

# Meta-analysis of Parkinson's Disease: Identification of a Novel Locus, *RIT2*

Nathan Pankratz, PhD,<sup>1</sup> Gary W. Beecham, PhD,<sup>2</sup> Anita L. DeStefano, PhD,<sup>3,4</sup> Ted M. Dawson, MD, PhD,<sup>5</sup> Kimberly F. Doherty, PhD,<sup>5</sup> Stewart A. Factor, DO,<sup>6</sup> Taye H. Hamza, PhD,<sup>7</sup> Albert Y. Hung, MD, PhD,<sup>8,9</sup> Bradley T. Hyman, MD, PhD,<sup>8,10</sup> Adrian J. Ivinson, PhD,<sup>10</sup> Dmitri Krainc, MD, PhD,<sup>8,9</sup> Jeanne C. Latourelle, DSc,<sup>3</sup> Lorraine N. Clark, PhD,<sup>11</sup> Karen Marder, MD,<sup>11</sup> Eden R. Martin, PhD,<sup>2</sup> Richard Mayeux, MD,<sup>11</sup> Owen A. Ross, PhD,<sup>12</sup> Clemens R. Scherzer, MD,<sup>10,13,14</sup> David K. Simon, MD, PhD,<sup>15</sup> Caroline Tanner, MD, PhD,<sup>16</sup> Jeffery M. Vance, MD, PhD,<sup>2</sup> Zbigniew K. Wszolek, MD,<sup>12</sup> Cyrus P. Zabetian, MD,<sup>17</sup> Richard H. Myers, PhD,<sup>3</sup> Haydeh Payami, PhD,<sup>7</sup> William K. Scott, PhD,<sup>2</sup> and Tatiana Foroud, PhD<sup>1</sup> and the PD GWAS Consortium

**Objective:** Genome-wide association (GWAS) methods have identified genes contributing to Parkinson's disease (PD); we sought to identify additional genes associated with PD susceptibility.

**Methods:** A 2-stage design was used. First, individual level genotypic data from 5 recent PD GWAS (Discovery Sample: 4,238 PD cases and 4,239 controls) were combined. Following imputation, a logistic regression model was employed in each dataset to test for association with PD susceptibility and results from each dataset were meta-analyzed. Second, 768 single-nucleotide polymorphisms (SNPs) were genotyped in an independent Replication Sample (3,738 cases and 2,111 controls).

**Results:** Genome-wide significance was reached for SNPs in *SNCA* (rs356165; G: odds ratio [OR] = 1.37;  $p = 9.3 \times 10^{-21}$ ), *MAPT* (rs242559; C: OR = 0.78;  $p = 1.5 \times 10^{-10}$ ), *GAK/DGKQ* (rs11248051; T: OR = 1.35;  $p = 8.2 \times 10^{-9}$ )/rs11248060; T: OR = 1.35;  $p = 2.0 \times 10^{-9}$ ), and the human leukocyte antigen (HLA) region (rs3129882; A: OR = 0.83;  $p = 1.2 \times 10^{-8}$ ), which were previously reported. The Replication Sample confirmed the associations with *SNCA*, *MAPT*, and the HLA region and also with *GBA* (E326K; OR = 1.71;  $p = 5 \times 10^{-8}$  Combined Sample) (N370; OR = 3.08;  $p = 7 \times 10^{-5}$  Replication sample). A novel PD susceptibility locus, *RIT2*, on chromosome 18 (rs12456492;  $p = 5 \times 10^{-5}$  Discovery Sample;  $p = 1.52 \times 10^{-7}$  Replication sample;  $p = 2 \times 10^{-10}$  Combined Sample) was replicated. Conditional analyses within each of the replicated regions identified distinct SNP associations within *GBA* and *SNCA*, suggesting that there may be multiple risk alleles within these genes.

**Interpretation:** We identified a novel PD susceptibility locus, *RIT2*, replicated several previously identified loci, and identified more than 1 risk allele within *SNCA* and *GBA*.

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Address correspondence to Dr Tatiana Foroud, Indiana University School of Medicine, 410 W. 10th Street (HS 4000), Indianapolis, IN 46202. E-mail: [tforoud@iupui.edu](mailto:tforoud@iupui.edu)

From the <sup>1</sup>Indiana University School of Medicine, Indianapolis, IN; <sup>2</sup>University of Miami Miller School of Medicine, Miami, FL; <sup>3</sup>Boston University School of Medicine, Boston, MA; <sup>4</sup>Boston University School of Public Health, Boston, MA; <sup>5</sup>Johns Hopkins University School of Medicine, Baltimore, MD; <sup>6</sup>Emory University, Atlanta, GA; <sup>7</sup>New York State Department of Health Wadsworth Center, Albany, NY; <sup>8</sup>Massachusetts General Hospital, Boston, MA; <sup>9</sup>Harvard Medical School, Harvard Medical School, Boston, MA; <sup>10</sup>Harvard NeuroDiscovery Center Biomarker Program, Harvard Medical School, Boston, MA; <sup>11</sup>Columbia University School of Medicine College of Physicians and Surgeons, New York, NY; <sup>12</sup>Mayo Clinic, Jacksonville, FL; <sup>13</sup>Partners Parkinson Center, Boston, MA; <sup>14</sup>Center for Neurologic Diseases, Brigham & Women's Hospital, Boston, MA; <sup>15</sup>Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA; <sup>16</sup>Parkinson's Institute, Sunnyvale, CA; <sup>17</sup>VA Puget Sound Health Care System and University of Washington, Seattle, WA.

Additional Supporting Information can be found in the online version of this article.

Members of the PD GWAS Consortium are listed in the Appendix.

Parkinson's disease (PD) is the second most common adult-onset neurodegenerative disorder worldwide.<sup>1</sup> Five genes have been identified with mutations that result in Mendelian forms of PD; however, mutations have been found in fewer than 5% of individuals with PD, suggesting additional genes contribute to disease risk.<sup>2</sup> Many candidate gene studies and several genome-wide association studies (GWASs) have been performed to identify risk factors for PD, with growing evidence for the role of *SNCA*, *MAPT*, *GBA*, *GAK/DGKQ*, and the human leukocyte antigen (HLA) region in disease susceptibility.<sup>3–12</sup> Two recent studies found evidence for association with additional loci, including *ACMSD*, *STK39*, *MCCC1/LAMP3*, *SYT11*, *CCDC62/HIP1R*, *STX1B*, *FGF20*, *STBD1*, *GPNMB*, and *PARK16*.<sup>11,13</sup> However, there is evidence that there are additional loci yet to be identified.

## Materials and Methods

### Discovery Sample

To identify additional genes associated with PD, we combined publicly available genotype level GWAS data obtained from dbGaP<sup>4,6</sup> along with 2 new datasets that are not yet publicly available and were obtained directly from the investigator who performed the GWAS.<sup>5,7,8</sup> All datasets employed standard UK Brain Bank criteria<sup>14</sup> for the diagnosis of PD, with a modification to allow the inclusion of cases that had a family history of PD. This modification was made because it is believed that familial PD cases may have a stronger genetic contribution than sporadic PD, making them potentially more informative for genetic studies. PD cases with a reported age of onset below 18 years of age were removed from the dataset ( $n = 17$ ). When data were available, any PD cases known to harbor a causative mutation, either 2 *parkin* mutations or a single *LRRK2* mutation, were excluded from further analysis ( $n = 57$ ).

