

## Penetrance Estimate of *LRRK2* p.G2019S Mutation in Individuals of Non-Ashkenazi Jewish Ancestry

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**ABSTRACT: Background:** Penetrance estimates of the leucine-rich repeat kinase 2 (*LRRK2*) p.G2019S mutation for PD vary widely (24%-100%). The p.G2019S penetrance in individuals of Ashkenazi Jewish ancestry has been estimated as 25%, adjusted for multiple covariates. It is

unknown whether penetrance varies among different ethnic groups. The objective of this study was to estimate the penetrance of p.G2019S in individuals of non-Ashkenazi Jewish ancestry and compare penetrance between Ashkenazi Jews and non-Ashkenazi Jews to age 80.

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**Methods:** The kin-cohort method was used to estimate penetrance in 474 first-degree relatives of 69 non-Ashkenazi Jewish *LRRK2* p.G2019S carrier probands at 8 sites from the Michael J. Fox *LRRK2* Cohort Consortium. An identical validated family history interview was administered to assess age at onset of PD, current age, or age at death for relatives in different ethnic groups at each site. Neurological examination and *LRRK2* genotype of relatives were included when available.

**Results:** Risk of PD in non-Ashkenazi Jewish relatives who carry a *LRRK2* p.G2019S mutation was 42.5% (95% confidence interval [CI]: 26.3%-65.8%) to age 80, which is not significantly higher than the previously estimated 25% (95% CI: 16.7%-34.2%) in Ashkenazi

Jewish carrier relatives. The penetrance of PD to age 80 in *LRRK2* p.G2019S mutation carrier relatives was significantly higher than the noncarrier relatives, as seen in Ashkenazi Jewish relatives.

**Conclusions:** The similar penetrance of *LRRK2* p.G2019S estimated in Ashkenazi Jewish carriers and non-Ashkenazi Jewish carriers confirms that p.G2019S penetrance is 25% to 42.5% at age 80 in all populations analyzed. © 2017 International Parkinson and Movement Disorder Society

**Key Words:** *LRRK2*; penetrance; Parkinson's disease

The p.Gly2019Ser (p.G2019S) is the most frequently reported mutation in leucine-rich repeat kinase 2 (*LRRK2*) and occurs in 1% of patients with sporadic PD and 4% of patients with familial PD<sup>1</sup> and is higher among Ashkenazi Jewish PD patients (14.3%-18.8%)<sup>2-5</sup> and North African Berbers (39.3%).<sup>6</sup> Estimating the risk of developing PD for individuals who carry the mutation p.G2019S, (penetrance) by a certain age has important implications for both genetic counseling and clinical trial planning. The penetrance estimates of *LRRK2* p.G2019S for the development of PD vary widely (24%-100%)<sup>7</sup> because of the use of different ethnic groups, gender, recruitment methods, statistical methods, and the presence of genetic or environmental modifiers of age at onset.

We previously estimated the penetrance of *LRRK2* p.G2019S in 2270 first-degree Ashkenazi Jewish relatives from the Michael J. Fox Ashkenazi Jewish *LRRK2* Consortium, including family history data on 652 Ashkenazi Jewish relatives of 129 PD probands with p.G2019S mutation and 1,618 Ashkenazi Jewish relatives of 345 PD probands without the p.G2019S mutation<sup>8</sup> without adjusting for covariates. We subsequently proposed statistical methods to account for multiple covariates simultaneously to refine the penetrance estimate.<sup>9</sup> We reestimated the adjusted penetrance of *LRRK2* p.G2019S in the Ashkenazi Jewish population as 25% (95% confidence interval 16.7%-34.2%)<sup>9</sup> to age 80 and found it to be similar to that reported initially.<sup>8</sup>

To determine whether the penetrance of *LRRK2* p.G2019S differs by ethnicity (ie, Ashkenazi Jews versus non-Ashkenazi Jews), we expanded the data collection to individuals without reported Ashkenazi Jewish ancestry (ie, non-Ashkenazi Jews) through the Michael J. Fox *LRRK2* consortium. We aimed to compare the penetrance of p.G2019S in individuals without reported Ashkenazi Jewish ancestry (ie, non-Ashkenazi Jews) to the penetrance estimate derived from the individuals of Ashkenazi Jewish ancestry, adjusted for multiple covariates. The penetrance

estimates were used to design a hypothetical PD protective trial testing disease modification.

