest that *PARK9* exists worldwide though rearrangements could not be excluded.[1-5] The role of a single heterozygous mutation remains unclear, although two symptomatic Italians and two asymptomatic Brazilian and four asymptomatic Japanese carriers have been reported.[5]

The clinical symptoms of our patient were similar to those reported previously.[1-5] However, there were also some different findings. Our patient was comparatively older at onset (22 years), without subacute onset like the Brazilian with homozygous missense mutation,[5] a slower progression rate compared with the Jordanian family (time of progression to bed-ridden state = 20 years vs 12 months),[1,3,4] and no apparent motor fluctuation. Our patient also showed inconsistent levodopa responsiveness with severe drug-induced psychosis and amyotrophy. These differences might be due to the different mutation types (such as missense/truncation mutations) or the different mutation localization.

A new interesting aspect of our report is neuroimaging in KRS. Although peripheral neuropathy was not apparent (e-Case report), our patient had generalized brain and spinal cord atrophy on MRI, which might reflect pyramidal tract degeneration and also multisystemic neurodegeneration in KRS by *ATP13A2* mutation. The pyramidal symptoms and weakness of the lower limbs, described previously in patients with KRS,[1] also could be caused by spinal cord atrophy.

PET findings of patients with levodopa-responsive autosomal recessive parkinsonism with *parkin*, *PINK1*, or *GBA* single heterozygous mutation indicate presynaptic dopaminergic dysfunction

Supplemental data at www.neurology.org

Table Clinical features of patients with Kufor-Rakeb syndrome[1-5]

Origin of family	Japanese	Chilean				Jordanian				Brazilian	Italian	Italian
Patient	A	II-8	II-9	II-10	II-11	V44	V48	V49	V53	BR-3042	VE-29	PK-69-01
Zygosity	Homo	Comp hetero				Homo				Homo	Hetero	Hetero
Mutation	F182L	1019GfsX1021/1306+5G→A				552LfsX788				G504R	T12M	G533R
Age at onset, y	22	18	17	15	12	12	15	13	12	12	30	40
Disease duration, y	21	27	26	26	26	24	19	18	11	10	5	16
Initial symptoms	G	B, M	B, R	B, M	D	B, M, R	B, R	M, R	B, R	B	N/A	N/A
Clinical signs												
Increased muscle tone	+	+	+	+	+	+	−	+	+	+	+	+
Babinski sign	+	+	−	+	+	+	+	+	+	−	−	−
Palmomental reflex	+	+	−	+	+	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Tremor	+	+	+	+	+	−	−	−	−	−	+	−
Rigidity	+	+	+	+	+	+	−	+	+	+	+	+
Bradykinesia	+	+	+	+	+	+	+	+	+	+	+	+
Slowed saccade eye movement												
Vertical	+	N/A	−	+	+	+	+	+	+	N/A	N/A	N/A
Horizontal	−	N/A	−	+	+	+	+	−	−	N/A	N/A	N/A
Supranuclear upgaze palsy	+	+	−	+	+	+	+	+	+	+	−	−
FFF mini-myoclonus	+	+	−	+	+	+	−	+	+	−	N/A	N/A
Hallucination	+	+	+	−	−	+	+	+	+	+	−	+
Dementia (MMSE)	15/30	N/A	19/30	15/30	9/28	14/30	2/30	13/30	2/30	−	−	−
Response to anti-PD drugs												
Trihexyphenidyl	N/A	+	+	+	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Levodopa	+	N/A	No tolerance	No tolerance	N/A	+	+	+	+	+	+	+

Homo = homozygous; Comp hetero = compound heterozygous; Hetero = heterozygous; B = bradykinesia; M = mental retardation; R = rigidity; D = developmental disturbance; G = gait disturbance; FFF mini-myoclonus = facial-faucial-finger mini-myoclonus; MMSE = Mini-Mental State Examination; PD = Parkinson disease; − = absent; + = present; N/A = not assessed.

in striatonigral system, in contrast to postsynaptic dysfunction in multiple system atrophy and progressive supranuclear palsy without levodopa responsiveness.[6] ^{18}F-dopa PET scan of our patient with levodopa-responsive parkinsonism with homozygous *ATP13A2* mutation also showed a presynaptic pattern often observed in idiopathic PD.

Intriguingly, the *GBA* gene encoding lysosomal enzyme was reported to be associated with synucleinopathies such as Lewy body diseases. Since the lysosomal degradation pathway can clear α-synuclein aggregates,[7] lysosomal dysfunction by *ATP13A2* or *GBA* mutation could be important in the pathogenesis of parkinsonism.