An Illumina genotyping array (Illumina, San Diego, CA) was used by all studies. Individual level genotypic data was available and reviewed across studies to identify sample duplicates (see Supplemental Methods). Prior to performing imputation, each study was subjected to rigorous quality review and data cleaning (see Supplemental Methods for more details) and principal component analysis was used to control for population stratification. Imputation was then performed for all autosomes using MACH 1.0 (Li and colleagues<sup>15</sup>). The 2.5 million HapMap2 single-nucleotide polymorphisms (SNPs) were analyzed using ProbABEL (<http://www.genabel.org/packages>) and a logistic regression model, that included sex and age, when appropriate (see Supplemental Methods). Meta-analysis was performed with METAL (<http://www.sph.umich.edu/csg/abecasis/Metal>) using an inverse-variance weighting scheme. This allowed an overall effect size to be estimated. Genomic control was employed so that results were down-weighted if the study's lambda exceeded 1.00. The Discovery Sample was large enough to have 80% power to detect relative risks as small as 1.14 to

1.18 with a relatively common risk allele (minor allele frequency [MAF] 0.2–0.35).

### SNP Selection for Replication Genotyping

A custom Illumina genotyping array was designed with 768 SNPs that included the following:

- *Known causative mutations*: SNPs that genotyped 2 common *LRRK2* mutations in European populations (G2019S and R1441H);
- *Known risk factors*: *GBA* (N370S, L444P, E326K, T369M);
- Previous GWAS associations: *PARK16*,<sup>6,12</sup> *LRRK2*,<sup>6,12</sup> *SNCA*,<sup>5–8,12</sup> *MAPT*,<sup>5–8</sup> *GAK*,<sup>5,8</sup> and the HLA region<sup>8</sup>;
- *Sex confirmation*: Three SNPs on the Y chromosome and 6 SNPs on the X chromosome in addition to the sex-specific probes included in the GoldenGate custom oligonucleotide pool;
- *Top priority association results from the meta-analysis*: SNPs were selected based on increasing  $p$ . A SNP was removed from consideration if it was in linkage disequilibrium (LD) ( $r^2 > 0.80$ ) with a SNP having a smaller  $p$  or had an Illumina design score less than 0.40 (if  $p < 1 \times 10^{-5}$ ) or 0.60 (if  $p \geq 1 \times 10^{-5}$ ). This approach identified 619 SNPs (all  $p < 3.2 \times 10^{-4}$ ). In addition, 28 additional SNPs were selected in the highest priority regions ( $p < 1 \times 10^{-5}$ ), in case 1 of the SNPs in these regions failed quality assessment after being genotyped on the replication array (eg, call rate  $< 0.98$ , divergence from Hardy-Weinberg equilibrium (HWE) in controls  $p < 0.0001$ ); and
- *Ancestry informative markers (AIMs)*: SNPs were selected based on fixation indices ( $F_{ST}$ ) between the Ashkenazi and British population clusters as defined using annotated results from Eigenstrat (see Supplemental Fig. 1). Markers were then ranked based on how well they differentiated between the 2 subpopulations, and 100 were selected in a manner similar to the 619 replication SNPs. A SNP was excluded from further consideration if it was in LD ( $r^2 > 0.05$ ) with any marker with a larger  $F_{ST}$  or if it had an Illumina design score less than 0.80. Samples were genotyped by the Genetic Resource Core Facility SNP Center at Johns Hopkins University using Illumina GoldenGate chemistry<sup>16</sup> and a custom panel of 768 SNPs (GS0012520-OPA) (see Supplemental Methods).

### Replication Sample

The independent Replication Sample included 3,738 PD cases and 2,111 controls. Samples were obtained either from an established repository (Coriell Repositories or National Cell Repository for Alzheimer Disease) that assured the samples had appropriate consent for sample and data sharing or directly from the investigator who had collected the sample, and whose study was approved by the appropriate Human Subject Committee at their institution. All samples included in the Replication Sample were reported as white, non-Hispanic. All cases underwent a neurological evaluation that employed PD

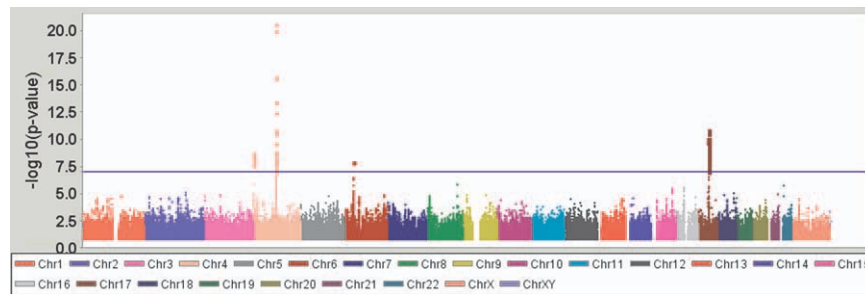


FIGURE 1: Genome-wide association results for PD susceptibility.

diagnostic criteria based broadly on the UK PD Society Brain Bank Criteria,<sup>17</sup> although modified to allow a positive family history of PD. Three cases reported an age of onset  $\leq 18$  years and were excluded from further study. When information was available, cases were excluded if they were known to harbor a causative mutation (either 2 *parkin* mutations or a single *LRRK2* mutation). Controls were selected, when possible, from the same study that also provided cases. Based on self-report, the control subjects did not have a personal history of PD.

The first level of data review focused on genotyping quality (SNP completeness). The second level focused on which samples and which SNPs would be included in analyses. The multidimensional scaling (MDS) algorithm implemented in PLINK (<http://pngu.mgh.harvard.edu/~purcell/plink>) was performed using the 100 AIMs and all other independent SNPs (SNPs with  $r^2 > 0.30$  were not included) to confirm that all samples were indeed white and non-Hispanic. Samples with an *LRRK2* mutation were removed from further analysis, as were any that were cryptically identical to an individual in the Discovery sample. More details are available in the Supplemental Methods.

We utilized the same logistic regression model used in the initial meta-analysis to analyze the Replication Sample. The initial analysis included the 619 SNPs designed to replicate our top priority association results. Unlike the Discovery Sample, in which each study included both cases and controls, the Replication Sample included some studies providing both cases and controls, while others provided only cases or only controls. Therefore, we could not analyze each sample separately as we had in the Discovery Sample analyses. Rather, the entire Replication Sample was analyzed together. The mean age at exam of the controls was later than the mean age at onset of the cases; therefore, we did not include age in the logistic regression model. There were statistically significant sex differences between the cases and controls. Therefore, the final analytic model included both sex as well as 1 principal component to adjust for the population stratification due to the disproportionate Ashkenazi Jewish ancestry of the cases. All analyses were performed using PLINK. Odds ratios (ORs) and  $p$  values were computed to assess the strength of the association. After excluding AIMs and considering LD between SNPs as implemented in SimpleM,<sup>18</sup> there were 530 effectively independent tests,

requiring a corrected threshold of  $p < 9 \times 10^{-5}$  for an association to be considered replicated in the Replication Sample

### Joint Analyses

We performed a meta-analysis to combine the results of the independent Discovery and Replication Samples only for the SNPs successfully genotyped in the Replication Sample. We used the same analytic approach as in the Discovery Sample. An association was considered statistically significant if the  $p$  value in the joint analyses exceeded genome-wide significance ( $p < 5 \times 10^{-8}$ ).

