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

Owen A. Ross, PhD,[1] Yih-Ru Wu, MD,[2] Mei-Ching Lee, MD,[3] Manabu Funayama, PhD,[4] Meng-Ling Chen, MSc,[5] Alexandra I. Soto, BSc,[1] Ignacio F. Mata, PhD,[6] Guey-Jen Lee-Chen, PhD,[7] Chiung Mei Chen, MD, PhD,[2] Michelle Tang, BSc,[8] Yi Zhao, MD, PhD,[8] Nobutaka Hattori, MD, PhD,[4,9] Matthew J. Farrer, PhD,[1] Eng-King Tan, MD,[8,10] and Ruey-Meei Wu, MD, PhD[5]

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-

From the [1]Department of Neuroscience, Mayo Clinic College of Medicine, Jacksonville, FL; [2]Department of Neurology, Chang Gung Memorial Hospital and Chang Gung University, College of Medicine; [3]Department of Neurology, Cathay General Hospital, Taipei, Taiwan; [4]Research Institute for Diseases of Old Ages, Juntendo University School of Medicine, Bunkyo, Tokyo, Japan; [5]Department of Neurology, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei, Taiwan; [6]Department of Neurology, University of Washington School of Medicine, Seattle, WA; [7]Department of Life Science, National Taiwan Normal University, Taipei, Taiwan; [8]Department of Neurology, Singapore General Hospital, National Neuroscience Institute of Singapore; [9]Department of Neurology, Juntendo University School of Medicine, Bunkyo, Tokyo, Japan; [10]Duke-NUS Graduate Medical School, Singapore.

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Address correspondence to Dr Wu, Department of Neurology, National Taiwan University Hospital, No. 7, Chung-Shan South Road, Taipei 100, Taiwan. E-mail: robinwu@ntu.edu.tw

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

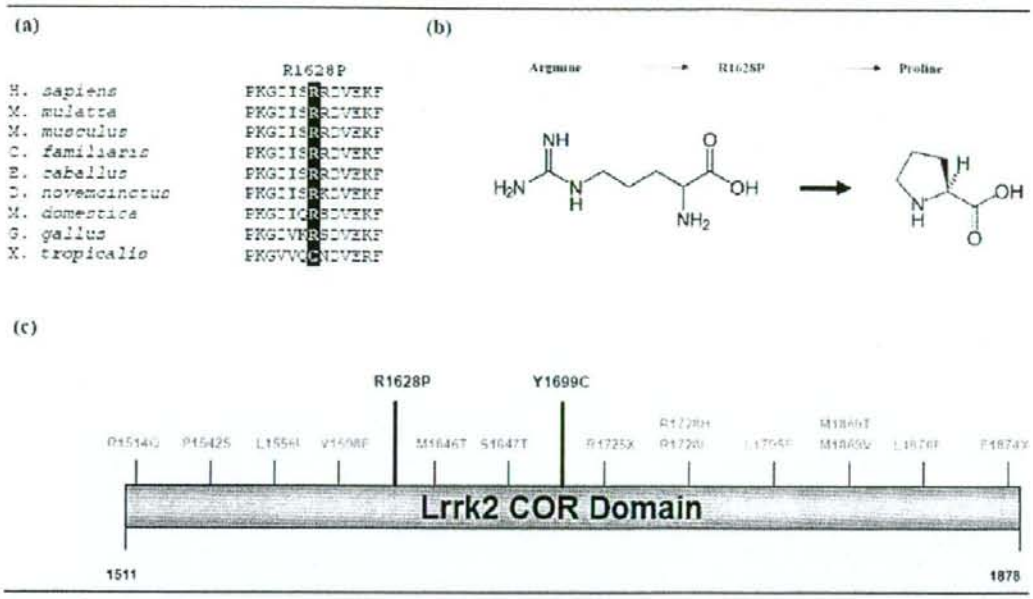

Fig. The COR domain (C) extends from amino acid 1511 to 1878 and contains 16 reported nonsynonymous changes (pathogenic variant Lrrk2 Y1699C is highlighted).[1] The Lrrk2 R1628P substitution (B) results in the replacement of an arginine with a cyclic proline residue. Given the conservation (A) at this 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

