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Validity and Utility of a *LRRK2* G2019S Mutation Test for the Diagnosis of Parkinson's Disease

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ABSTRACT

The G2019S mutation in the *LRRK2* gene, the most common known cause of Parkinson's disease (PD), will soon be widely available as a molecular clinical test for PD. The objective of this study was to assess performance characteristics of G2019S as a clinical test for PD in the setting of typical movement disorder clinics in the United States. Subjects included 1518 sequentially recruited PD patients from seven movement disorder clinics in the United States, and 1733 unaffected subjects. All 3251 subjects were genotyped for the G2019S mutation using a TaqMan assay, and mutations were verified by direct sequencing. Test validity estimates were calculated using standard methods. A total of 20/1518 patients and 1/1733 controls carried the G2019S mutation. Specificity was 99.9% (95% CI, 99.6–100%), sensitivity was 1.3% (0.8–2.1%), and the positive likelihood ratio was 22.8. A positive family history of PD increased the positive likelihood ratio to 82.5. Information on gender, age at disease onset, or age at testing did not improve test performance. The gene test was highly accurate in classifying mutation carriers as PD, but it performed poorly in predicting the phenotype of non-mutation carriers. A G2019S molecular test for PD would be highly specific, technically simple, and inexpensive. Test interpretation is straightforward when used for diagnosis of symptomatic individuals, but is more complex for risk assessment and predictive testing in asymptomatic individuals. Test results can have psychological, social, and economical ramifications; thus, proper counseling is essential.

INTRODUCTION

PARKINSON'S DISEASE (PD) is a progressive neurodegenerative disorder that affects 1–2% of the population over the age of 60 (Tanner and Goldman 1996). The diagnosis of PD is based on the presence of the cardinal signs of resting tremor, muscle rigidity and slowed movement, and the exclusion of known causes of parkinsonism (Hughes *et al*. 1992). Differential diagnosis of PD can be complicated, especially early in the course of disease, because other neurodegenerative disorders can mimic clinical signs of PD. For more than one quarter of patients with parkinsonism, the initial diagnosis changes during the course of the disease, and a final clinical diagnosis is, at best, 90% accurate (Hughes *et al*. 2001). At present, a definitive diagnosis of PD can only be achieved after death by neuropathological examination of the brain for the presence of Lewy bodies and neuronal loss in the substantia nigra. A gene test, if sufficiently specific to PD, can help physicians achieve a definitive molecular diagnosis early in the course of the disease.

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PD is a multifactorial disorder involving both genetic and environmental factors. Several causative genes for PD have been identified (Polymeropoulos *et al*. 1997; Kitada *et al*. 1998; Leroy *et al*. 1998; Bonifati *et al*. 2003; Le *et al*. 2003; Paisan-Ruiz *et al*. 2004; Valente *et al*. 2004; Zimprich *et al*. 2004). A subset of PD is autosomal dominant and can result from mutations in the *α-synuclein* (Polymeropoulos *et al*. 1997) or leucine-rich repeat kinase 2 (*LRRK2*) genes (Paisan-Ruiz *et al*. 2004; Zimprich *et al*. 2004). Another subset is autosomal recessive and due to mutations in *parkin* (Kitada *et al*. 1998), *DJ-1* (Bonifati *et al*. 2003), or *PINK1* (Valente *et al*. 2004). Still, the majority of PD is currently of unknown cause. With the exception of *parkin*, which is responsible for approximately 50% of autosomal recessive juvenile parkinsonism (Lucking *et al*. 2000), and *LRRK2*, which is associated with the more common forms of PD (Paisan-Ruiz *et al*. 2004; Zimprich *et al*. 2004), mutations in other PD-linked genes reported to date are rare. More than 20 putatively pathogenic *LRRK2* variants have been identified; the most common is G2019S (Di Fonzo *et al*. 2005; Gilks *et al*. 2005; Kachergus *et al*. 2005; Nichols *et al*. 2005). The G2019S mutation results from a 6055 G → A transition in exon 41, causing an amino acid substitution (Gly to Ser) in the conserved kinase domain of the protein.