To test the hypothesis that there might be more than 1 risk variant in a particular gene or gene region contributing to the association, we performed conditional analyses. For each statistically significant gene/region, we identified the SNP with the most extreme  $p$  value in the combined Discovery and Replication samples. We then modified the logistic regression model to include not only sex and the principal component covariate, but also the genotype at the most significant SNP. We then reviewed the  $p$  value for the other SNPs in the gene/region to determine if any other SNPs remained statistically significant (gene-wide empirical  $p < 0.05$  using permutation testing) after adjusting for the effect of the most significant SNP. In this way, we could identify genes/regions in which more than 1 SNP provided distinct evidence of association with PD susceptibility.

Ingenuity Pathway Analysis (IPA) software was used to search for biological relationships among the genes meeting genome-wide significance. A gene list (*DGKQ*, *GAK*, the HLA region, *MAPT*, *SNCA*, and *RIT2*) was entered into a "My Pathway" analysis in IPA. Restricting species to human and allowing for findings among chemicals, the Path Explorer tool under the Build tab was used to search among the Ingenuity knowledge base and external databases to identify the shortest pathways among the genes with either no or 1 intervening molecule. Links between genes represent protein-protein interactions or indicate that 1 gene influences phosphorylation of the connected gene.

## Results

### Discovery Sample

The final Discovery Sample used in the meta-analysis included 4,238 PD cases and 4,239 controls (Table 1).

TABLE 1: Summary Properties of the Studies Included in the Meta-analysis

Variable	PROGENI/ GenePD <sup>5</sup>	NIA Phase I <sup>4</sup>	NIA Phase II <sup>6</sup>	HHHG <sup>7</sup>	NGRC <sup>8</sup>	All Studies
Platform	Illumina 370	Illumina 250+300	Illumina 550	Illumina 610/1M/550	Illumina Omni1	
SNPs (n) <sup>a</sup>	324,989	514,260	521,070	484,712	788,882	
Total available (n)	1,739	523	1,206	1,262	3,986	8,716
Cases used in analyses	840	245	618	579	1,956	4,238
Controls used in analyses	862	256	520	619	1,982	4,239
Lambda (genomic inflation)	1.008	1.012	1.015	1.001	1.041	
Cases with family history of PD (%)	100.0	25.5	35.5	25.2	21.75	
Age (direction) <sup>b</sup>	<b>4 × 10<sup>-29</sup></b> (+)	4 × 10 <sup>-6</sup> (-)	<b>0.003</b> (+)	9 × 10 <sup>-50</sup> (-)	8 × 10 <sup>-128</sup> (-)	8 × 10 <sup>-86</sup> (-)
Male (direction) <sup>c</sup>	<b>6 × 10<sup>-16</sup></b> (+)	<b>0.003</b> (+)	<b>5 × 10<sup>-12</sup></b> (+)	<b>3 × 10<sup>-22</sup></b> (+)	<b>3 × 10<sup>-72</sup></b> (+)	<b>2 × 10<sup>-115</sup></b> (+)

The individual level genotypes for PROGENI/GenePD, the NIA Phase I and II, and NGRC are all available through dbGaP.

<sup>a</sup>Number of SNPs in filtered dataset prior to imputation. This is the number of unambiguous (ie, no A/T or C/G pairings) SNPs passing all quality assessment.

<sup>b</sup>A plus sign (+) for age indicates an association between PD risk and older age. Those studies in which the age at onset of the cases was significantly older than the age at exam of the controls are in bold; age was included as a covariate in these studies only.

<sup>c</sup>A plus sign (+) for male indicates an association between higher PD risk and male sex; sex was included as a covariate for all studies. PD = Parkinson's disease; SNP = single-nucleotide polymorphism.

TABLE 2: Five Loci Associated with PD at Genome-wide Significance in the Discovery Set

Locus	Chr	SNP	bp	A1/A2	A1 Freq	Imputed/ Genotyped <sup>a</sup>	Odds Ratio	$p^b$	Direction of Effect in 5 Studies <sup>c</sup>
<i>SNCA</i>	4q	rs356165	90856624	G/A	0.4099	IIII	1.37	$9 \times 10^{-21}$	+++++-----
<i>MAPT</i>	17q	rs242559	42198305	C/A	0.2165	IIII	0.78	$1 \times 10^{-10}$	-----
<i>GAK</i>	4p	rs11248051	848332	T/C	0.1071	GIIG	1.35	$8 \times 10^{-9}$	+++++
<i>DGKQ</i>	4p	rs11248060	954359	T/C	0.1237	GGGG	1.35	$2 \times 10^{-9}$	+++++
HLA region	6p	rs3129882	32517508	A/G	0.4275	GGGG	0.83	$1 \times 10^{-8}$	-+----

<sup>a</sup>Values for imputed (I) or genotyped (G) status.  
<sup>b</sup>Bold indicates genome-wide significance ( $p < 5 \times 10^{-8}$ ).  
<sup>c</sup>Direction of effects are listed in the following order: PROGNI/GenePD, NIA Phase I, NIA Phase II, HIHG, and NGRC.  
Chr = chromosome; HLA = human leukocyte antigen; PD = Parkinson's disease; SNP = single-nucleotide polymorphism.

Meta-analysis was performed combining the results from each dataset to identify SNPs associated with PD susceptibility (Fig. 1, Table 2). Genome-wide significance ( $p < 5 \times 10^{-8}$ ) was reached for SNPs in *SNCA* (rs356165; OR = 1.37;  $p = 9.3 \times 10^{-21}$ ), *MAPT* (rs242559; OR = 0.77;  $p = 1.5 \times 10^{-10}$ ), *GAK* (rs11248051; OR = 1.35;  $p = 8.2 \times 10^{-9}$ )/*DGKQ* (rs11248060; OR = 1.35;  $p = 2.0 \times 10^{-9}$ ), and the HLA region (rs3129882; OR = 1.21;  $p = 1.2 \times 10^{-8}$ ), which have been previously established in PD susceptibility. No other regions exceeded genome-wide thresholds of significance; however, 28 SNPs had association results with  $p < 10^{-5}$  (see Supplemental Table 1 for complete results).

Distinct clusters could be identified based on ancestry (see Supplemental Results). However, none of the genome-wide significant findings could be explained by ancestry. These were tested in 3 ways: (1) adjusting for principal components; (2) adjusting for cluster membership; and (3) stratifying by cluster membership.