## Methods

### Participants

Our study builds on the Michael J. Fox *LRRK2* Cohort Consortium established in 2009<sup>10</sup> to examine the genetic and environmental factors associated with disease onset in first-degree relatives of PD probands with p.G2019S mutations (relatives of carrier probands) and relatives of PD probands without p.G2019S mutations (relatives of noncarrier probands). Written informed consent was obtained from each proband and institutional review boards at each site approved the protocol. All PD probands were required to have 4 non-Ashkenazi Jewish grandparents. We used the same valid and reliable structured family history questionnaires<sup>11</sup> across sites and the ascertainment scheme did not depend on sampling based on reporting a positive family history of PD. The PD probands were contacted once and completed a valid family history interview, either in person or over the telephone, and provided information on each of their first-degree relatives. PD probands were genotyped for *LRRK2* p.G2019S. Most of the relatives were not genotyped due to lack of resources to collect blood samples in all family members or death of older relatives (eg, parents). Key information for each relative included demographics such as year of birth, current age or age at death, gender, ethnicity, PD status, age at onset of PD, and genotype if known. The PD proband in each family was excluded from the penetrance estimation to avoid ascertainment bias.<sup>12</sup>

A total of 69 non-Ashkenazi Jewish PD probands were recruited at 8 sites and family history interviews were obtained on 474 first-degree non-Ashkenazi Jewish relatives including the French PD Genetic

**TABLE 1.** Demographics and disease characteristics of non-Ashkenazi Jewish first-degree relatives of *LRRK2* p.G2019S carrier probands

Relatives, n = 474	Relatives of p.G2019S carrier probands
Age, years (SD), n	57.0 (20.4), 429
Age at onset PD, years (SD), n	67.6 (10.7), 45
Male, n = 228	
Age, years (SD), n	57.1 (21.0), 211
Age at onset PD, years (SD), n	68.3 (9.2), 17
Female, n = 246	
Age, years (SD), n	56.9 (19.8), 218
Age at onset PD, years (SD), n	67.1 (11.7), 28
Parents, n = 127	
Age, years (SD), n	75.4 (13.5), 105
Age at onset PD, years (SD), n	69.4 (12.2), 22
Siblings, n = 220	
Age, years (SD), n	58.4 (18.0), 197
Age at onset PD, years (SD), n	65.8 (8.9), 23
Children, n = 127	
Age, years (SD), n	39.6 (12.8), 127
Age at onset PD, years (SD), n	– (–), 0

*LRRK2*, leucine-rich repeat kinase 2; SD, standard deviation.

network, France (n = 173 relatives from 21 PD probands); Barcelona, Spain (n = 151 relatives from 24 PD probands); and 6 other smaller sites contributing 150 relatives from 24 PD probands (California and Indiana; Tübingen, Germany; Milan, Italy; San Sebastian, Spain; Toronto, Canada; see Supplementary Tables 1 and 2 and Supplementary Figure 1). Probands recruited from Indiana University were seen at multiple sites within the United States. There were 389 relatives recruited from clinic-based samples and 85 from population-based or community-based samples. The characteristics of relatives and PD probands are presented in Table 1, Supplementary Tables 1, 2, and 3.

We estimated the penetrance of *LRRK2* p.G2019S in the non-Ashkenazi Jewish population using 474 relatives of 69 carrier probands, adjusting for multiple covariates.<sup>9</sup> We compared the penetrance estimate of *LRRK2* p.G2019S in first-degree relatives of non-Ashkenazi Jewish PD probands to Ashkenazi Jewish PD probands and used a newly developed statistical method that accounted for covariates (eg, demographics or risk factors of PD) simultaneously,<sup>9</sup> thereby improving the precision of the estimates.