A direct causal relationship between G2019S and PD has not yet been established. However, the fact that numerous studies worldwide have shown that the mutation is common in PD, nearly absent in controls, and segregates closely with PD in families, leaves little doubt that the mutation is associated with PD. The frequency of the G2019S mutation in PD ranges from 1% to over 30%, depending primarily on the ethnic background of the patients. Studies performed in the United States and Europe have consistently found a mutation frequency of 1–6.6% in Caucasian PD patients (Di Fonzo *et al*. 2005; Gilks *et al*. 2005; Kachergus *et al*. 2005; Kay *et al*. 2006; Nichols *et al*. 2005). In Ashkenazi Jews (Ozelius *et al*. 2006) and north African Arabs (Lesage *et al*. 2006), the mutation frequency is as high as 18% and 30%, respectively. The mutation frequency is higher in patients who report a family history of PD than in those with apparently sporadic disease (Kay *et al*. 2006). G2019S is an old mutation that appears on few ancestral haplotypes, one of which dates back to 2,250 years ago (Goldwurm *et al.* 2005; Kachergus *et al.* 2005; Lesage *et al.* 2005; Zabetian *et al.* 2006b.) Many PD patients with this mutation have no known family history of PD (Gilks *et al*. 2005). Age at onset in patients with G2019S has ranged from 28 to 86 years (Kay *et al*. 2006; Lesage *et al*. 2005a; Zabetian *et al*. 2005). The factors responsible for the variation in age at onset are unknown.

G2019S is rare in non-PD populations. Among several thousand controls who have been screened to date, only 4 unaffected individuals over the age of 60 with G2019S have been documented (Kay *et al*. 2005; Lesage *et al*. 2005a; Ross *et al*. 2006). There are also reports of unaffected G2019S carriers in familial PD kindreds (Aasly *et al*. 2005; Di Fonzo *et al*. 2005; Kachergus *et al*. 2005; Kay *et al*. 2006; Khan *et al*. 2005; Lesage *et al*. 2005a; Paisan-Ruiz *et al*. 2005; Gaig *et al*. 2006). Although most asymptomatic G2019S carriers are younger than the average age at onset in the family, there are a few cases that have remained unaffected to advanced age (Kachergus *et al*. 2005; Lesage *et al*. 2005a; Paisan-Ruiz *et al*. 2005; Gaig *et al*. 2006). G2019S has also been tested in patients with Alzheimer's disease (AD) and other neurodegenerative disorders (Hernandez *et al*. 2005; Toft *et al*. 2005; Zabetian *et al*. 2006a). AD was the most likely to be associated with G2019S because AD and PD overlap in their clinical and pathological presentations and a genetic link between the two disorders has long been suspected. Studies of G2019S in AD, with a combined sample size of over 2000 patients, failed to detect a single G2019S mutation. One of the studies alone had greater than 95% power to detect a mutation, even if the frequency of G2019S in AD was 1/10 of its frequency in PD (Zabetian *et al*. 2006a).

Due to its relatively high frequency in PD and virtual absence in controls and other neurodegenerative disorders, G2019S has been proposed as a gene test for PD. The gene test is now available in Europe (http://www.genetests.org) and is imminent in the United States. The aim of the present study was to assess clinical validity and utility of a G2019S molecular test for the diagnosis of PD in symptomatic individuals in the setting of movement disorder clinics. We calculate sensitivity, specificity, positive predictive value, and positive likelihood ratio of the G2019S test overall, and, stratified by family history, age at disease onset, age at testing and gender. We also address the utility of a G2019S test for risk assessment and predictive diagnosis of asymptomatic individuals, which relies not only on the clinical validity estimates presented here, but also on age-specific penetrance, which is not yet well established. Finally, we discuss the social, ethical, and legal implications of a gene test when there is no prevention or improved treatment.