### Replication Sample

The Replication Sample is summarized in Table 3. Genotypes were successfully generated for 705 of 768 attempted SNPs (92%). The only notable SNP loss was the *GBA* L444P SNP, which failed to genotype presumably because of the homology with the neighboring pseudogene.

Data was released for 5,794 study samples (>99% of attempted samples) and 123 blinded duplicate study samples. Detailed review of samples was performed to remove samples that were unexpected duplicates, poorly performing, or did not cluster as Caucasian, non-Hispanic (see Supplemental Results). All samples identified with a causative *LRRK2* mutation were eliminated from further analysis (n = 61 cases, 1 control).

Analysis of the Replication Sample confirmed the previously identified associations with *SNCA*, *MAPT*, the HLA region, and *GBA* (Fig. 2; complete results in Supplemental Table 2). Only the *GAK/DGKQ* region was not statistically significant ( $p = 0.01$ ). We replicated a novel locus on chromosome 18 within the *RIT2* gene that is in LD with markers in nearby *SYT4* (rs12456492;  $p = 2 \times 10^{-7}$ ; Fig. 3). Given the regional LD, determining if the underlying functional variation affects 1 gene product vs the other can be difficult to discern, as is the case with the *GAK/DGKQ* locus.

### Joint Analysis

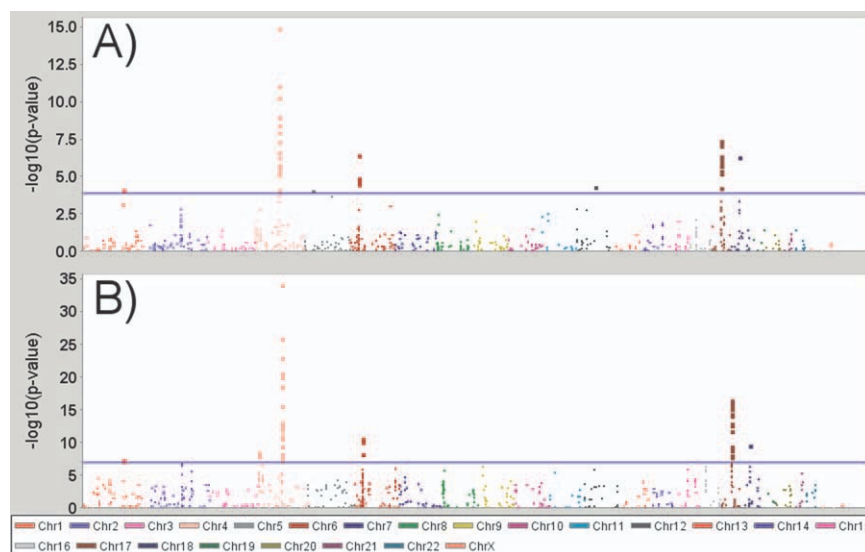
With the power of the joint analysis of the Discovery and Replication Samples, *GBA* now reached genome-wide significance. Many additional SNPs in *GAK/DGKQ*, *SNCA*, the HLA region, and *MAPT* reached significance. Our newly identified locus, *RIT2*, also met genome-wide criteria in this joint analysis (OR = 1.19;  $p = 2 \times 10^{-10}$ ) (Table 4).

To further explore the association results in each gene, we performed conditional analyses in the combined samples. We detected 2 distinct effects within the *GBA* locus (see Table 4). The SNPs with the most extreme  $p$  values in the Combined Sample were rs12726330 and the E326K variant, which both reached genome-wide significance ( $p = 5 \times 10^{-8}$ ). These 2 SNPs are in high LD with each other, so when 1 genotype is included in a logistic regression model, the other becomes nonsignificant. When E326K is included in the logistic model, another SNP remained statistically significant (N370S;  $p < 7 \times 10^{-5}$ ). All results included the principal component that accounts for Ashkenazi ancestry, which controls for the increased incidence of *GBA* mutations in the Ashkenazi population. Moreover, when individuals within

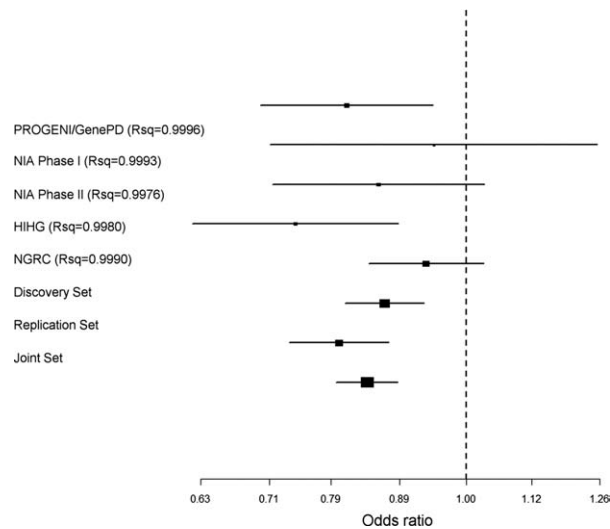
**TABLE 3: Replication Sample**

Study	PD cases (n)	Controls (n)	Total
Harvard NeuroDiscovery Center Biomarker Study <sup>26</sup>	441	247	658
GenePD <sup>27-29</sup>	276	269	545
PROGENI <sup>30</sup>	311	197	508
Search <sup>31</sup>	357	150	507
DATATOP <sup>32</sup>	359	0	359
Partners	358	0	358
LOAD Study <sup>33</sup>	0	450	450
Postcept <sup>34</sup>	318	2	320
Core PD <sup>35</sup>	536	0	536
JHU Udall	125	0	125
NetPD <sup>36</sup>	427	0	427
Mayo Clinic Jacksonville	74	87	161
Other samples (from Coriell)	186	709	895
Total recruited	3,738	2,111	5,849
	<b>Cases</b>	<b>Controls</b>	<b>p</b>
Total number analyzed (n)	3,223	2,035	
Male:female ratio	2,069:1,154	897:1,138	$1 \times 10^{-46}$
Age at onset (case)	$56.3 \pm 12.2$		$1 \times 10^{-78a}$
Age at evaluation	$65.6 \pm 10.2$	$64.1 \pm 15.2$	0.0003

<sup>a</sup>Comparison made to age at evaluation of controls.  
PD = Parkinson's disease.



**FIGURE 2: Manhattan plot of results. (A) Replication sample results alone (B) meta-analyzed with the discovery sample; the blue line indicates the study-wide significance level ( $p < 9.4 \times 10^{-5}$  for the replication stage alone;  $p < 5 \times 10^{-8}$  for the meta-analysis).**



**FIGURE 3: Forest plot of the novel RIT2 SNP (rs12456492).** Rsq values are a measure of imputation quality generated by MACH that range from 0 to 1, with 1 being highly accurate.

the Ashkenazi cluster were excluded from the Discovery Sample and the Replication Sample, the association to rs12726330 and to E326K remained at genome-wide significance (Discovery Sample: 3,792 cases, 3,842 controls,  $p = 2 \times 10^{-5}$ ; Replication Sample: 3,025 cases, 1,931 controls,  $p = 0.0005$ ; Combined Sample:  $p < 5 \times 10^{-8}$ ).