Giovanni Coppola, MD,[1] Anna Karyda, BA,[2] Rosa Rademakers, PhD,[3] Qing Wang, PhD,[1] Matt Baker, BSc,[3] Mike Hutton, PhD,[3] Bruce L. Miller, MD,[2] and Daniel H. Geschwind, MD, PhD[1]

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.

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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])

From the [1]Department of Neurology, Program in Neurogenetics, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles; [2]Department of Neurology, Memory and Aging Center, University of California, San Francisco, San Francisco, CA; and [3]Department of Neuroscience, Mayo Clinic College of Medicine, Jacksonville, FL.

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Address correspondence to Dr Geschwind, Department of Neurology, David Geffen School of Medicine at UCLA, 695 Charles E. Young Drive South, Los Angeles, CA 90095. E-mail: dhg@ucla.edu

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.

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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

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.

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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

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

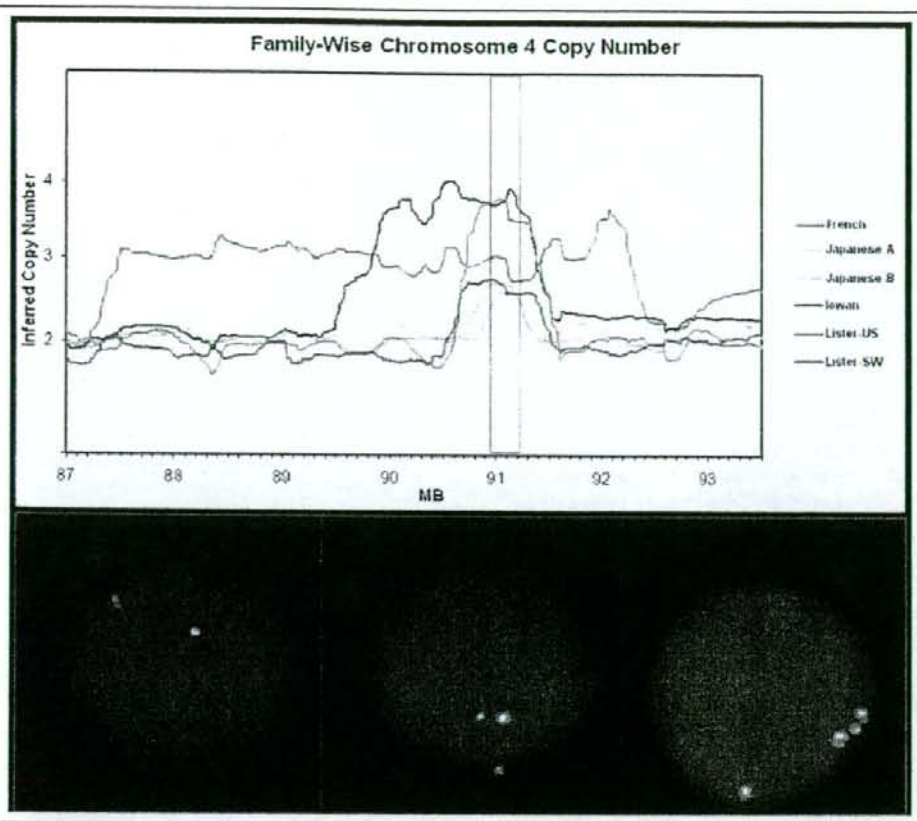

Fig 1. A representation of fluorescent in situ hybridization (FISH) and Affymetrix 250k SNP microarrays (CHIPS) that were used to examine the region of multiplication in the proband of each family. (A) Relative copy number estimates were plotted against physical genomic position on chromosome 4. Raw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