MATERIALS AND METHODS

Study subjects

Subjects studied included 1518 individuals with PD and 1733 individuals without PD. Patients were recruited from seven movement disorder clinics at academic institutions and their affiliated clinics in Portland, OR, Seattle, WA, and Albany, NY. As participants in the NeuroGenetics Research Consortia, these clinics have used standardized protocols for diagnosis, patient selection, and enrollment (Kay *et al*. 2006). All patients carried a clinical diagnosis of PD by a movement disorder neurologist according to the UK Parkinson's Disease Brain Bank criteria, except that family history was not used as an exclusion criterion (Hughes *et al*. 1992). Patients were enrolled sequentially, regardless of the presence or absence of a family history or age at disease onset. Approximately 85% of subjects who were invited to participate gave consent and were enrolled. Age at onset was defined as the age at which the subject noticed the first PD symptom. Late-onset PD was defined as onset after 50 years and early-onset as onset at age 50 or younger. Unaffected subjects were recruited as controls for genetic studies of PD from the same settings as patients. All controls were free of neurodegenerative disease by self-report. A subset of controls (25%) was personally examined and confirmed to be free of any neurological or neurodegenerative disease. None of the subjects used in the present study was genetically related as far as could be determined. Ethnicity and race categories were defined according to the National Institutes of Health (NIH) guidelines

and presented to subjects for self-assignment. Most PD patients and most controls have been described previously (Kay *et al*. 2006; Zabetian *et al*. 2005). Informed consent, approved by the Institutional Review Board of each participating institution, was obtained from all subjects prior to enrollment.

Molecular analysis

Genomic DNA was extracted from peripheral blood using standard methods. All samples were genotyped using a TaqMan assay on an ABI PRISM 7900HT sequence detection system (Applied Biosystems, Foster City, CA). Primer and probe sequences and assay conditions are available upon request from the corresponding author. Twenty six percent of the subjects (546 consecutive PD patients and 281 consecutive controls) were analyzed by direct sequencing of exon 41 as well as by the TaqMan method (Zabetian *et al*. 2005). In addition, all mutations detected by the TaqMan assay were verified by direct sequencing.

Statistical analysis

Sensitivity, defined as the percentage of PD patients who are detected by the test, was calculated as the number of G2019S mutation carriers with PD (true-positives) divided by the total number of patients with or without G2019S (sum of true-positives and false-negatives). Specificity, defined as the percentage of unaffected individuals (controls) who were correctly classified by the test as not having the disease, was calculated as the number of unaffected cases who lacked G2019S (true-negatives) divided by the total number of unaffected individuals (sum of false-positives and true-negatives). Positive predictive value, defined as the likelihood that an individual with a positive test result has PD, was calculated as the number of true-positives divided by the sum of true-positives and false-positives. Positive likelihood ratio was defined as the ratio of the probability of G2019S-positive tests among those who have PD to the probability of G2019S positive tests among those who do not have PD. Positive likelihood ratio was calculated as sensitivity/(1-specificity). Confidence intervals were calculated using the efficient-score method corrected for continuity and described by Newcombe (1998).

RESULTS

In the total sample of 3251 subjects analyzed, 22/6502 chromosomes had a G2019S mutation. All 22 mutations were confirmed by sequencing. Twenty-one of the mutations were in 20 PD patients, and one was in an unaffected individual. One patient was homozygous with onset at age 62, a positive family history of PD, and typical PD with no obvious distinguishing features (Kay *et al*. 2006). One unaffected individual had the mutation; he was determined by neurological examination to be free of PD at age 89 (Kay *et al*. 2005) and remains unaffected at age 90. Characteristics of the PD patients who were mutation carriers did not differ significantly from the overall clinic PD population by gender, race/ethnicity, age, or age at onset, but a larger proportion of the mutation carriers had a family history of PD than the clinic series (55% vs. 23.4%; Table 1). The mutation was observed in nearly equal frequencies in early-onset and late-onset PD (1.7% vs. 1.2%; Table 2). The highest mutation frequency was seen in patients with an affected first degree relative (4.8%). The odds ratio for patients with a familial first degree history of PD was 86.6 (Table 2).