We detected 2 distinct associations at the *SNCA* locus (see Table 4); 1 association that is tagged by rs356220 and the other tagged by rs356198. The second association, tagged by rs356198, still exceeded genome-wide significance when conditioning on rs356220 ( $p = 5 \times 10^{-9}$ ). Our results are corroborated by other studies which identified independent associations within *SNCA*.<sup>9,10,12</sup> See Supplementary Methods and Supplementary Table 6 for more information.

We assessed the biological relationships among the genome-wide significant genes identified in our study (Fig. 4). Paths between genes represent protein–protein interactions or phosphorylation. This network suggests that *GAK* and *RIT2* may be part of the same disease pathway as *MAPT* and *SNCA*, while *DGKQ* and the HLA region may influence risk of PD via another mechanism.

## Discussion

We performed a large meta-analysis including 2 studies not included in any reported meta-analysis. The Discovery and Replication samples were well characterized and established criteria were utilized for the diagnosis of PD. Both sporadic and familial PD cases were included. Cases with a known causative mutation were excluded (ie,

*LRRK2* mutation; 2 *parkin* mutations). Using a rigorous 2-stage design, we identified a novel locus, *RIT2*, associated with PD susceptibility. In addition, we also replicated loci previously associated with PD, including *GAK*, *SNCA*, the HLA region, and *MAPT*. Pathway analyses suggest that *GAK* and *RIT2* may be part of the same disease pathway as *MAPT* and *SNCA*, while *DGKQ* and the HLA region may influence risk via another mechanism.

We detected genome-wide significant evidence of association to *RIT2*, a gene proposed in previous studies but which did not meet stringent statistical criteria as a risk factor for PD. The protein encoded by human *RIT2* binds to the product of human calmodulin 1 (phosphorylase kinase, delta) *CALM1*.<sup>19</sup> Of note, *CALM1* binds to human *SNCA* and *MAPT*.<sup>20,21</sup> Comparison of gene expression in brain tissue from neuropathologically confirmed PD cases and controls demonstrates reduced expression of *RIT2* in the remaining portion of the substantia nigra.<sup>22</sup> Results from our GWAS, pathway analysis, and expression studies provide supporting biological evidence that *RIT2* acts as a PD gene and suggest a starting point for functional analysis.

We also explored the role of *GBA* variants in PD susceptibility. E326K is sometimes considered a benign polymorphism, since in the homozygous or compound heterozygous state it is not sufficient to cause Gaucher disease. However, results of this study and a previous study<sup>23</sup> indicate that E326K may be a susceptibility allele for PD. Most previous GWAS have not included all known *GBA* mutations in their analyses; for example, N370S is not included or tagged by GWAS arrays. However, we did ensure that this mutation was genotyped in our Replication Sample. Therefore, we were able to test in our Replication Sample for the association of *GBA* mutations and variants with PD susceptibility and then could utilize conditional analyses to determine that it was likely that there is more than 1 genetic factor in *GBA* influencing disease risk. Thus, our results suggest that additional analyses and potential functional studies are warranted to better delineate the role of *GBA* in PD susceptibility.

We also detected evidence of at least 2 distinct genetic effects within *SNCA*, a well-known PD susceptibility gene. While the SNP with the most extreme  $p$  value in the Discovery Sample (rs356165) failed to genotype in the Replication Sample, it is in complete LD ( $r^2 = 1.0$ ) with the most extreme  $p$  value in the Replication sample (rs356220). Moreover, they belong to the same LD block as the top SNP in other studies (rs356219;  $r^2 = 0.96$ ).<sup>11</sup> Two previous studies have reported high LD ( $D' = 0.90$ ) but low intermarker correlation ( $r^2 < 0.10$ ) between the primary *SNCA* finding, rs356220, and the

**TABLE 4: Summary of the Statistically Significant SNPs from the Meta-analysis of the Discovery and Replication Samples and for Conditional Analyses of the Combined Sample**

Region	Markers Tested (n)	Marker	Chr	Position	Alleles (Ref/Other)	Discovery <i>p</i>	Replication			Combined Sample		
							Ref Freq	<i>p</i>	OR (95% CI)	SNPs Tagged (n)	<i>p</i>	
<i>GBA</i>	7	E326K	1	153472791	A/G	$2 \times 10^{-5}$	0.017	0.0009	1.71 (1.55–1.89)	2	$5 \times 10^{-8}$	
		N370S <sup>a</sup>	1	153472258	C/T	NA <sup>b</sup>	0.009	$7 \times 10^{-5}$	3.08 (2.32–4.09)	2	$7 \times 10^{-5}$	
<i>GAK</i>	13	rs11248060	4	954359	T/C	$1 \times 10^{-9}$	0.131	0.045	1.26 (1.21–1.31)	7	$3 \times 10^{-9}$	
<i>SNCA</i>	33	rs356220	4	90860363	T/C	$9 \times 10^{-21}$	0.414	$1 \times 10^{-15}$	1.38 (1.34–1.42)	12	$8 \times 10^{-35}$	
		rs356198 <sup>a</sup>	4	90901527	A/G	$4 \times 10^{-5}$	0.175	$2 \times 10^{-5}$	0.82 (0.79–0.84)	3	$5 \times 10^{-9}$	
HLA region	24	rs2395163	6	32495787	C/T	$3 \times 10^{-7}$	0.197	$1 \times 10^{-5}$	0.81 (0.78–0.84)	11	$3 \times 10^{-11}$	
<i>MAPT</i>	40	rs199515	17	42211804	G/C	$2 \times 10^{-11}$	0.187	$4 \times 10^{-7}$	0.76 (0.74–0.79)	29	$3 \times 10^{-17}$	
<i>RIT2</i>	8	rs12456492	18	38927378	G/A	$4 \times 10^{-5}$	0.340	$5 \times 10^{-7}$	1.19 (1.16–1.22)	6	$2 \times 10^{-10}$	

<sup>a</sup>Indicates a conditional analysis that includes the most significant SNPs in the region as a covariate (additive model); odds ratios and *p* for these SNPs are from the conditional analysis. The same conditional analysis was then performed in the Discovery Sample and meta-analyzed.

<sup>b</sup>N370S was not tagged in the discovery set and could not be included in a conditional analysis of that dataset.

Chr = chromosome; CI = confidence interval; Freq = frequency; HLA = human leukocyte antigen; OR = odds ratio; Ref = reference; SNP = single-nucleotide polymorphism. Bold indicates study-wide significance for each phase of analysis:  $p < 5 \times 10^{-8}$  for the Discovery phase,  $p < 9 \times 10^{-5}$  for the replication phase, and  $p < 5 \times 10^{-8}$  for the Combined analyses. SNPs tagged (n) refers to the number of SNPs in the region where the *p*-value was less than 0.05 in the Combined Sample before conditioning on the index SNP and where the *p*-value was greater than 0.05 in the conditional analyses.