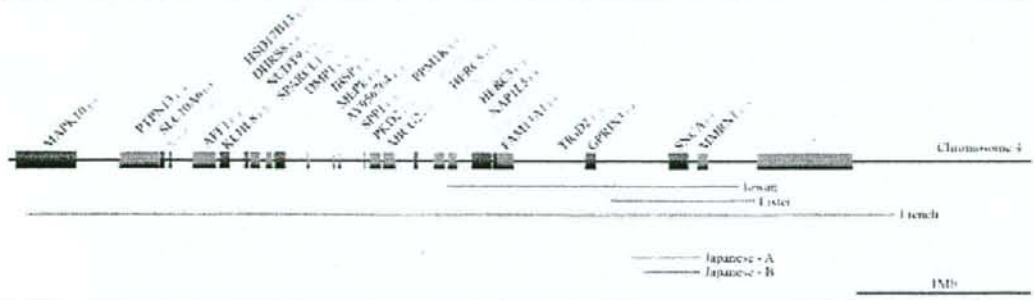

Fig 2. Representation of the genes in the multiplication region in each family. Displays the genes that are present in each of the multiplied regions of the families. Figure is drawn approximately to scale. The coding genomic DNA strand of each gene is indicated by (+) or (−). Genes are colored to represent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

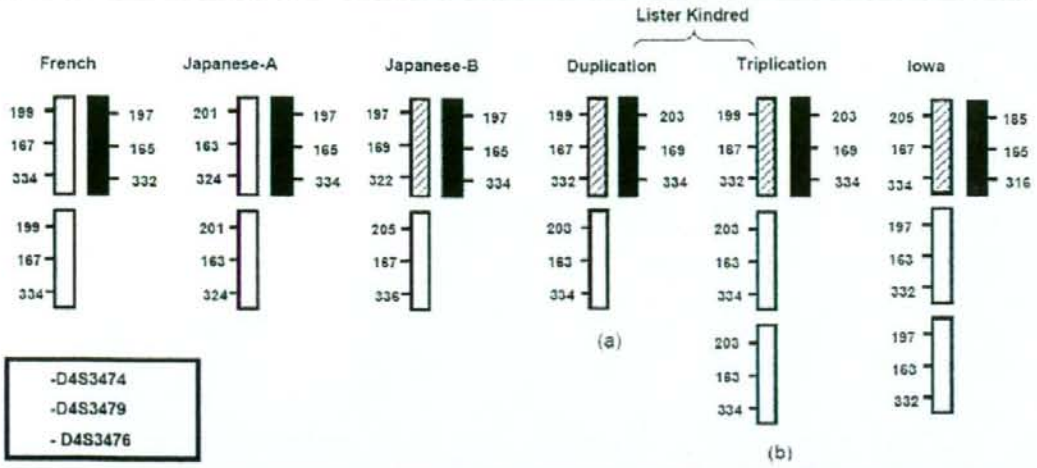

Fig 3. The allele sizes and dosage for the chromosome 4 markers D4S3474, D4S3479, and D4S3476 are shown for each family. These data demonstrate an intraallelic, segmental duplication in the French and Japanese A families. However, interallelic recombination occurred initially in Japanese B and the in 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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Mutation Analyses in Amyotrophic Lateral Sclerosis/ Parkinsonism–Dementia Complex of the Kii Peninsula, Japan

Hiroyuki Tomiyama, MD,[1] Yasumasa Kokubo, MD,[2] Ryogen Sasaki, MD,[2] Yuanzhe Li, MD,[1] Yoko Imamichi,[1] Manabu Funayama, PhD,[3] Yoshikuni Mizuno, MD,[3] Nobutaka Hattori, MD, PhD,[1] and Shigeki Kuzuhara, MD[2,4]*