Altogether, our findings expand the phenotypic spectrum associated with *PARK9*-linked parkinsonism into multiple-system disorders. Furthermore, functional analysis of *ATP13A2* could open a new therapeutic window in widespread neurodegenerative disorders.

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NEUROFERRITINOPATHY IN A JAPANESE FAMILY WITH A DUPLICATION IN THE FERRITIN LIGHT CHAIN GENE

Neuroferritinopathy is a rare autosomal dominant movement disorder with the deposition of iron and ferritin within the basal ganglia. Four different pathogenic mutations in the ferritin light polypeptide (FTL) gene have been reported.[1-4] The variety of its clinical features makes the diagnosis of neuroferritinopathy difficult. In this study we investigated a Japanese family with neuroferritinopathy to clarify the phenotypic and genetic spectrum of neuroferritinopathy.

Proband. A 42-year-old Japanese man first developed hand tremors in his middle teens. He noticed his right foot dragging at age 35, and generalized hypotonia, hyperextensibility, aphonia, micrographia, hyperreflexia, and cognitive impairment (IQ = 66) at age 42. His unsteady gait with long steps, with his arms and legs dangling, seemed to be due mainly to hypotonus. Rigidity, spasticity, dystonia, or chorea were not observed. His serum ferritin concentration was 5 μg/L (normal = 33 to 330). A brain MRI revealed bilateral symmetric cystic changes of the pallidum and the striatum. Hyperintense lesions in the T2-weighted imaging involved the thalamus, dentate nucleus, and substantia nigra.

The proband's mother had developed hand tremors at age 10. She presented with difficulty walking at age 35 and developed cognitive impairment and akinetic mutism, and died at age 64. Her CT imaging showed cystic changes of the pallidum and the striatum. None of the proband's relatives, except for his mother, had any neurologic symptoms.

Methods. After informed consent was obtained, genomic DNA was extracted from a blood sample of the proband and was amplified by PCR. The entire coding region of the FTL gene was sequenced using a BigDye Terminator Cycle Sequencing Kit according to the manufacturer's protocol. In order to confirm the mutation, the PCR-RFLP assay was developed with AciI. We have not performed genetic testing in any asymptomatic family member because informed consent was not obtained.

Results. In exon 4 of the *FTL* gene, duplication of the 469–484 sequence was found (figure, A). The mutation replaces the C-terminal 14 amino acid residues with a novel 23 amino acid sequence (figure, B). This mutation is described as c.469_484dup16nt (p.Leu162Arg*fs*X185) in standard genetic nomenclature. The mutation was not found in 20 control chromosomes and after BLASTN searching of International Nucleotide Sequence Database Collaboration (INSDC). The mutation creates the gain of an AciI restriction site, proven by PCR–restriction fragment length polymorphism analysis (figure, C).

Discussion. Neuroferritinopathy was first reported in 2001. The original mutation, an insertion of adenine in position 460–461 (460InsA), has been found mainly in cases of neuroferritinopathy of the north of England.[1] The insertion of a dinucleotide, thymine and cytosine, in position 498–499 was detected in a French family.[2] The insertion of a cytosine in position 646–647 was reported in a family of French Canadian and Dutch ancestry.[3] A missense mutation in position 474 of guanine to adenine was found in a family of Gypsy ancestry.[4] In this study, we found a novel mutation, the duplication of the 469–484 sequence of the *FTL* gene in a Japanese family. This is the first family with neuroferritinopathy of non-European origin. The deceased proband's mother was undoubtedly affected by neuroferritinopathy based on her clinical features and CT findings. All of her relatives, except for the proband, had no neurologic symptoms. Considering the high penetration of neuroferritinopathy,[5] we suspect that a new genetic mutation in the *FTL*

PARK9-LINKED PARKINSONISM IN EASTERN ASIA: MUTATION DETECTION IN ATP13A2 AND CLINICAL PHENOTYPE

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Analysis of Lrrk2 R1628P as a Risk Factor for Parkinson's Disease

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Common genetic variants that increase the risk for Parkinson's disease may differentiate patient subgroups and influence future individualized therapeutic strategies. Herein we show evidence for *leucine-rich repeat kinase 2 (LRRK2)* c.4883G>C (R1628P) as a risk factor in ethnic Chinese populations. A study of 1,986 individuals from 3 independent centers in Taiwan and Singapore demonstrates that Lrrk2 R1628P increases risk for Parkinson's disease (odds ratio, 1.84; 95% confidence interval, 1.20–2.83; $p = 0.006$). Haplotype analysis suggests an ancestral founder for carriers approximately 2,500 years ago. These findings support the importance of *LRRK2* variants in sporadic Parkinson's disease.