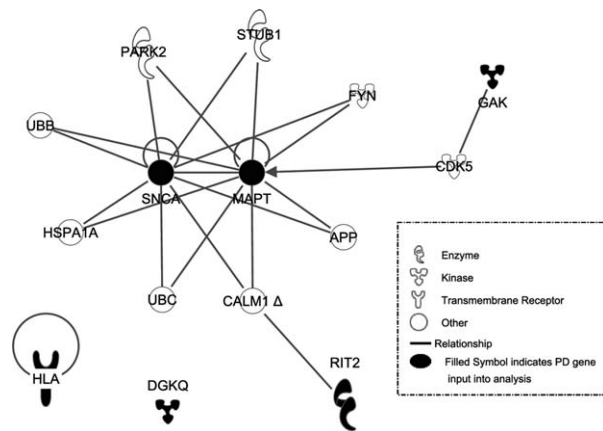


FIGURE 4: Ingenuity analysis.

deleterious Rep1-263 allele.<sup>24,25</sup> Rep1 is a microsatellite marker with 3 predominant alleles (259, 261, and 263) that has consistently been associated with PD risk and often with age at onset. The independent signal reported here, rs356198, is in high LD with the inversely associated Rep1-259 allele (in the PROGENI dataset:  $D' = 0.92$ ,  $r^2 = 0.48$ ). It is possible that the 2 independent SNPs are tagging a functional effect of Rep1 or that Rep1 is not functional, but merely tagging the same underlying causal variant(s) as the 2 SNPs.

The SNP with the most extreme  $p$  value in the HLA region (rs3129882) was the same SNP identified in the NGRC sample that initially reported this association.<sup>8</sup> This is to be expected, because that study is included in our meta-analysis. This SNP was successfully genotyped in the Replication Sample, but was not statistically significant ( $p = 0.92$ ). Rather, a different SNP was statistically significant in the Replication Sample (rs2395163;  $p = 1 \times 10^{-5}$ ) and reached genome-wide significance in the Combined Sample ( $p = 3 \times 10^{-11}$ ). LD is high between the 2 SNPs ( $D' = 0.92$ ), but the correlation was low due to differing allele frequencies (MAF for rs3129882 = 0.433; MAF for rs2395163 = 0.197;  $r^2 = 0.25$ ). The allele frequency of rs2395163 is closer to that seen in the variant with the most extreme  $p$  value in the another recent meta-analysis of PD<sup>11</sup> (MAF for chr6:32588205 = 0.15) and is in moderate to high LD with that SNP in the 1000 Genomes data ( $D' = 1.00$ ;  $r^2 = 0.71$ ). There is evidence that rs2395163 and chr6:32588205 tag the same LD block and that the association with these SNPs is independent of the original rs3129882 finding (see Supplementary Table 5 and Supplementary Methods).

Recently, another group reported results from a meta-analysis of several existing GWAS.<sup>11,13</sup> Two of the Discovery Samples are in common in both studies and

there is some overlap among our Replication samples, although the extent is difficult to quantify. There are several regions in common between studies. For example, both our study and theirs confirmed the association of *GAK*, *SNCA*, the HLA region, and *MAPT*. The 2 recent meta-analyses reported 10 new loci: *ACMSD*, *STK39*, *MCCC1/LAMP3*, *SYT11*, *CCDC62/HIP1R*, *PARK16*, *NMD3*, *STBD1*, *GNPMB*, *FGF20*, *MMP16*, and *STX1B*. Of note, *SYT11* is within the same LD block on chromosome 1 as *RIT1*, whereas *RIT2* is within the same LD block on chromosome 18 as *SYT4*. It remains to be seen which of these genes harbors the true susceptibility alleles and if they have an interaction within a common pathway leading to PD pathogenesis. Supplemental Table 3 summarizes the results in our study for the SNPs in the 10 new loci. We have nominal significance ( $p < 0.05$ ) for all but 1 of these SNPs. Similar ORs in the same direction and for the same allele as presented in the original article was observed when analyses were limited to the 2 datasets not included in the original manuscript (Husman Institute for Human Genomics [HIHG] and NeuroGenetics Research Consortium [NGRC]), and all but 3 SNPs remained nominally significant.

Comparing our results to those of recent GWASs, 1 other previously reported locus could be replicated by our analyses. *BST1* has been seen in multiple datasets.<sup>8,9,12</sup> Although association to this locus did not meet our genome-wide criteria in either the Discovery or Combined Sample analyses, our results for SNPs in this gene did meet established criteria for replication of a previously reported association. The SNP rs4698412 had a  $p$  value of 0.002 in our Discovery Sample,  $5 \times 10^{-5}$  in our Replication Sample, and  $3 \times 10^{-7}$  in the Combined Sample.

In summary, we completed a meta-analysis of existing available PD GWAS datasets and identified a novel susceptibility locus, *RIT2*, and confirmed the association of several known genes. Using our Replication and Discovery Samples, conditional analyses confirmed that in 2 genes, there are multiple risk alleles that have distinct effects on disease risk. These results have important implications as studies are being designed to sequence these regions to identify all potentially functional disease-associated variants.

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### Potential Conflicts of Interest

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## Appendix: PD GWAS Consortium

### **PSG-PROGENI Investigators and Coordinators**

Albany Medical College: S Factor, D Higgins, S Evans; Barrow Neurological Institute: H Shill, M Stacy, J Danielson, L Marlor, K Williamson; Baylor College of Medicine: J Jankovic, C Hunter; Beth Israel Deaconess Medical Center: D Simon, P Ryan, L Scollins; Beth Israel Medical Center: R Saunders-Pullman, K Boyar, C Costan-Toth, E Ohmann; Brigham & Women's Hospital: L Sudarsky, C Joubert; Brown University (Memorial Hospital of RI): J Friedman, K Chou, H Fernandez, M Lannon; Cleveland Clinic Florida-Weston: N Galvez-Jimenez, A Podichetty, K Thompson; Clinical Neuroscience Center: P Lewitt, M DeAngelis; Colorado Neurological Institute: C O'Brien, L Seeberger, C Dingmann, D Judd; Columbia University Medical Center: K Marder, J Fraser, J Harris; Creighton University: J Bertoni, C Peterson; Evanston Northwestern Healthcare: M Rezak, G Medalle; Hotel-Dieu Hospital-Chum: S Chouinard, M Panisset, J Hall, H Poiffaut; Hunter Homes McGuire Veterans Medical Center: V Calabrese, P Roberge; Indiana University School of Medicine: J Wojcieszek, J Belden; Institute For Neurodegenerative Disorders: D Jennings, K Marek, S Mendick; Johns Hopkins University: S Reich, B Dunlop; London Health Sciences Centre: M Jog, C Horn; Mayo Clinic Jacksonville: R Uitti, M Turk; McFarland Neurosciences: T Ajax, J Mannerter; Medical College of Georgia: K Sethi, J Carpenter, B Dill, L Hatch, K Ligon, S Narayan; Medical College of Wisconsin: K Blindauer, K Abou-Samra, J Petit; Medical University of Ohio: L Elmer, E Aiken, K Davis, C Schell, S Wilson; Mount Sinai School of Medicine: M Velickovic, W Koller (deceased), S Phipps; North Shore-LIJ Health System: A Feigin, M Gordon, J Hamann, E Licari, M Marotta-Kollarus, B Shannon, R Winnick; Northwestern University: T Simuni, A Vide-