Using a clinical diagnosis of PD as the outcome, we investigated the ability of a G2019S mutation test to predict the outcome correctly. The first level of analysis was performed regardless of age, family history, or gender. Specificity was high (99.9%), but sensitivity was poor (1.3%; Table 3A). The positive predictive value was 95.2% and the positive likelihood ratio was 22.8. These data demonstrate excellent test ability to predict disease when the mutation is present, but poor distinguishing power between PD and unaffected individuals when the mutation is not found. The second level of analysis was performed by stratifying the subjects by family history of PD (Table 3B), age at testing (Table 3C), age at onset (Table 3D), or gender (Table 3E). Only family history improved test performance. Positive likelihood ratio increased from 13.4 for in-

Table 1. Subject Characteristics

	PD	*Unaffected*
All subjects		
Number of individuals studied	1518	1733
Male	67.4%	39.4%
Caucasian	94.7%	93.7%
Positive family history (PD in 1st or 2nd degree relative)	23.4%	—
Age at onset, mean years ± SD (range)	58.0 ± 12.0 (14–90)	—
Age at blood draw for testing, mean years ± SD (range)	67.9 ± 10.5 (30–93)	70.7 ± 16.1 (21–109)
G2019S mutation carriers		
N	20	1
Male	65%	100%
Caucasian	90%[a]	100%
Positive family history (PD in 1st or 2nd degree relative)	55%	0%
Age at onset, mean years ± SD (range)	53.9 ± 12.1 (28–71)	—
Age at blood draw, mean years ± SD (range)	66.3 ± 9.6 (49–82)	85

[a]One subject was Caucasian/Hispanic, and another was of mixed race.

Table 2. G2019S Carrier Frequency and Odds Ratios

	G2019S carriers/Total[a]	*OR (95% CI)*	p
Unaffected Subjects			
All	1/1733	Reference for A-G	—
Age ≤50 years	0/211	Reference for H	—
Age >50 years	1/1521	Reference for I	—
Male	1/682	Reference for J	—
Female	0/1049	Reference for K	—
PD patients			
A. All	20/1518	23.1 (3.1–172.5)	$<10^{-4}$
B. Familial PD (1st or 2nd degree)	11/355	55.4 (7.1–430.4)	$<10^{-4}$
C. Familial PD (1st degree)	11/231	86.6 (11.1–674.0)	$<10^{-4}$
D. Familial PD (2nd degree)	0/124	—	—
E. Non-familial PD	9/1163	13.5 (1.7–106.8)	0.002
F. Onset Age ≤50 years	7/419	29.4 (3.6–239.8)	$<10^{-4}$
G. Onset Age >50 years	13/1095	20.8 (2.7–159.3)	$<10^{-4}$
H. Age ≤50 years	1/107	—	—
I. Age >50 years	19/1411	20.7 (2.8–155.2)	$<10^{-4}$
J. Male	13/1023	8.8 (1.1–67.2)	0.01
K. Female	7/495	—	—

[a]Age at blood draw was unknown for 1 control subject, gender was unknown for 2 controls, and age at onset was unknown for 4 patients.

OR, odds ratio; CI, 95% confidence interval.

dividuals without a family history of PD to 82.5 for individuals who had a parent or sibling with PD.

DISCUSSION

Molecular testing for G2019S is simple, inexpensive, highly specific, and straightforward to interpret when used for diagnosis in symptomatic individuals. As a diagnostic test for PD, G2019S will have excellent specificity, but poor sensitivity. The high specificity reflects the high penetrance of the mutation. Through the stratification of groups, the G2019S genetic test was shown to be most useful for individuals who have a family history of PD in a first-degree relative, as was demonstrated by a six-fold increase in positive likelihood ratio. Stratification by gender, age at onset of disease, or by age at the time of test-

Table 3. Sensitivity, Specificity, Positive Predictive Value, and Positive Likelihood Ratio of the G2019S Test[a]