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The discovery of *leucine-rich repeat kinase 2 (LRRK2)* mutations in both familial and sporadic forms of Parkinson's disease (PD) has caused a paradigm shift in the field. Six Lrrk2 substitutions have been proven to play a role in PD pathogenesis or susceptibility, and are distributed throughout the different protein domains, suggesting that each domain is critical for normal physiological Lrrk2 function (Roc, C-terminal of ROC [COR], mitogen-activated protein kinase kinase kinase, and WD40).[1] However, the *LRRK2* gene harbors numerous other nonsynonymous variants (>70), and the functional role of these variants, whether they are benign single nucleotide polymorphisms (SNPs) pathogenic mutations, or risk factors for disease, remains unresolved.

The recently identified genetic risk factor Lrrk2 G2385R is observed in approximately 5% of the healthy Asian population, increasing to approximately 10% in populations with sporadic, late-onset PD.[2–8] Lrrk2 G2385R is located in the WD40 domain and is hypothesized to impair Lrrk2 dimerization/scaffold formation and to promote apoptosis.[3,6] Herein we provide evidence to support Lrrk2 R1628P (rs33949390), within the COR domain, as the second genetic risk factor for PD identified in the ethnic Chinese population.

Subjects and Methods

Subjects

A total of 1,079 ethnic Han Chinese patients (average age at onset, 62 years) from Taiwan and Singapore have been examined clinically and are being longitudinally observed by neurologists at 3 centers (R.-M.W., Y.-R.W., C.M.C., and E.-K.T.) (Table 1). Of the 1,079 patients, 44 reported a family history of disease (defined as 1 or more relatives with parkinsonism within 3 meiosis of relationship), 179 presented with early-onset PD (<50 years), and 900 patients had typical late-onset PD (≥50 years). All patients fulfilled criteria for a clinical diagnosis of PD with at least two of three cardinal signs (tremor, rigidity, and bradykinesia) and a positive response to L-dopa therapy.[9] A total of 907 ethnically matched Han Chinese control subjects (average age, 57 years) without evidence of neurological disorder were also recruited from participating centers. Population stratification is minimized because these study participants are all of Han Chinese descent. In addition, 151 PD patients and 95 control subjects from the Japanese population, diagnosed at Juntendo University (by M.F. or N.H.), were included in the study. Research protocols were reviewed by the institutional ethics board committee of each center, and all subjects gave informed consent.

Genetic Analysis

LRRK2 c.4883G>C (R1628P; rs33949390) was genotyped by restriction fragment length polymorphism (RFLP) or ABI Taqman (Applied Biosystems, Foster City, CA) "by-design" oligonucleotide probes and positives confirmed by direct DNA sequencing of exon 34, as described previously.[10]

Haplotype analysis was performed on 32 Lrrk2 R1628P carriers with chromosome 12q12 polymorphic markers amplified by polymerase chain reaction using fluorescently la-

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Table 1. **Allele and Genotype Frequencies of *LRRK2* c.4883G>C (R1628P; rs33949390)**

Series	Affection Status	Genotype GG (n)	Genotype GC (n)	Genotype CC (n)	Carrier Frequency	G Allele (n)	C Allele Frequency	Allelic *p*	OR (95% CI)
1. R.-M. Wu	Patients (n = 484)	452	31	1	6.6%	935	33 (3.4%)	0.025	2.15 (1.08–4.29)
	Control Subjects (n = 341)	330	11	0	3.2%	671	11 (1.6%)		
2. Y.-R. Wu	Patients (n = 345)	324	21	0	6.1%	669	21 (3.0%)	0.179	1.39 (0.70–2.75)
	Control Subjects (n = 316)	302	14	0	4.4%	618	14 (2.2%)		
3. E.-K. Tan	Patients (n = 250)	237	13	0	5.2%	487	13 (2.6%)	0.163	2.20 (0.83–5.83)
	Control Subjects (n = 250)	244	6	0	3.0%	494	6 (1.2%)		
Overall	Patients (n = 1,079)	1,013	65	1	6.1%	2091	67 (3.1%)	0.006	1.84 (1.20–2.83)
	Control Subjects (n = 907)	876	31	0	3.4%	1783	31 (1.7%)		

Displays the frequencies observed for the *leucine-rich repeat kinase 2 (LRRK2)* c.4883G>C (R1628P; rs33949390) variant in each of the three series. Series 1 and 2 are from Taiwan, an island of the east coast of China, and Series 3 uses subjects from Singapore, an island of the south coast of Malaysia. *p* values are calculated by χ^2 with Yates correction. Power calculations suggest that for replication studies in the ethnic Chinese population given a disease allele frequency in cases of 0.061 and odds ratio (OR) of 1.84, a sample size of 614 patients and an equal number of matched control subjects would be required to have 80% power to observe a statistically significant difference ($p < 0.05$).