### Statistical Analysis

Demographics and disease characteristics of first-degree non-Ashkenazi Jewish relatives were compared among recruitment sites (France, Spain, and all other sites combined). Demographic and disease characteristics of families and PD probands with and without *LRRK2* p.G2019S mutations were compared using the Student *t* tests, Wilcoxon-Mann-Whitney test,  $\chi^2$  test,

Fisher's exact test, and Kruskal Wallis test, where appropriate. The difference in age at onset of PD between clinic-based and population or community-based samples was tested by log-rank test.

Our method allows incorporation of information such as genotypes of relatives in the model. A total of 51 relatives from 31 families were known to carry *LRRK2* p.G2019S mutations, and 38 relatives from 26 families were known to be noncarriers of *LRRK2* p.G2019S. The genotypes of the remaining 385 relatives were unknown. When most of the genotypes in relatives are not observed, the kin-cohort method<sup>12</sup> estimates the probability of missing *LRRK2* p.G2019S carrier status in the relatives using the mutation status in PD probands and Mendelian inheritance patterns with the prevalence of mutation in relatives. The method incorporates this estimated probability with age at PD diagnosis in the first-degree relatives to estimate the age-specific cumulative risk of PD in p.G2019S carriers and noncarriers using an expectation-maximization algorithm<sup>13</sup> developed to handle missing relatives' genotype data. We assumed healthy non-Ashkenazi Jewish relatives have 0.4% prevalence of *LRRK2* p.G2019S mutation.<sup>4</sup> Covariates in PD probands and relatives such as PD proband's sex, relative's sex, site of enrollment, and recruitment method were adjusted simultaneously to improve the accuracy of the penetrance estimation through a Cox proportional hazard model<sup>9</sup>. A bootstrap resampling method was used to compute confidence intervals of the estimated age-specific penetrance accounting for correlation among relatives in the same family.<sup>14</sup>

We conducted several comparisons based on the estimated penetrance in the non-Ashkenazi Jewish relatives. First, we compared the penetrance of *LRRK2* p.G2019S to age 80 with the PD risk in noncarrier relatives. Second, we examined whether the penetrance of *LRRK2* p.G2019S differed by sex. Third, we estimated the penetrance in parents and siblings. Fourth, we examined the effect of covariates on PD risk.

Next, we compared the penetrance at age 80 in non-Ashkenazi Jewish carrier relatives with previously obtained penetrance in Ashkenazi Jewish carrier relatives<sup>9</sup> using the Wald test at a 5% significance level.

Last, we use penetrance estimates to calculate the sample size needed for a hypothetical 2-arm PD prevention trial comparing the likelihood of diagnosis of PD (phenoconversion). Our penetrance estimates provided design parameters for the placebo arm by computing the probability of developing PD within the next 5 years for individuals who had not developed PD by a given age. Assuming a certain effect size, we estimated the sample size to test the null hypothesis of no difference in the proportion of PD phenoconverters between the placebo arm and the intervention arm to

**TABLE 2.** Cumulative risk of PD to age 80 in non-Ashkenazi Jewish first-degree relatives of *LRRK2* p.G2019S carrier probands

Relatives <sup>a</sup>	Cumulative risk in p.G2019S carrier relatives to age 80, %	Cumulative risk in p.G2019S noncarrier relatives to age 80, %	Cumulative risk in p.G2019S carrier relatives to age 80 compared to noncarrier relatives to age 80	Relatives with PD, n
Total, n = 474	42.5 (26.3-65.8)	2.7 (0.1-10.7)	$P < .001$	45
Male relatives, n = 228	35.2 (17.8-58.4)	2.1 (0.1-7.8)	$P = .001$	17
Female relatives, n = 246	49.3 (30.3-74.3)	3.2 (0.1-13.4)	$P < .001$	28

<sup>a</sup>A total of 89 non-Ashkenazi Jewish relatives were genotyped for the *LRRK2* G2019S mutations with 51 carrier relatives from 31 families and 38 noncarriers from 26 families. The genotype for the rest of 385 relatives were unknown. Abbreviations: *LRRK2*, leucine-rich repeat kinase 2.

achieve 80% power (a 2-sided test with significance level = .05).<sup>9</sup> We considered 2 scenarios for the effect size of the intervention. In scenario 1, the intervention was assumed to reduce the risk of PD to that observed in the noncarrier relatives, essentially eliminating the effect of the mutation completely (100%) and assuming the intervention has no effect on noncarrier relatives. In scenario 2, the intervention was assumed to manifest half of the effect as in scenario 1 (50%).

