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R1514Q Substitution in Lrrk2 Is Not a Pathogenic Parkinson's Disease Mutation

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Abstract: **Mutations in *LRRK2* were first reported as causing Parkinson's disease (PD) in late 2004. Since then, approximately a dozen *LRRK2* substitutions have been identified that are believed to be pathogenic mutations. The substitution of adenine for guanine at nucleotide 4541 (4541G>A) in *LRRK2* was recently reported. This substitution resulted in the replacement of an arginine at position 1514 with a glutamine (R1514Q). Although this substitution was not found in a large cohort of controls, its pathogenicity could not be verified. We have now genotyped the R1514Q substitution in a sample of 954 PD patients from 429 multiplex PD families. This substitution was identified in 1.8% of the PD patients; however, the majority of the PD sibships segregating this substitution were discordant for this putative mutation. In addition, the R1514Q substitution was detected in 1.4% of neurologically evaluated, control individuals. These data suggest that the R1514Q variant is not a pathogenic *LRRK2* mutation. We believe it is imperative that the causative nature of any newly identified genetic variant be determined before it is included in any panel for diagnostic testing.**

Key words: Parkinson's disease; genetics; LRRK2; mutation

Parkinson's disease (PD) is the second most common neurodegenerative disorder. Clinical features of PD include resting tremor, rigidity, bradykinesia, and postural instability. Although quite variable, the average age of onset is 60 years. In addition, there is a slight preponderance of affected men. Pathologically, PD is characterized by the presence of Lewy bodies and progressive degeneration of neurons in the substantia nigra, pars compacta, and other brain regions.[1]

Five genes have been identified with a confirmed role in PD etiology.[2] Four of these genes are alpha-synuclein (*SNCA*), parkin (*PRKN*), PTEN-induced kinase 1 (*PINK1*), and DJ-1. Typically, mutations in these four genes result in early-onset PD. The fifth and most recently identified gene is leucine-rich repeat kinase 2 or *LRRK2*[3,4] Alterations in *LRRK2* appear to result in phenotypically typical PD with mutations having been identified in patients with later, more typical, age of onset. Over a dozen pathogenic *LRRK2* mutations have been identified in both autosomal dominant and idiopathic PD patients with the G2019S mutation being the most common genetic mutation identified in all of PD to date.[5,6] The pathological spectrum of *LRRK2* mutations includes individuals with Lewy bodies that are restricted to the brainstem, but also diffuse Lewy bodies, and at least in 1 case, neurofibrillary tangles and abnormal tau deposits.[7]

Recent reports have identified as many as 25 coding sequence variants in *LRRK2* during the analysis of PD patients.[7–10] In a recent study by Mata and colleagues,[7] 100 affected probands with a family history of Parkinsonism were sequenced for all 51 exons of *LRRK2*. Twenty-six coding variants, including 15 nonsynonymous amino acid substitutions, were identified. One such coding sequence change, resulting in the substitution of an arginine at amino acid 1514 with glutamine (R1514Q), was identified in a family with 2 affected monozygotic twin sisters. DNA was not available from the deceased, affected mother. Ethnicity of this family was not specified but reportedly was North American, Irish, Norwegian, Spanish, or Taiwanese. As reported, this change was not found in an ethnically diverse set of 1,000 control samples. Because segregation analysis was precluded in this family, the authors concluded that pathogenicity for this mutation could

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not be determined. However, an additional study[4] reported this change as a nonpathogenetic change because it was observed to have a frequency of 1% in at least 1,200 control chromosomes.

In our ongoing effort to identify additional PD susceptibility genes, we have recruited a large cohort of PD families.[6] The sample consists of 954 PD patients from 429 families recruited through 59 Parkinson Study Group sites located throughout North America. Families were ascertained through an affected sibling pair, although additional affected relatives were collected, if available. All participants underwent a uniform neurological evaluation that included the Unified Parkinson's Disease Rating Scale (UPDRS) Parts II and III. The genotyped sample was 58% male and primarily Caucasian (94%), although Hispanic subjects also participated (5%). Age of PD onset in the sample averaged 61.2 years and ranged from 18 to 87 years. Peripheral blood was obtained from all individuals after appropriate written informed consent approved by each individual institution's institutional review board was completed.

The control sample was collected through three sources and provided appropriate written informed consent. One sample of controls (n = 52) was ascertained in Indiana, and all control subjects were examined by a single Parkinson Study Group movement disorder specialist. These control subjects completed the identical clinical evaluation as the PD sample. Individuals were considered controls if they met the following criteria: did not have a diagnosis or symptoms of PD, Alzheimer's disease (AD), stroke, or other neurological disorder; no tremor; no other first-degree family members reported to be diagnosed with PD; and no history of polio. The average age at examination of these first control subjects was 68.3 years, with a range of 55 to 82 years. All individuals were non-Hispanic Caucasians. A second control sample (n = 40) was obtained from the National Cell Repository for Alzheimer's Disease. The subjects were recruited as part of an ongoing genetic initiative to make available to the research community a sample of rigorously evaluated individuals without any evidence of neurological disease. All control subjects were evaluated, and there was no evidence for either PD or dementia. The average age at examination of the second control cohort was 76.9 years, with a range of 58 to 91 years. As was the case with the first control set, all subjects from the second control set were non-Hispanic Caucasians. DNA was prepared from peripheral blood samples collected from the PD families and control subjects. The third control sample (n = 276) is composed of three neurologically normal Caucasian control panels (NDPT002, NDPT006, NDPT009) obtained from the NINDS Human Genetics Resource Center at the Coriell Institute Coriell Cell Repositories (Camden, NJ). This third control sample contains 132 males and 144 females. The average age at examination of the subjects was 69.7 years, with a range of 55 to 88 years. In total, 368 neurologically normal control samples were evaluated.