beled primers (sequences are available on request). DNA products were run on an ABI3730 and analyzed using GeneMapper software (Applied Biosystems, Foster City, CA) alongside standard controls (CEPH 1331-01 and -02). Physical map positions are given with reference to the March 2006 human reference sequence (National Center for Biotechnology Information Build 36.1). Using marker allele frequencies in the putative, mutation-bearing ancestral haplotype in comparison with the noncarrier population, we estimated the age of the Lrrk2 R1628P variant. In brief, under the assumption of an ancestral haplotype, marker frequencies were referenced as 0.99 in carriers and empirically determined in noncarriers (n = 80). Linkage disequilibrium index (δ) between each marker and mutation was calculated.[11] Average genetic distances and recombination fractions (θ) were estimated between each marker and *LRRK2* using the Marshfield recombination map. The age of the mutation in generations (g) was derived from the equation $g = \ln \delta / \ln(1 - \theta)$ for each marker.[12]

Results

The Lrrk2 R1628P variant is approximately twice as frequent in affected individuals as control subjects (odds ratio, 1.84; 95% confidence interval, 1.20–2.83; $p = 0.006$) (see Table 1). Independently the same trend was observed in each ethnic Chinese series although statistical significance was not reached in two cohorts given their size and relatively low frequency of the 1628P allele (see Table 1). Unaffected carriers in Series 2 (Y.-R.W.) are approximately 10 years younger than affected carriers (51 vs 61 years of age), which may affect statistical significance. The Lrrk2 R1628P variant was not observed in our 246 Japanese subjects.

One Lrrk2 R1628P carrier was sequenced for all exons and exon-intron boundaries of *LRRK2* in our previous study.[10] No other variant was observed that could account for the associated risk. Even though all Lrrk2 R1628P carriers also harbor Lrrk2 S1647T and two additional synonymous changes (G1624G and K1637K) in exon 34, their relatively high allele frequency, global dispersion, and lack of significance in previous PD association studies indicates they are unlikely to influence disease risk.[13,14] These data support the hypothesis that the Lrrk2 R1628P substitution is the functional risk factor in carriers.

Haplotype Analysis

Our haplotype results suggest that Lrrk2 R1628P carriers are related to a single common founder (Table 2). We observed SNP alleles located in exon 34 adjacent to *LRRK2* c.4883G>C (R1628P; rs33949390), which cosegregates with the mutation. Population stratification does not appear to influence our association because these shared SNP alleles are present in both affected and unaffected carriers demonstrating a shared genetic background. Data from adjacent microsatellite

Table 2. **Chromosome 12q12 Haplotype Analysis of *LRRK2* c.4883G>C (R1628P; rs33949390) Carriers**

Marker Name	Position[a]	A	B	C	D	E	F	G	H	I	J	Shared Alleles
D12S2080	33,305,718	184/188	196/196	196/200	184/196	184/188	192/196	188/196	188/188	184/188	192/196	
D12S2194	38,738,008	249/253	249	249	249/253	249	249	249	249	249/257	245/249	249
rs11175964	38,989,254	GG	GG	GA	GG	GG	GG	GG	GG	GG	GA	G
D12S2516	38,989,339	252/254	254	254	252/254	252/254	252/254	252/254	252/254	252/254	254	254
rs1896252	39,000,026	TC	CC	CC	TC	TC	TC	TC	TC	TC	CC	C
rs1427263	39,000,101	CA	AA	AA	CA	CA	CA	CA	CA	CA	AA	A
rs33949390	39,000,112	GC	GC	GC	GC	GC	GC	GC	GC	GC	GC	C
rs11176013	39,000,140	GA	GG	GG	GA	GA	GA	GA	GA	GA	GG	G
rs11564148	39,000,168	TA	AA	TA	TA	TA	TA	TA	TA	TA	TA	A
rs10878405	39,028,521	GA	AA	GA	GA	GA	GA	GA	GA	GA	GA	A
rs11176143	39,028,630	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	G
D12S2519	39,116,885	132/138	132	132	132	134/140	132/138	134/138	132/140	132/134	132/140	132
D12S2521	39,128,754	323/351	351/367	351/367	351/363	319/375	323/351	327/379	319/351	363/379	319/351	351
D12S2522	39,132,267	281/297	297	297/299	297	283/297	281/297	281/297	283/297	297	285/297	297
D12S2517	39,282,898	188	182/188	188/192	182/188	188/190	188/190	186/188	188/190	182/188	188/202	188
D12S1301	42,348,809	116/120	100/116	100/116	112/124	112/116	100/120	104/120	104/116	116/116	116/120	

We examined 32 carriers for haplotype analysis on the surrounding chromosome 12q12 region with results indicative of a common ancestral founder in carriers.