	SS	*SP*	*PPV*	*PLR*
A. Clinic population overall				
PD overall	1.3 (0.8–2.1)	99.9 (99.6–>99.9)	95.2 (74.1–99.8)	22.8 (3.1–169.9)
B. Stratified by family history[b]				
Familial PD, 1st degree	4.8 (2.5–8.6)	99.9 (99.6–>99.9)	91.7 (59.8–99.6)	82.5 (10.7–636.2)
Nonfamilial PD	0.8 (0.4–1.5)	99.9 (99.6–>99.9)	90.0 (54.1–99.5)	13.4 (1.7–105.7)
C. Stratified by age[c]				
≤50 years	0.9 (0.05–5.8)	100 (97.8–100)	100 (5.5–100)	∞
>50 years	1.3 (0.8–2.1)	99.9 (99.6–>99.9)	95.0 (73.1–99.7)	20.5 (2.7–152.8)
D. Stratified by age at onset[d]				
≤50 years	1.7 (0.7–3.6)	99.9 (99.6–>99.9)	87.5 (46.7–99.3)	29.0 (3.6–234.7)
>50 years	1.2 (0.7–2.1)	99.9 (99.6–>99.9)	92.9 (64.2–99.6)	20.6 (2.7–157.1)
E. Stratified by gender[e]				
Male	1.3 (0.7–2.2)	99.9 (99.1–>99.9)	92.9 (64.2–99.6)	8.7 (1.1–66.1)
Female	1.4 (0.6–3.0)	100 (99.5–100)	100 (56.1–100)	∞

[a]SS, sensitivity; SP, specificity; PPV, positive predictive value; PLR, positive likelihood ratio. SS, SP, and PPV are shown as percentages. 95% confidence intervals are in parentheses.

[b]Patients were classified as familial first degree or nonfamilial, and each group was compared to all controls.

[c]Patients and controls were classified by age at blood draw, and the comparisons were made between young patients versus young controls, and older patients versus older controls.

[d]Patients were classified by their age at onset, and each group was compared to all controls.

[e]Male controls were compared to male patients, and female controls were compared to female patients.

ing did not improve the test outcome. Sensitivity of the assay was consistently low in all analyses. Poor sensitivity is due to the fact that the vast majority of PD cases are caused by factors that remain unknown. We suspect that sensitivity would be significantly higher in Ashkenazi Jews and Arabs, because up to 30% of PD in these ethnic groups may be due to G2019S (Lesage *et al*. 2006; Ozelius *et al*. 2006).

This study was intended to provide a general indication for the validity and utility of a G2019S test in a movement disorder clinic setting. Caution should be exercised in applying the values from this paper to other populations because the emerging evidence suggests that G2019S frequency may vary significantly across ethnic groups and geographic locations (Kay *et al*. 2006; Tan *et al*. 2005; Lesage *et al*. 2006; Ozelius *et al*. 2006). The estimates presented here were calculated using patient populations from several movement disorder clinics on the east coast and the west coast of the United States. Despite the geographic distance between the clinics, the findings were similar across the sites. Our clinic populations are mostly Caucasian of mixed European origin. None of the clinics sees a preponderance of any specific ethnic group.

Diagnostic testing

One potential utility of a genetic test is in improved accuracy of diagnosis, especially in complicated cases. Using pathological diagnosis as the gold standard, the accuracy of clinical diagnosis of idiopathic PD by movement disorder specialists is between 70% and 98% (Hughes *et al*. 2002; Litvan *et al*. 2003). Hughes *et al*. (2002) summarized the literature and concluded that a clinical accuracy of 90% may approximate the maximum that can be expected. They also make a crucial point: 30% of the initial diagnoses of parkinsonism, including 25% of initial diagnoses of idiopathic PD, change to a different diagnosis during the course of the disease. Even for the skilled movement disorder specialist, it may take years before a final clinical diagnosis can be established. Pathological diagnosis, which is the current gold standard, defines a heterogeneous syndrome known as idiopathic PD, but cannot distinguish the different etiologic entities that comprise idiopathic PD. Molecular gene tests like G2019S can identify the individual disease entities at their specific molecular roots. A simple blood test for G2019S, if positive, can confirm the diagnosis of PD within the first week of presentation. Early and accurate diagnosis can be important for the well-being of the patient and their families, because it will spare them the uncertainty and the anguish of a changing diagnosis. A gene test may also improve prognostic prediction for patients. It has been suggested that *LRRK2*-related PD might represent a more benign form of PD (Nichols *et al*. 2005). If confirmed to be generally true, physicians could provide G2019S patients with some optimism. At this time, the gene test would not help to improve efficacy of treatment, patient care, or prognosis, although this may change as the function of the gene is better understood and targeted treatments are developed.