[1]*Department of Neurology, Juntendo University School of Medicine, Tokyo, Japan*
[2]*Department of Neurology, Mie University School of Medicine, Tsu, Japan*
[3]*Research Institute for Diseases of Old Ages, Juntendo University School of Medicine, Tokyo, Japan*
[4]*Department of Neurology, National Center Hospital of Neurology and Psychiatry, Tokyo, Japan*

Abstract: To clarify the genetic background of amyotrophic lateral sclerosis (ALS)/parkinsonism–dementia complex (PDC) of the Kii peninsula, Japan (Kii ALS/PDC), we performed extended mutation analyses of three patients with pathologically diagnosed Kii ALS/PDC. Direct sequencing analyses were performed in 19 genes, including ALS/frontotemporal lobar degeneration (FTLD)-related genes (*SOD2*, *SOD3*, *ALS2/alsin*, *SMN1*, *PGRN*, *ANG*, *VEGF*, *VCP*, *VAPB*, *DCTN1*, *CHMP2B*, and *TARDBP* or *TDP-43*), tauopathy-related gene (*GSK3*β), and parkinsonism-related genes (*alpha-synuclein*, *LRRK2*, *parkin*, *DJ-1*, *PINK1*, and *ATP13A2*). Gene dosage analyses were conducted in screening of *MAPT*, *alpha-synuclein*, *TDP-43* (or *TARDBP*), *GSK3*β, and *parkin*. We found no mutation in the 19 genes. We found a homozygous nonsynonymous SNP (*ALS2/alsin* V368M) shared by all the three patients. Gene dosage was normal in *MAPT*, *alpha-synuclein*, *TDP-43*, *GSK3*β, and *parkin*. The present findings, together with a previous negative study on *MAPT* and *SOD1* mutation, further elucidated the lack of causative mutations in all exons, exon–intron boundaries, or some rearrangements of the reported major causative or susceptible genes related to ALS, FTLD, parkinsonism, synucleinopathy, TDP-43 proteinopathy, and tauopathy. However, the familial aggregation and lack of any environment factors suggest that Kii ALS/PDC is caused by other yet unidentified genetic factors. © 2008 Movement Disorder Society

Key words: Kii ALS/PDC; amyotrophic lateral sclerosis; parkinsonism; dementia; genetics

The Western Pacific amyotrophic lateral sclerosis (ALS)/parkinsonism–dementia complex (PDC) is a progressive and fatal neurodegenerative disorder with high incidence among the indigenous people of three areas on the Pacific volcanic belt; Chamorros on Guam and Mariana Islands, Papuans in the coastal plain of West New Guinea, and Japanese in the Kii peninsula of Japan.[1] Clinically, ALS and PDC occur in isolation or in combination. Neuropathologically, ALS and PDC on Guam and Kii are characterized by abundant neurofibrillary tangles (NFTs) throughout the entire central nervous system, most markedly in the brainstem and temporal lobe, together with selective involvement of the upper and lower motor neurons.[2,3] Most, but not all, investigators consider ALS and PDC to be different manifestations of a single disease entity (ALS/PDC).[1,4]

In the 1980s, the disappearance of high incidence of ALS and marked decline in PDC were reported in Guam possibly related to changes in the environment and westernization of the lifestyle of Chamorros.[5] Although various environment factors, such as consumption of cycad and fruit bats, and deficiency of various minerals, have been suspected in Chamorros of Guam,[6–8] none has been experimentally verified so far. With regard to genetic factors, although *Tau (MAPT)*[9]

H. Tomiyama and Y. Kokubo contributed equally to this work.

*Correspondence to: Dr. Shigeki Kuzuhara, Department of Neurology, National Center Hospital of Neurology and Psychiatry, 4-1-1 Ogawahigashimachi, Kodaira, Tokyo 187-8551, Japan.
E-mail: kuzuhara@ncnp.go.jp