[a]Microsatellite allele sizes were normalized using CEPH-control DNA (1331-01 and 1331-02), and approximate positions are determined from the National Center for Biotechnology Information (NCBI) March 2006 human genome assembly. The shared alleles between markers D12S2194 and D12S2517 indicate a minimum ancestral haplotype of approximately 500kb and are highlighted in the last column. Allele 351 for marker D12S2521 is found in 74% (n = 32) of Lrrk2 R1628P carriers and is rare in noncarriers (n = 80; 1%). Nonsharing was observed for markers D12S2519 (n = 3; 9.3%) and D12S2521 (n = 8; 25%). However, given the distance from the mutation of approximately 100Kb and the shared single nucleotide polymorphism (SNP) data, these are most likely due to recombination events.

markers are in good agreement; allele 351 of D12S2521 is rare in the general population (n = 1/80; 1%) but frequent in Lrrk2 R1628P carriers (n = 23/31; 74%), consistent with one ancestral haplotype. However, for a number of carriers, historical recombination may have occurred between markers D12S2519 and D12S2522. Allele sharing between markers D12S2194 to D12S2517 indicates a minimum ancestral haplotype of approximately 500kb.

It is possible to generate an estimate of the age of the mutational event using the allele frequencies of the markers in Lrrk2 R1628P carriers and noncarriers, and the excess of linkage disequilibrium. However, low sample numbers and unphased haplotypes are two caveats of this approach. It should also be noted that this calculation is under the assumption of a major ancestral haplotype, and that nonallele sharing is due to recombination and not independent founders, thus biasing the estimate toward a more recent event. From our calculations assuming each generation to approximate 30 years, *LRRK2* c.4883G>C (R1628P; rs33949390) occurred 89 generations ago (95% confidence interval, 85–92) or approximately 2,500 years ago.

Discussion

Lrrk2 R1628P is the second major risk variant identified in the ethnic Chinese populations of Taiwan and Singapore. The clinical phenotype of affected Lrrk2 R1628P carriers is typical late-onset L-dopa–responsive PD. The average age at onset in our affected Lrrk2 R1628P carriers is 60 years. Of note, the average age in our unaffected carriers is 5 years younger (55 years) and suggests some may yet develop PD symptoms.

Lrrk2 R1628P appears to be restricted to the ethnic Chinese population. We did not observe the Lrrk2 R1628P in 246 Japanese subjects, and this absence is supported by a *LRRK2* sequencing project that did not observe the variant in 36 probands with familial PD of Japanese descent (Dr Cyrus Zabetian, personal communication). Our estimation of the mutation's age (approximately 2,500 years) coupled with population-specific mutation frequencies in Taiwan, Singapore, and Japan provides evidence that the Lrrk2 R1628P substitution occurred some 2,000 years later than Lrrk2 G2385R. Given the global ethnic Chinese Diaspora, it is likely both variants will be observed in communities outside of the Asian continent. Of note, the dbSNP database does record one carrier of European