## Results

### Demographics

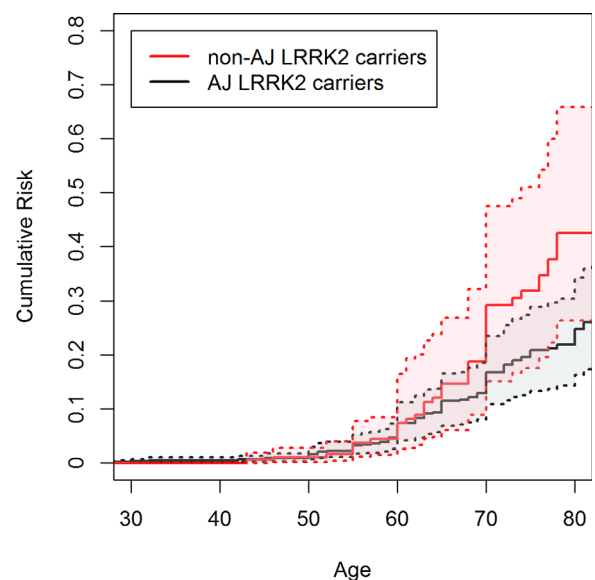
The demographic and clinical characteristics of first-degree non-Ashkenazi Jewish relatives stratified by the recruitment site and the PD proband's mutation status are reported in Table 1 and Supplementary Tables 2 and 3, respectively. The flowchart and distribution of the study population are reported in Supplementary Figure 1 and Supplementary Table 4.

Among the 474 non-Ashkenazi Jewish first-degree relatives of p.G2019S carrier PD probands (ie, 127 parents, 220 siblings, and 127 children), 45 had PD (ie, 22 parents, 23 siblings, and 0 children). The mean age at onset of PD, defined as onset of motor symptoms of PD, was similar in male and female relatives. Gender distribution was similar across the 3 non-Ashkenazi Jewish site groups. There was no significant difference between clinic-based and community- or population-based samples on the age at onset of PD. Among 69 carrier PD probands in the non-Ashkenazi Jewish cohort, 9 PD probands (13%) had families with multiple PD-affected individuals. When adjusted for family size, 11% of family members of carrier PD probands were affected by PD on average (standard deviation = 0.14), which is similar to the Ashkenazi Jewish cohort where 10% of family members of Ashkenazi Jewish carrier PD probands were affected by PD on average (standard deviation = 0.17).

### Penetrance of *LRRK2* p.G2019S

Penetrance estimates (cumulative risk of PD) of *LRRK2* p.G2019S in the non-Ashkenazi Jewish relatives are presented in Table 2 and Supplementary Table 5. The penetrance of p.G2019S to age 80 in

non-Ashkenazi Jewish carrier relatives (42.5%; 95% CI: 26.3%-65.8%) was significantly higher than the noncarrier relatives (2.7%; 95% CI: 0.1%-10.7%,  $P < .001$ ; Fig. 1; hazard ratio of carriers to noncarriers: 20.85, 95% CI: 4.75-829.19,  $P = .005$ ), similar to what we have seen in the Ashkenazi Jewish population. The large confidence interval for the hazard ratio is due to the low hazard rate in the noncarrier relatives. When the male and female relatives were examined separately, the p.G2019S penetrance to age 80 in carrier male relatives (35.2%; 95% CI: 17.8%-58.4%) was not significantly different from female carrier relatives (49.3%; 95% CI: 30.3-74.3%; Supplementary Fig. 3A; hazard ratio of male to female: 0.64, 95% CI: 0.32-1.14,  $P = .11$ ; Table 3). When non-Ashkenazi Jewish parents and siblings of probands were examined separately, the penetrance of p.G2019S to age 80 in carrier parents (39.6%; 95% CI: 21.9%-67.9%) was not significantly different from carrier siblings (45.7%; 95% CI: 26.9%-67.2%,  $P = 0.52$ ; Supplementary Fig. 4A).