novic, A Kaczmarek, K Williams, M Wolff; Ochsner Clinic Foundation: J Rao, M Cook; Ohio State University: M Fernandez, S Kostyk, J Hubble, A Campbell, C Reider, A Seward; Oregon Health & Science University: R Camicioli, J Carter, J Nutt, P Andrews, S Morehouse, C Stone; Ottawa Hospital Civic Site: T Mendis, D Grimes, C Alcorn-Costa, P Gray, K Haas, J Vendette; Pacific Neuroscience Medical Group: J Sutton, B Hutchinson, J Young; Saskatoon Dist Health Board Royal Univ Hosp: A Rajput, A Rajput, L Klassen, T Shirley; Scott & White Hospital/Texas A&M University: B Manyam, P Simpson, J Whetteckey, B Wulbrecht; The Parkinson's & Movement Disorder Institute: D Truong, M Pathak, K Frei, N Luong, T Tra, A Tran, J Vo; Toronto Western Hospital, University Health: A Lang, G Kleiner-Fisman, A Nieves, L Johnston, J So; UMDNJ-School of Osteopathic Medicine: G Podskalny, L Giffin; University of Alabama at Birmingham: P Atchison, C Allen; University of Alberta: W Martin, M Wieler; University of Calgary: O Suchowersky, S Furtado, M Klimek; University of California Irvine: N Hermanowicz, S Niswonger; University of California San Diego: C Shults (deceased), D Fontaine; University of California San Francisco: M Aminoff, C Christine, M Diminno, J Hevezi; University of Chicago: A Dalvi, U Kang, J Richman, S Uy, J Young; University of Cincinnati: A Dalvi, A Sahay, M Gartner, D Schwieterman; University of Colorado Health Sciences Center: D Hall, M Leehey, S Culver, T Derian; University of Connecticut: T Demarcaida, S Thurlow; University of Iowa: R Rodnitzky, J Dobson; University of Kansas Medical Center: K Lyons, R Pahwa, T Gales, S Thomas; University of Maryland School of Medicine: L Shulman, S Reich, W Weiner, K Dustin; University of Miami: K Lyons, C Singer, W Koller (deceased), W Weiner, L Zelaya; University of Minnesota: P Tuite, V Hagen, S Rolandelli, R Schacherer, J Kosowicz; University of New Mexico: P Gordon, J Werner; University of Puerto Rico School of Medicine: C Serrano, S Roque; University of Rochester: R Kurlan, D Berry, I Gardiner; University of South Florida: R Hauser, J Sanchez-Ramos, T Zesiewicz, H Delgado, K Price, P Rodriguez, S Wolf-rath; University of Tennessee Health Science Center: R Pfeiffer, L Davis, B Pfeiffer; University of Texas Southwestern Medical Center: R Dewey, B Hayward, A Johnson, M Meacham, B Estes; Wake Forest University School of Medicine: F Walker, V Hunt, C O'Neill; Washington University: B Racette, L Good, M Rundle.

### **GenePD Investigators and Coordinators**

University Southern California School of Medicine: M Lew; University of Calgary: O Suchowersky; University

of Lübeck, Germany: C Klein; UMDNJ-Robert Wood Johnson Medical School: L Golbe, MH Mark; Massachusetts General Hospital, Harvard Medical School: J Growdon, N Huggins; University of Virginia Health System: GF Wooten; University of Alabama at Birmingham: R Watts; University of Toronto: M Guttman; Washington University School of Medicine: B Racette, J Perlmutter; Barrow Neurological Institute: L Marlor; Sun Health Research Institute: H Shill; University of Miami: C Singer; Parkinson Institute, Istituti Clinici di Perfezionamento, Milano, Italy: S Goldwurm, G Pezzoli; Boston University School of Medicine: MH Saint-Hilaire, T Massood; Cleveland Clinic Foundation: K Baker, I Itin; University of Louisville School of Medicine: I Litvan; University of Sydney ANZAC Research Institute, Concord Hospital, Sydney, Australia: G Nicholson, A Corbett; Struthers Parkinson's Center, Minneapolis: M Nance; Port City Neurology, Scarborough, ME: E Drasby; Parkinson's Disease and Movement Disorder Center of Boca Raton: S Isaacson; Newcastle University, Newcastle upon Tyne, UK: D Burn, P Chinnery; General Regional Hospital Bolzano, Bolzano, Italy: P Pramstaller; University of Arkansas for Medical Sciences: J Al-hinti; Aarhus University Hospital, Aarhus, Denmark: A Moller, K Ostergaard; University of Arizona: S Sherman; Auckland City Hospital, Auckland, New Zealand: R Roxburgh, B Snow; University of Kentucky College of Medicine: J Slevin, F Cambi.

#### **NGRC Investigators and Coordinators**

New York State Department of Health Wadsworth Center: D Kay, J Montimurro, V Kusel; VA Puget Sound Health Care System and University of Washington: A Samii, E Martinez, D Yearout; Oregon Health and Sciences University: J Nutt; Evergreen Hospital Medical Center: P Agarwal, A Griffith; Virginia Mason Medical Center: JW Roberts; Samuel Stratton VA Medical Center and Albany Medical Center: DS Higgins. Albany Medical Center: Eric Molho, Emory University: Ami Rosen.

#### **HIHG Investigators and Coordinators**

CA Jauregui, MA Nance, RL Watts, JP Hubble, WC Koller, K Lyons, R Pahwa, MB Stern, A Colcher, BC Hiner, J Jankovic, WG Ondo, FH Allen, Jr., CG Goetz, GW Small, D Masterman, F Mastaglia, BL Scott, C Singer, F Nahab, MA Pericak-Vance, and JL Haines who contributed to this study. Some of the samples used in this study were collected while the Udall PDRCE was at Duke University.