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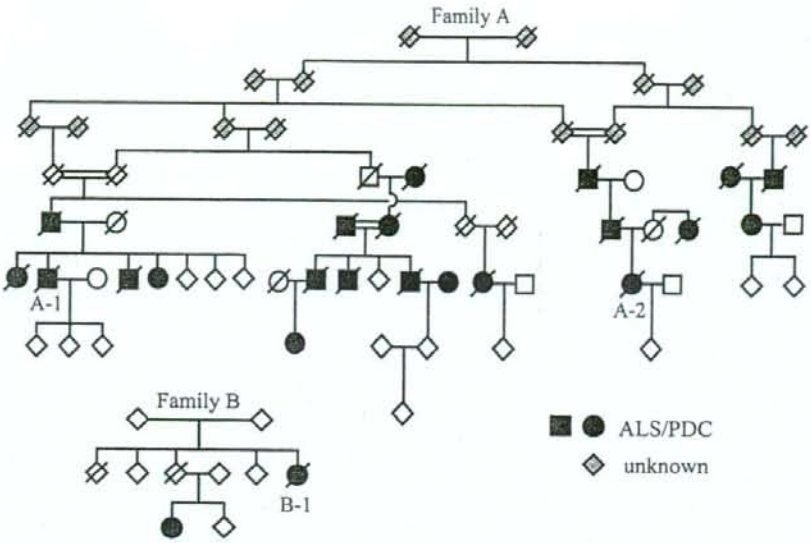

FIG. 1. Family trees (Family A and Family B) of patients with ALS/PDC from the Kii peninsula. Squares, men; circles, women; solid symbols, patients with amyotrophic lateral sclerosis/parkinsonism-dementia complex (ALS/PDC); open symbols, healthy individuals; gray symbols, unknown (precise information was not available); /(slash mark), deceased. The sexes are concealed (diamond symbols) to safeguard the confidentiality of the family members.

might be a modifier gene that increases the risk for Guam ALS, Guam PDC, and Guam neurodegenerative disorders in the presence of other unidentified gene(s) or by regulating *Tau* expression,[10,11] no causative mutation in *Tau* was detected in both Guam and Kii ALS/PDC with abundant NFTs pathology.[4] In addition, a previous genome-wide association study could not identify a single gene locus for Guam PDC, suggesting a geographic disease isolate with a complex genetic, genetic/environmental, or purely environmental etiology.[12] On the other hand, other studies proposed a mixture of other factors in the pathogenesis of ALS/PDC on Guam, including prolonged exposure to an environment severely deficient in Ca^{2+} and Mg^{2+} concurrent with a susceptibility genotype of *TRPM7* T1482I allele,[13] as well as neurotoxicity associated with β-methylamino-L-alanine in the cycads.[6]

In contrast to Guam ALS/PDC, high average annual incidence rates (417.9/100,000 in 1995–1998, unpublished data) of ALS/PDC in Hohara area of Kii continuing even after dramatic changes in foods and drinking water, and the much higher aggregation in the same family with a family history of approximately 80% in patients with Kii (40% in Guam ALS/PDC) strongly suggest major contribution of genetic factors.[11,14,15] And no customs of eating cycad or fruit bats exist in Japanese people living in Kii. Thus, further genetic analyses for Kii ALS/PDC might help disclose the pathogenesis of ALS/PDC.[4,16] Backed with this background, we performed mutation analysis of genes related to ALS, frontotemporal lobar degeneration (FTLD), tauopathy, and parkinsonism, and gene dosage analyses of *MAPT*, *alpha-synuclein*, *TDP-43* (or *TARDBP*), *GSK3*β, and *parkin* in three Kii patients with neuropathologically verified ALS/PDC.

METHODS

Patients

The study was approved by the Ethics Review Committees of Mie and Juntendo Universities. We analyzed DNA samples of three patients with Kii ALS/PDC from two families in the Kii peninsula. Consanguinity was seen in Family A but not in Family B (Fig. 1). The clinical diagnoses were based on features of typical ALS and PDC occurring singularly or in combination as shown in Table 1. The clinical diagnosis was verified as ALS/PDC in each patient at postmortem examination. All patients showed cardinal neuropathological findings of ALS/PDC including abundant NFTs associated with loss of nerve cells in the cerebral cor-

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.