**FIG. 1.** Estimated age-specific risk of PD in non-Ashkenazi Jewish *LRRK2* p.G2019S carriers (red solid line) and Ashkenazi Jewish *LRRK2* p.G2019S carriers (black solid line) and their confidence intervals (dashed lines). [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

**TABLE 3.** Estimated hazard ratios of PD onset in non-Ashkenazi Jewish first-degree relatives of *LRRK2* p.G2019S carrier probands

Variable	Estimated hazard ratio	Lower limit	Upper limit	P value
Relative's sex, male versus female	0.635	0.322	1.137	.114
Prognostic factors				
Proband's sex, male versus female	0.900	0.456	1.691	.695
Sites				
France versus others	0.885	0.331	2.254	.776
Barcelona-Spain versus others	0.707	0.259	1.696	.388
France versus Barcelona-Spain	1.252	0.498	3.110	.576
Recruitment scheme				
Clinic versus community- or population-based sample	0.887	0.271	3.667	.955

The Cox proportional hazards model is  $\lambda$  (Carrier status, relative's sex, proband's sex, site, recruitment scheme) =  $\lambda_0(t)$  exp( $\beta$  carrier status +  $\eta$  I(relative's sex = male) +  $\gamma_1$  I(proband's sex = male) +  $\gamma_2$  I(site = France) +  $\gamma_3$  I(site = Barcelona-Spain) +  $\gamma_4$  I(recruitment scheme = clinic-based)). Abbreviations: *LRRK2*, leucine-rich repeat kinase 2.

When examining the effect of the PD proband's sex, site of enrollment, and recruitment method that may modify the PD risk, none of the prognostic factors that we controlled significantly influenced PD risk. The risk of PD was similar between male and female probands, and there was no difference between sites. Moreover, the risk of PD was similar between the clinic-based sample and the population-based or community-based samples (see Table 3). The penetrance estimates in non-Ashkenazi Jewish French and Spanish relatives (the 2 largest sites) were similar to one another and to the rest of the non-Ashkenazi Jewish (see Supplementary Table 5 and Supplementary Fig. 5A).

Last, we compared the penetrance estimates in non-Ashkenazi Jewish relatives to Ashkenazi Jewish relatives (Table 4 and Supplementary Table 6). The penetrance of *LRRK2* p.G2019S for the development of PD to age 80 in non-Ashkenazi Jewish carrier relatives (42.5%; 95% CI: 26.3%-65.8%) was not significantly different from previously estimated in Ashkenazi Jewish carrier relatives (25%; 95% CI: 16.7%-34.2%;  $P = .947$ ).<sup>9</sup> There was no significant difference in penetrance comparing non-Ashkenazi Jewish to Ashkenazi Jewish when stratified by sex.

### Sample Size Estimation for Clinical Trial Testing Disease Modification

The effects size and sample size estimates are presented in supplementary Table 7. When we considered a study of 5 year duration, in scenario 1, a sample size of 111 per arm is required to detect a risk difference in PD conversion of 7.5% (2.3%-12.7%) between placebo arm and intervention arm with a baseline age of 60. In scenario 2, a sample size of 632 per arm is required to detect the difference of 3.8% (1.1%-6.4%) to detect half of the risk reduction between placebo arm and intervention arm with a baseline age of 60. Therefore, a larger sample size is needed for a prevention trial to detect a smaller risk differences with sufficient power.

When we considered a study of 3-year duration at age 60, a sample size of 211 per arm was required to detect a risk difference of 4.0% in scenario 1 and 1,216 per arm was required to detect the difference of 2.0% in scenario 2, which required a larger sample size. We also considered the same sample size calculation with an average recruitment baseline age of 70, which led to a larger required sample size to detect the smaller risk difference.