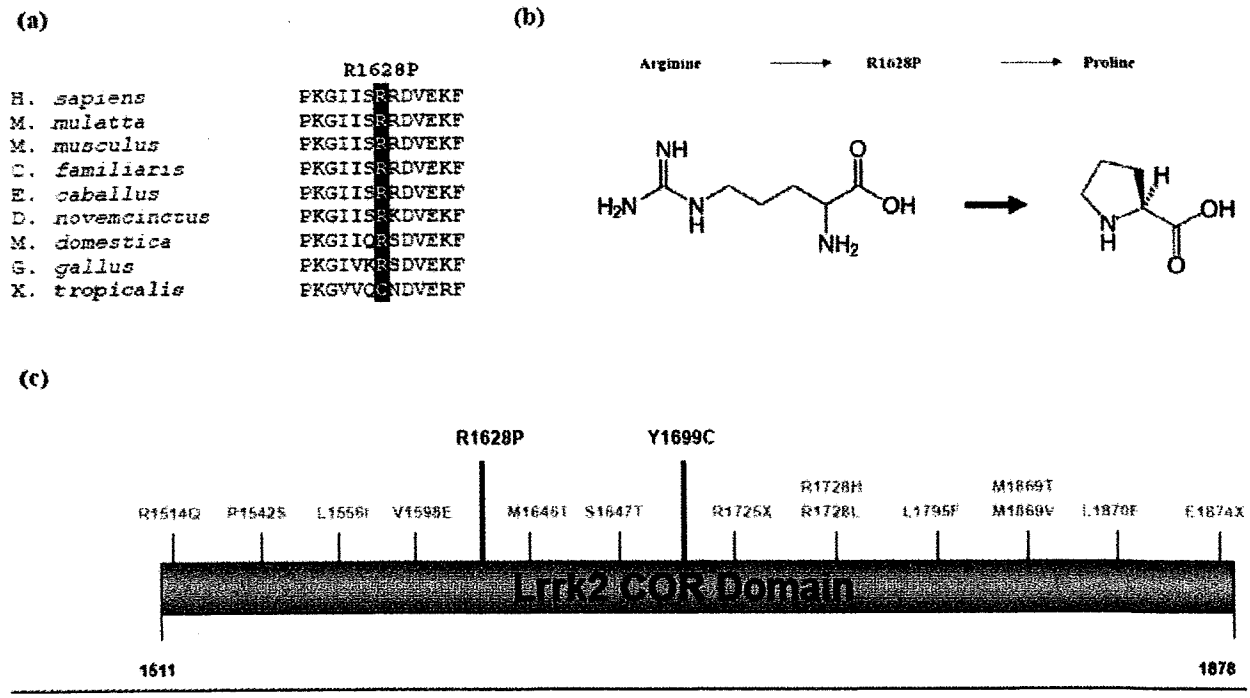

Fig. The COR domain (C) extends from amino acid 1511 to 1878 and contains 16 reported nonsynonymous changes (pathogenic variant Lrrk2 Y1699C is highlighted).[1] The Lrrk2 R1628P substitution (B) results in the replacement of an arginine with a cyclic proline residue. Given the conservation (A) at this amino acid position across species, this substitution may disrupt an important protein-protein interaction or the observed dimerization of the Lrrk2 protein.

descent; this may be a rare independent event, but previous studies have failed to identify any non-Asian carriers ($n > 2{,}500$), and it is equally likely this individual has some Asian genetic background.

To date, genome-wide association studies have not found such risk factors in US PD patients. A question remains whether multiple variants with small effect sizes contribute to complex disorders such as PD. Lrrk2 R1628P and G2385R in ethnic Chinese samples provide support for this hypothesis. Although no subjects with Lrrk2 R1628P and G2385R were observed in our study, no doubt carriers with digenic inheritance will be identified, and it will be interesting to assess whether a potential increased level of susceptibility exists in such individuals. However, it should be noted that homozygous Lrrk2 G2019S carriers do not appear to present with a more severe phenotype than heterozygous carriers.[15]

Lrrk2 R1628P is located in the COR domain and is evolutionarily conserved across species highlighting the importance of the residue to protein function (Fig). Indeed, the substitution of a highly basic polar arginine (R) with a neutral nonpolar proline (P) is likely to cause a conformational change in Lrrk2 secondary structure; proline is considered an α-helix breaker that introduces a β-hairpin turn. We postulate this substitution affects the dynamic interaction among the Roc, COR, and mitogen-activated protein kinase kinase kinase domains critical for activity, and may disrupt Lrrk2 dimerization.

Herein we present the first evidence to support Lrrk2 R1628P as the second common genetic risk factor for PD in the ethnic Chinese population. Moreover, we have reproduced the risk effect in a multicenter approach with combined pooled analysis of other ethnic Chinese series (see Table 1). This collaborative approach will be crucial in determining the pathogenicity of other *LRRK2* variants. Future therapeutic interventions will most likely be determined by the genomic background of the individual; thus, identification of common risk factors in PD (odds ratio, ≤ 2) will have a profound effect on diagnosis and treatment.

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Gene Expression Study on Peripheral Blood Identifies Progranulin Mutations

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Peripheral blood is a readily available tissue source allowing relatively noninvasive screening for a host of medical conditions. We screened total-blood progranulin *(PGRN)* levels in 107 patients with neurodegenerative dementias and related conditions, and 36 control subjects, and report the following findings: (1) confirmation of high progranulin expression levels in peripheral blood; (2) two subjects with reduced progranulin levels and mutations in the *PGRN* gene confirmed by direct sequencing; and (3) greater *PGRN* messenger RNA levels in patients with clinical diagnosis of Alzheimer's disease. This proof-of-principle report supports the use of gene quantification as diagnostic screen for *PGRN* mutations and suggests a potential role for progranulin in Alzheimer's disease.