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SHORT COMMUNICATION

LRRK2 P755L variant in sporadic Parkinson's disease

Hiroyuki Tomiyama · Ikuko Mizuta · Yuanzhe Li · Manabu Funayama · Hiroyo Yoshino · Lin Li · Miho Murata · Mitsutoshi Yamamoto · Shin-ichiro Kubo · Yoshikuni Mizuno · Tatsushi Toda · Nobutaka Hattori

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Abstract Parkinson's disease (PD) is a neurodegenerative disorder of unknown etiology with probable involvement of genetic-environmental factors. The majority of PD cases (approximately 90–95%) are sporadic, while familial cases account for approximately 5–10% of PD. In a recent report, a heterozygous *LRRK2* P755L mutation within *LRRK2* exon 19 was found in 2% of Chinese sporadic PD patients and in 0% of normal controls or Caucasians, suggesting that the mutation is disease-associated with ethnic specificity. To further evaluate the role of *LRRK2* P755L variant in sporadic PD, we performed direct sequencing of *LRRK2* exon 19 in 501 Japanese sporadic PD patients (male 249, female 252, aged 28–92 years, mean 65.0 years) and 583 controls of the Japanese general population as an extended association study. In this group, we found six patients (6/501 = 1.2%) and eight controls of the general population (8/583 = 1.6%) with a heterozygous P755L variant ($P = 0.80$, $\chi^2 = 0.064$). No other variants were found in exon 19. Together with previous reports, our extended case-controlled study of large sample size suggests that *LRRK2* P755L is a non-disease-associated polymorphism in PD patients.

Keywords Parkinson's disease · Genetics · *PARK8* · *Leucine-rich repeat kinase 2 (LRRK2)* · Polymorphism · Association study · Japanese · Ethnic background

H. Tomiyama · Y. Li · L. Li · S.-i. Kubo · N. Hattori (✉)
Department of Neurology,
Juntendo University School of Medicine,
2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan
e-mail: nhattori@med.juntendo.ac.jp

I. Mizuta · T. Toda
Division of Clinical Genetics,
Osaka University Graduate School of Medicine,
Suita, Japan

I. Mizuta · M. Murata · M. Yamamoto · T. Toda · N. Hattori
Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency,
Saitama, Japan

M. Funayama · H. Yoshino · Y. Mizuno
Research Institute for Diseases of Old Age,
Juntendo University School of Medicine, Tokyo, Japan

M. Murata
Department of Neurology, Musashi Hospital,
National Center of Neurology and Psychiatry, Tokyo, Japan

M. Yamamoto
Department of Neurology,
Kagawa Prefectural Central Hospital, Takamatsu, Japan

Introduction

Parkinson's disease (PD, OMIM #168600) is the second most common neurodegenerative disorder next to Alzheimer's disease. The clinical features are characterized by levodopa-responsive parkinsonism, such as rigidity, resting tremor, bradykinesia, and postural instability. Although the cause of PD remains unclear, genetic-environmental interaction is suggested for the development of the disease. One of the autosomal-dominant forms of PD, *PARK8*, was originally mapped from a Japanese Sagamihara family (Funayama et al. 2002) and *LRRK2* (*PARK8*; *leucine-rich repeat kinase 2*, OMIM *609007) was identified as the causative gene for *PARK8*-linked PD (Paisán-Ruíz et al. 2004; Zimprich et al. 2004). Among *LRRK2* mutations, the most common *LRRK2* G2019S mutation in North Africans and Ashkenazi Jews has shown ethnic differences among Caucasian, Japanese, and Chinese (Nichols et al. 2005; Gilks et al. 2005; Lesage et al. 2006; Tomiyama et al.