**TABLE 4.** Comparing cumulative risk of PD to age 80 between non-Ashkenazi Jewish and Ashkenazi Jewish first-degree relatives of *LRRK2* p.G2019S carrier probands

Relatives	G20109S mutation carrier status	Cumulative risk in non-Ashkenazi Jewish <sup>a</sup> relatives to age 80, %	Cumulative risk in Ashkenazi Jewish <sup>b</sup> relatives to age 80, % <sup>9</sup>	Cumulative risk in non-Ashkenazi Jewish relatives to age 80 compared to Ashkenazi Jewish relatives to age 80
All	Carriers	42.5 (26.3-65.8)	25.0 (16.7-34.2)	$P = .106$
	Noncarriers	2.7 (0.1-10.7)	11.0 (8.0-14.7)	$P = .013$
Male	Carriers	35.2 (17.8-58.4)	21.5 (9.0-35.6)	$P = .268$
	Noncarriers	2.1 (0.1-7.8)	15.2 (10.5-20.6)	$P < .001$
Female	Carriers	49.3 (30.3-74.3)	28.5 (18.8-39.4)	$P = .098$
	Noncarriers	3.2 (0.1-13.4)	6.6 (4.0-9.7)	$P = .394$

<sup>a</sup>A total of 89 non-Ashkenazi Jewish relatives were genotyped for the *LRRK2* G2019S mutations with 51 carrier relatives from 31 families and 38 noncarriers from 26 families. The genotype for the rest of the 385 relatives were unknown.

<sup>b</sup>A total of 158 Ashkenazi Jewish relatives were genotyped for the *LRRK2* G2019S mutations with 90 carrier relatives from 59 families and 68 noncarriers from 47 families. The genotype for the rest of the 2,112 relatives were unknown. Abbreviations: *LRRK2*, leucine-rich repeat kinase 2.

## Discussion

We have determined that the p.G2019S penetrance for the development of PD to age 80 in non-Ashkenazi Jewish carrier relatives is 42.5% (95% CI: 26.3%-65.8%), which is similar to previously estimated in Ashkenazi Jewish carrier relatives 25% (95% CI: 16.7%-34.2%).<sup>9</sup> Estimates of *LRRK2* p.G2019S penetrance have been reviewed in previous studies and range from 24% to 100%.<sup>7</sup> Clark and colleagues<sup>3</sup> reported a lifetime penetrance of 24% up to age 80 (95% CI: 13.5%-43.7%) in 2,975 carrier and noncarrier relatives of 459 PD cases and 2,044 relatives of 310 control probands using the kin cohort method,<sup>15</sup> similar in Ashkenazi Jewish and non-Ashkenazi Jewish cases. In contrast, Healy and colleagues<sup>1</sup> reported a risk of 28% at age 59, 51% at 69, and 74% at 79 for the p.G2019S mutation in 1,045 mutation carriers from 133 families from 24 populations worldwide. The upper bound of the confidence interval for the current study is 65.8%, which is lower than the 74% reported by Healy and colleagues. Our recent study of *LRRK2* p.G2019S penetrance in Ashkenazi Jews included 474 PD probands from 3 site groups. The lifetime risk of PD was estimated to be 26% by age 80,<sup>8</sup> and after adjusting for demographic or clinical characteristics of PD probands or relatives, the lifetime risk of PD was estimated to be 25% by age 80,<sup>9</sup> almost identical to the earlier report in Clark and colleagues.<sup>3</sup> A recent study in Tunisia,<sup>16</sup> North Africa, known to have a high burden of *LRRK2* p.G2019S parkinsonism, estimated the penetrance in *LRRK2* p.G2019S carriers to be 91% by age 80. The high *LRRK2* p.G2019S penetrance may have arisen due to the exclusion of asymptomatic carrier relatives from their estimation.<sup>1,16</sup>