Predictive testing

The utility of a G2019S molecular test for predictive testing and risk assessment in asymptomatic individuals is less straightforward than its use for symptomatic diagnosis. The power of G2019S as a predictive test will rely not only on the sensitivity and specificity, but also on the age-dependent penetrance, which is not yet well established. Currently, risk assessment for PD is based on empirical data. The lifetime risk of developing PD is 1–2% (Elbaz *et al*. 2002). The risk is increased three-fold for individuals who have a positive family history of late-onset PD, and by nearly eight-fold for those with a family history of early-onset PD (Payami *et al*. 2002). Hence, the empirical lifetime risk for an individual assessed on a family-by-family basis can range from 1% to 16%. For families in which PD is caused by an autosomal dominant mutation (such as G2019S), the *a priori* risk for first-degree relatives of a mutation carrier is 50%. In such families, a molecular gene test can determine if an at-risk family member is a mutation carrier, in which case PD risk increases from 50% to up to possibly 100%, depending on age and penetrance. In a person who lacks the mutation, risk will decrease from 50% to the population baseline of 1–2%.

Accurate estimates of age-dependent penetrance of the G2019S mutation are required to determine the age-specific probability of developing PD if the mutation is present. Early studies estimated penetrance as 15% at age 40, rising to 85% by age 70, and nearly 100% by age 80 (Kachergus *et al*. 2005; Lesage *et al*. 2005a; Di Fonzo *et al*. 2006). The sample sizes, however, were small and confidence intervals were large. There have since been reports of 3 individuals with G2019S who have lived past age 80 without developing signs of PD (Kay *et al*. 2005; Gaig *et al*. 2006), hence penetrance is not 100% by age 80. Another challenge in predictive testing is the age at PD onset in G2019S carriers, which can vary by more than half a century. There is currently no explanation for the wide variation in time of symptom onset. In sum, the presence of G2019S in an asymptomatic individual will predict a substantially increased risk of PD, but it does not guarantee that the individual will develop PD, nor can it predict age at onset.

Ethical, legal, and social implications

The ethical, legal, and social implications of the G2019S test are in many ways similar to those that have been well documented for Huntington's Disease (Paulson and Prior 1997) and AD (Steinbart *et al*. 2001), which are often seen as the paradigm for genetic testing for late-onset neurodegenerative disorders. These issues include, but are not limited to, the requirements for individual and familial consent and concerns about medical privacy and confidentiality of genetic data with respect to employment and insurability. Although a G2019S genetic test may be straightforward for diagnostic purposes in symptomatic individuals, a positive test result for one person may reveal crucial information about others who are not involved in the testing process. For example, a positive test result for one individual may reveal that a parent is an obligate mutation carrier, and thus every sibling would have a 50% chance of carrying the mutation. Some families may resent such information, as they may not have considered the disease to be hereditary and did not seek or give consent to receive such information. Other families may embrace the opportunity and seek presymptomatic predictive testing. Some families may become divided, with some relatives seeking testing and others not. Test results, both positive and negative, may have a profound psychological impact on the individual and family mem-

bers, particularly given the high specificity but low sensitivity of the assay. If positive, the main concerns become the unknown penetrance, the wide variability and unpredictability of age at onset of symptoms, and the current lack of prevention or disease-modifying therapies. This may change as targeted therapies against G2019S dysfunction are developed, but currently, no special treatment or cure can be offered. A main benefit of asymptomatic testing is for research to identify asymptomatic mutation carriers for follow up to study the disease process by imaging and searching for biomarkers. Another benefit of the G2019S test would be to allow individuals believed to be at high risk for hereditary PD to plan for the future, armed with the knowledge of whether or not they carry this autosomal dominant mutation. Carriers of the mutation, for example, may wish to undergo prenatal or even preimplantation genetic testing to avoid passing the G2019S mutation to future generations. Of course, the selective termination of a fetus that may develop a progressive neurodegenerative disorder in adulthood is itself a subject of considerable debate. Careful consideration of these and other ethical issues and proper counseling with the individual and family prior to testing is therefore essential.

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