The guanine to adenine substitution at nucleotide 4541 of the *LRRK2* cDNA that results in the R1514Q Lrrk2 (dardarin) protein variant was screened for using a newly developed TaqMan allelic-discrimination assay (Applied Biosystems). The assay was performed with 30 ng of genomic DNA from each PD subject and control individual using conditions recommended by the manufacturer and an Applied Biosystems 7300 Real Time PCR System. Of 954 affected individuals from 12 different families, 16 (1.8%) were shown to be heterozygous carriers of the R1514Q variant. In addition, 5 (1.4%) of 368 control subjects were also found to be heterozygous for the same variant similar to the frequency observed by Zimprich and associates.[4] The proportion of affected individuals determined to carry this variant is not significantly different than that of the controls ($P = 0.336$).

Discordance for the mutation among affected individuals was observed in 10 of the 13 families in which the variant was segregating. Of the 28 affected individuals in these 12 families for whom DNA was available for study, 12 of them do not carry the R1514Q variant. This finding suggests that the R1514Q variant is not segregating with PD in these families. No statistically significant difference between the R1514Q carrier group (16) and the noncarrier group (938) was detected in our analyses of numerous parameters, including age of disease onset (61.75 years in R1514Q carriers vs. 60.9 years in noncarriers), disease duration (7.18 years carriers vs. 9.53 years noncarriers), Mini-Mental State Examination score (25.62 carriers vs. 26.48 noncarriers), Blessed Functional Activity Scale (3.66 carriers vs. 4.41 noncarriers), Hoehn & Yahr (2.2 carriers vs. 2.48 noncarriers), and ethnicity. Taken together, these data suggest that the R1514Q variant is likely a nonpathogenic variant in Lrrk2 that does not contribute to the development of Parkinson disease, confirming the report of Zimprich and coworkers.[4]

Understandably, there is an increased interest in genetic testing for PD with diagnostic testing already available for several of the previously identified genes contributing to PD susceptibility. However, we believe that diagnostic testing must proceed with caution. It is imperative that the causative nature of any newly identified genetic variant be determined before it is included in any panel for diagnostic testing. The low frequency of many of the identified *LRRK2* mutations has prevented estimation of penetrance in most cases. We believe data regard-

ing reduced penetrance and consequent variability in age of onset for putative mutations should be available to the clinician to enable proper genetic counseling to those patients undergoing genetic testing. This is especially critical for at-risk individuals undergoing presymptomatic testing. Therefore, we urge caution and due diligence in implementation of *LRRK2* genetic testing to ensure that the patients' best interests are realized and the risks of misinterpretation and potential harm are minimized.

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Mortality in Patients with Parkinson's Disease Treated by Stimulation of the Subthalamic Nucleus

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Abstract: **Subthalamic nucleus (STN) stimulation improves motor disability and quality of life in patients with advanced Parkinson's disease (PD). Short-term mortality is low, but little is known about long-term mortality. We assessed mortality and causes of death in 171 consecutive PD patients treated by STN stimulation. Surgery was performed after a median lagtime of 13 years from PD onset at a median age of 57 years. The median follow-up after surgery was 41 months. Sixteen patients died 8 to 83 months after neurosurgery. Poorer cognitive function was the only predictive factor for mortality (standardized mortality ratio = 2.9; 95% confidence interval [CI], 1.6–4.7; $P < 0.0001$). Based on a historical comparison of 118 operated patients with 39 nonoperated patients from a different population, survival among operated patients was not better (hazard ratio = 1.2; 95% CI, 0.7–2.1).** © 2006 Movement Disorder Society

Key words: mortality; deep brain stimulation; subthalamic nucleus; Parkinson's disease

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Stimulation of the subthalamic nucleus (STN) is an effective treatment for advanced Parkinson's disease (PD) that improves both motor disability[1] and quality of life.[2] Perioperative mortality is low,[3] but little is known about longer-term mortality. We investigated the patterns of mortality in the PD patients treated with STN stimulation in our center.

PATIENTS AND METHODS

The charts of 171 consecutive patients treated by STN stimulation at Salpêtrière-Hospital (February 1996 to December 2004) were reviewed. All patients had long-standing PD with severe levodopa-related motor complications. At the time of surgery, they were under 75 years of age, had no contraindications for neurosurgery, no active severe psychiatric illness, and were not demented.

Patients were assessed before neurosurgery and 6 months afterward.[4] Mentation, behavior, mood, activities of daily living, motor disability, and complications from L-dopa were assessed using Unified Parkinson's Disease Rating Scale (UPDRS, Parts I–IV).[5] Cognition was tested by the Mattis Dementia Scale.[6] Psychiatric assessment was based on an interview and the Montgomery–Asberg Depression Rating Scale (MADRS).[7] Procedures for localizing the STN by magnetic resonance imaging and surgery are described elsewhere.[8]

Statistical Analyses

Patients were followed from surgery until death or July 2005. Kaplan–Meier survival curves were constructed using death (any cause) as the outcome. We compared the number of deaths observed among operated patients to the expected number based on French mortality rates (2000–2002),[9] and calculated a standardized mortality ratio (SMR). Its 95% confidence interval (CI) was based on a Poisson distribution of the number of deaths.

We assessed relationships between survival and baseline characteristics (age at PD onset, age at surgery, sex, disease duration, doses of L-dopa equivalent, UPDRS, MADRS, Mattis Dementia Scale) using univariate Cox models. Because no death occurred between surgery and