The strengths of our study include, first, the use of the sampling scheme that recruits probands regardless of their family history of PD. This differs from other studies that included only families with multiple relatives who developed PD. Second, to date this is the first and largest study to use the identical family history interview in different ethnic groups at multiple sites to assess the age-specific risk of PD.<sup>8</sup> By using a valid and reliable family history interview,<sup>11</sup> we were able to estimate the non-Ashkenazi Jewish *LRRK2* p.G2019S penetrance among several ethnic groups, including 5 sites in Europe and 2 in the United States. This aids in the comparison of penetrance estimates to previous PD populations. Third, the probands were recruited from clinic-based, population-based, or community-based cohorts, but most of the patients were sampled through clinical centers. The additional recruitment of patients in population-based or community-based samples would allow the extension of penetrance estimates to broader groups. Last, we controlled for covariates

simultaneously. This methodology facilitates obtaining more accurate and refined penetrance estimates.

One limitation of this study is the relatively small number of non-Ashkenazi Jewish participants, leading to a wide confidence interval for the penetrance estimates. The upper limit of the 95% confidence interval for the penetrance in Ashkenazi Jewish carrier relatives is 34.2%, which is slightly below the estimated penetrance in non-Ashkenazi Jewish carrier relatives (42.5%). It is conceivable that with a larger sample size, a lower penetrance in Ashkenazi Jews when compared with non-Ashkenazi Jews could be significant. For example, we would need at least 1,806 non-Ashkenazi Jewish relatives to detect a significant difference in penetrance between non-Ashkenazi Jewish carrier relatives (42.5%) and Ashkenazi Jewish carrier relatives (the upper confidence limit of Ashkenazi Jewish carrier relatives reaches 34.2%). The PD probands were recontacted for the family history interview sometimes years after initial recruitment. The validated family history was administered only once. We were unable to contact all PD probands in the previous study. The reasons for a small number of non-Ashkenazi Jewish PD probands and relatives in our study includes the short recruitment period and practical difficulties in reaching probands and relatives. Another limitation is that the actual genotype information was not available in all relatives. Although we used our new kin-cohort method to include the actual genotypes in relatives, when available, higher precision would be expected if the estimation were based on the actual relatives' genotype. An additional limitation is that we did not control for environmental risk factors in the relatives (eg, cigarette smoking<sup>17</sup>) or common mutations that are associated with PD such as mutations in the glucocerebrosidase (*GBA*)<sup>18</sup> gene in the non-Ashkenazi Jews. However, none of the non-Ashkenazi Jewish PD probands who were genotyped for the *GBA* mutation carried the *GBA* mutations ( $n = 36$ ). Excluding the remaining 33 probands with missing *GBA* mutation status would lead to a small sample size; therefore, we included all of the probands into the analysis. In terms of the noncarrier group, our cumulative risk of *LRRK2* p.G2019S to age 80 in non-Ashkenazi Jewish noncarriers (2.7%; 95% CI: 0.1%-10.7%) was slightly but not significantly higher than the general population (1.7%),<sup>19</sup> and lower than Ashkenazi Jewish noncarrier relatives (11.0%; 95% CI: 8.0%-14.7%,  $P = .008$ ).<sup>9</sup> We did not include 22 non-Ashkenazi Jewish relatives of 3 noncarrier probands because we did not have sufficient samples to represent relatives from noncarriers. Our estimation of the PD risk in the non-carrier group is obtained solely from relatives of *LRRK2* carrier probands, which may explain a slightly higher PD risk when compared with the general population. The significantly higher PD

risk in Ashkenazi Jewish noncarriers than non-Ashkenazi Jewish noncarriers ( $P = .013$ ) may indicate that there are other genetic modifiers in the Ashkenazi Jewish population that may increase the PD risk.