Ann Neurol 2008;64:92–96

Frontotemporal lobar degeneration (FTLD) comprises a group of dementias with related clinical and neuropathological characteristics.[1] FTLD is the second most common cause of presenile dementia after Alzheimer's disease (AD)[1–3] and accounts for 5 to 10% of neurodegenerative dementias in epidemiological samples and between 9 and 16% in autopsy series.[1] Clinical subtypes of FTLD include (1) a behavioral variant with predominant frontotemporal involvement, (2) semantic dementia, and (3) primary progressive aphasia. A family history is present in about 40% of the FTLD patients, and four genes have been discovered as genetic causes. Mutations in *MAPT* have been identified in more than 100 families, and 2 other causative genes (*VCP*[4] and *CHMP2B*[5])

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Genomic Investigation of α-Synuclein Multiplication and Parkinsonism

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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.

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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

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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

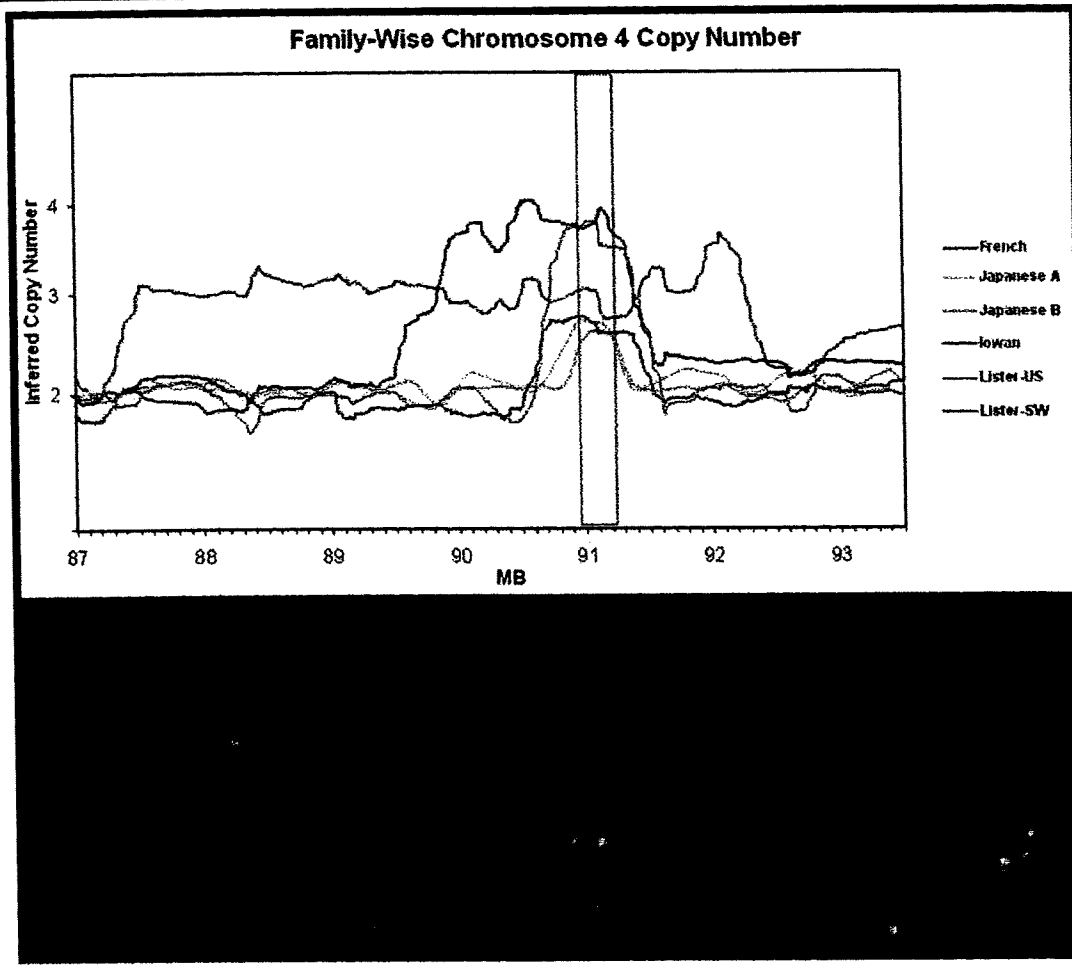

Fig 1. A representation of fluorescent in situ hybridization (FISH) and Affymetrix 250k SNP microarrays (CHIPS) that were used to examine the region of multiplication in the proband of each family. (A) Relative copy number estimates were plotted against physical genomic position on chromosome 4. Raw 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.*