The number of non-Ashkenazi Jewish samples required to achieve a power of 80% for a 3-year prevention trial is large (scenario 2). To design an efficient trial, investigators need to enrich samples based on other risk factors in addition to the *LRRK2* p.G2019S mutation. International studies such as the Parkinson's Progression Markers Initiative<sup>20</sup> were launched to identify additional biomarkers for predicting PD susceptibility and progression in the prodromal phase so that individuals at the highest risk for PD can be identified and recruited. ■

## References

1. Healy DG, Falchi M, O'Sullivan SS, et al. Phenotype, genotype, and worldwide genetic penetrance of *LRRK2*-associated Parkinson's disease: a case-control study. *Lancet Neurol* 2008;7:583-590.
2. Ozelius LJ, Senthil G, Saunders-Pullman R, et al. *LRRK2* G2019S as a cause of Parkinson's disease in Ashkenazi Jews. *N Engl J Med* 2006;354:424-425.
3. Clark LN, Wang Y, Karlins E, et al. Frequency of *LRRK2* mutations in early- and late-onset Parkinson disease. *Neurology* 2006;67:1786-1791.
4. Orr-Urtreger A, Shifrin C, Rozovski U, et al. The *LRRK2* G2019S mutation in Ashkenazi Jews with Parkinson disease: is there a gender effect? *Neurology* 2007;69:1595-1602.
5. Alcalay RN, Mirelman A, Saunders-Pullman R, et al. Parkinson disease phenotype in Ashkenazi Jews with and without *LRRK2* G2019S mutations. *Mov Disord* 2013;28:1966-1971.
6. Hulihan MM, Ishihara-Paul L, Kachergus J, et al. *LRRK2* Gly2019Ser penetrance in Arab-Berber patients from Tunisia: a case-control genetic study. *Lancet Neurol* 2008;7:591-594.
7. Goldwurm S, Tunesi S, Tesei S, et al. Kin-cohort analysis of *LRRK2*-G2019S penetrance in Parkinson's disease. *Mov Disord* 2011;26:2144-2145.
8. Marder K, Wang Y, Alcalay RN, Mejia-Santana H, et al. Age-specific penetrance of *LRRK2* G2019S in the Michael J. Fox Ashkenazi Jewish *LRRK2* Consortium. *Neurology* 2015;85:89-95.
9. Lee A, Marder K, Alcalay RN, et al. Estimation of genetic risk function with covariates in the presence of missing genotypes. *Stat Med* 2017 (in press).
10. Marras C, Alcalay RN, Caspell-Garcia C, et al. Motor and non-motor heterogeneity of *LRRK2*-related and idiopathic Parkinson's disease. *Mov Disord* 2016;31:1192-1202.
11. Marder K, Levy G, Louis ED, et al. Accuracy of family history data on Parkinson's disease. *Neurology* 2003;61:18-23.
12. Wang Y, Liang B, Tong X, et al. Efficient estimation of nonparametric genetic risk function with censored data. *Biometrika* 2015;102:515-532.
13. Laird NM, Ware JH. Random effects models for longitudinal data. *Biometrics* 1982;38:963-974.
14. Liang B, Wang Y, Zeng D. GSSE: Genotype-Specific Survival Estimation. R package. <https://cran.r-project.org/web/packages/GSSE/index.html>. 2015. Accessed on October 20, 2015.
15. Wacholder S, Hartge P, Struwing JP, et al. The kin-cohort study for estimating penetrance. *Am J Epidemiol* 1998;148:623-630.
16. Trinh J, Amouri R, Duda JE, et al. A comparative study of Parkinson's disease and leucine-rich repeat kinase 2 p.G2019S parkinsonism. *Neurobiol Aging* 2014;35:1125-1131.
17. Ritz B, Ascherio A, Checkoway H, et al. Pooled analysis of tobacco use and risk of Parkinson disease. *Arch Neurol* 2007;64:990-997.
18. Sidransky E, Lopez G. The link between the GBA gene and parkinsonism. *Lancet Neurol* 2012;11:986-998.
19. Pringsheim T, Jette N, Frolkis A, Steeves TD. The prevalence of Parkinson's disease: a systematic review and meta-analysis. *Mov Disord* 2014;29:1583-1590.
20. Marek K. The Parkinson Progression Marker Initiative (PPMI). *Prog Neurobiol* 2011;95:629-635.