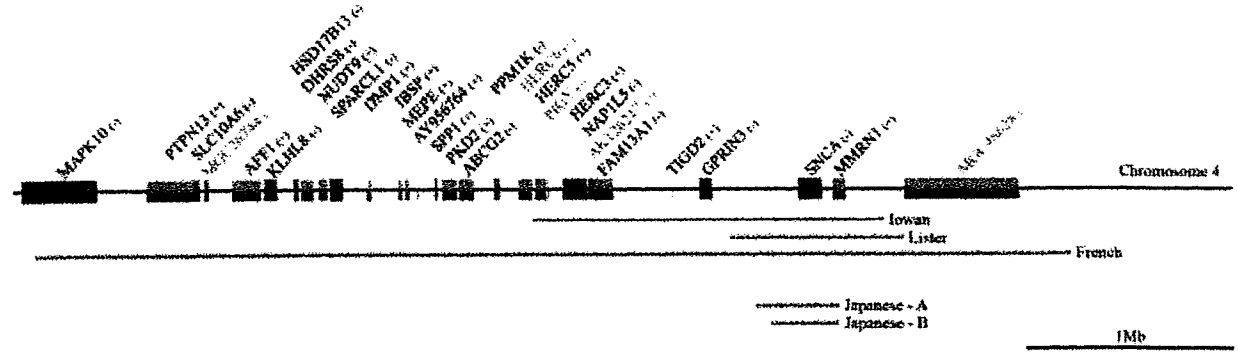

Fig 2. Representation of the genes in the multiplication region in each family. Displays the genes that are present in each of the multiplied regions of the families. Figure is drawn approximately to scale. The coding genomic DNA strand of each gene is indicated by (+) or (−). Genes are colored to represent 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

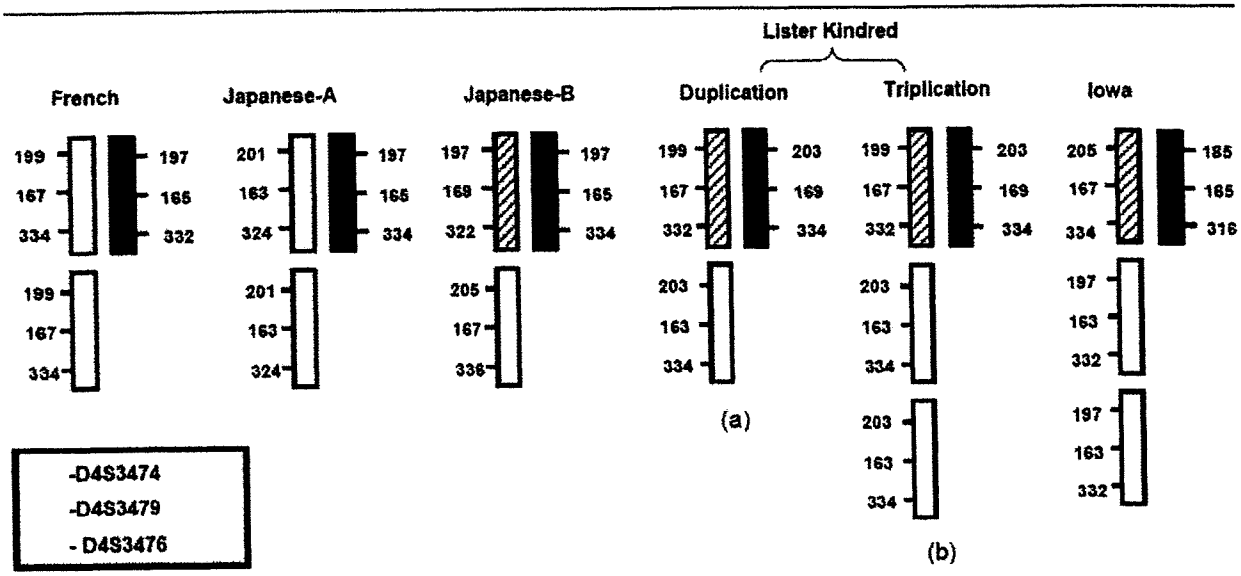

Fig 3. The allele sizes and dosage for the chromosome 4 markers D4S3474, D4S3479, and D4S3476 are shown for each family. These data demonstrate an intraallelic, segmental duplication in the French and Japanese A families. However, interallelic recombination occurred initially in Japanese B and the in 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.

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Review

Progress in the pathogenesis and genetics of Parkinson's disease

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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.

Keywords: Parkinson's disease; familial Parkinson's disease; pathogenesis; genetics

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.

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.

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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.