#### **NetPD Investigators and Coordinators**

University of Michigan: K Chou, K Wernette; Rush-Presbyterian-St. Luke's Medical Center: K Shannon, J

Jaglin; Oregon Health Sciences University: J Carter, P Andrews; Baylor College of Medicine: J Jankovic, J Shahed, C Hunter; University of Virginia: FG Wooten, MF Keller; The Parkinson's Institute: C Tanner, T Stewart, C Lawrence, K Smith; University of Miami: C Singer, M Quesada; University of Pennsylvania: A Siderowf, S Reichwein; University of South Florida: R Hauser, S Wolfrath, G Piper; Washington University: B Racette, P Deppen, M Ammel; Johns Hopkins University School of Medicine: T Dawson, B Dunlop; University of Calgary: O Suchowersky, L Derwent; University of Southern California: MF Lew, M Villa; Georgia Health Sciences University: K Sethi, B Dill, S Narayan; Barrow Neurological Institute: R Burns, L Marlor; University of Alabama: RL Watts, R McMurray; Brigham & Women's Hospital: L Sudarsky, G Hage; The Parkinson's & Movement Disorder Institute: D Truong, AH Tran, M Tran; University of California San Francisco: MJ Aminoff, J Roth; Beth Israel Deaconess Medical Center: DK Simon, L Scollins, L Paul, P Rose; Northwestern University: T Simuni, K Williams; University of Texas Southwestern Medical Center: R Dewey, B Hayward, A Johnson, A Gode; University of Colorado Health Sciences Center: M Leehey, J Bainbridge; Duke University Medical Center: B Scott, J Field; LSU Health Science Center Shreveport: R Zweig, C Hilliard; SUNY Downstate Medical Center: I Bodis-Wollner, S Glzman; University of Vermont: R Hamill, C Homan, S Lennox, C Potter; Southern Illinois University: R Elble, C Young, B Lokaitis, D Kelly; Pacific Health Research & Education Institute: GW Ross, S Terashita; Thomas Jefferson University: J Schneider, S Sendek; Thomas Jefferson University/Llankenau Hospital: S Gollomp, R Gollomp, S Raza; Dartmouth Hitchcock Medical Center: DJ Coffey, PR Leblanc; University of Kentucky: F Cambi, R Wagner; Medical University of South Carolina: K Bergman, V Hinson, V Salak; LSU Medical Center: J Rao, M Cook.

#### **CorePD Investigators and Coordinators**

Columbia University: RN Alcalay, E Caccappolo, H Mejia-Santana, M-X Tang, L Rosado, MO Reilly, D Ruiz, B Ross, M Verbitsky, S Kisselev, ED Louis, H Andrews, C Waters, S Fahn, L Cote, S Frucht, B Ford, R Ottman, LN Clark; Rush University: C Comella; University of Pennsylvania: A Colcher, A Siderowf; Institute of Neurodegenerative Disorders: D Jennings; Park Nicollet Clinic: M Nance; Beth Israel: S Bressman; University of Miami: WK Scott; Parkinson's Institute: C Tanner; Marshfield Clinic: S Mickel; Neuroscience Institute at Central DuPage Hospital: M Rezak; Northwestern University: K Novak; Butler Hospital: JH

Friedman; University of Tennessee: R Pfeiffer; Johns Hopkins University: L Marsh; Medical College of Wisconsin: B Hiner.

**LOAD Investigators and Coordinators**

The following investigators and Alzheimer's Disease Centers participated in the Study: Boston University: R Green, N Kowall, L Farrer; Columbia University: J Williamson, V Santana; Duke University: D Schmechel, P Gaskell, K Welsh-Bohmer, M Pericak-Vance; Indiana University: B Ghetti, MR Farlow, K Horner; Massachusetts General Hospital JH Growdon, D Blacker, RE Tanzi, BT Hyman; Mayo Clinic-Rochester: B Boeve, K Kuntz, L Norgaard, N Larson; Mayo Clinic-Jacksonville: D Kistler, F Parfitt, J Haddow; Mount Sinai School of Medicine: J Silverman, M Schnaider Beerl, M Sano, J Wang, R Lally; Northwestern University: N Johnson, M Mesulum, S Weintraub, E Bigio; Oregon Health and Science University: J Kaye, P Kramer, J Payne-Murphy; Rush University: D Bennett, H Jacobs, J-S Chang, D Arends; University of Alabama at Birmingham: L Harrell; University of California, Los Angeles: G Bartzokis, J Cummings, PH Lu, U Toland; University of Kentucky: W Markesbery, C Smith, A Brickhouse; University of Pennsylvania: J Trojanowski, V Van Deerlin, EM Wood; University of Pittsburgh: S DeKosky, R Sweet, E Weamer; University of Southern California: IH Chui, A Varpertian; University of Texas Southwestern: R Diaz-Arrastia, R Rosenberg, B Davis; University of Washington: T Bird, M Rumbaugh, GD Schellenberg, M Raskind; Washington University at St Louis: A Goate, J Morris, J Norton, D Levitch, B Grant, M Coats.

**DATATOP Investigators and Coordinators**

Participating Investigators—University of Kansas: W Koller; University of South Florida: CW Olanow; University of Iowa: R Rodnitzky; Massachusetts General Hospital: JS Fink, JH Growdon; Ohio State University: G Paulson; University of Rochester: R Kurlan; Roger Williams General Hospital: JH Friedman; Oregon Health Sciences University: S Ganchar, J Nutt; University of Saskatchewan: AH Rajput; University of Virginia: JB Bennett, GF Wooten; Sinai Hospital, Detroit: P LeWitt; Rush-Presbyterian-St Luke's Medical Center: C Goetz, C Tanner, K Shannon; University of Calgary: O Suchowersky; Columbia-Presbyterian Medical Center: MF Brin, SB Bressman; University of Miami: WJ Weiner and J Sanchez-Ramos; Baylor College of Medicine: J Jankovic; University of Michigan: JB Penney; Toronto Hospital: A Lang; St Luke's Hospital, Denver: M Hoehn; California Parkinson's Foundation: J Tetrud;

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Site Coordinators—R Barter and B Vetere-Overfield, Kansas City, Kans; L Gauger and T Malapira, Tampa, Fla; J Dobson, Iowa City, Iowa; S Atamian, M Tennis, JB Cohen, G Desclos, and E Hoffman, Boston; L Denio, S Huber, T Woike, K Zoog, R Mendell, and K Dudte, Columbus, Ohio; J Behr, IF Gardiner, Rochester, NY; M Lannon, Providence, RI; J Carter and S Northrup, Portland, Oreg; B Kanigan, Saskatoon, Sask; M Turk and E Landow, Charlottesville, Va; P Schlick and K Mistura, Detroit; VS Carroll and JA Thelen, Chicago; C Demong, Calgary, Alta.; L Winfield and C Moskowitz, New York; A Ingenito, C Sheldon, and L Cornelius, Miami; D Heiberg and C Dunne, Houston; J Brady, Ann Arbor, Mich; C Kierans, L Belle-Scantlebury, and J Duff, Toronto; H Weber, Denver; D Savoini, P Lewis, and SJ Kutner, San Jose, Calif; P Gray, Ottawa; C Glaeske and R Hofman, Omaha, Neb; MM Pay and D Salmon, San Diego, Calif; F McFaul and D Amyot, Montreal; M Bergen, New Brunswick, NJ; L McGee-Minnich, St Louis; P O'Donnell, Baltimore; and S Ferrise and K Shallow, Philadelphia.

Coordination and Data Center—University of Rochester Medical Center: RM Pelusio (program manager); A Rudolph (senior study coordinator); C Miller (nurse clinician); M Linsner, J Connorton, J Nusbaum, and C Casaceli (analyst-programmers); C Irvine, C Orme, and GJ Wixsom (information analysts); M Schirazi, J Sotack, and H Randolph (data-control clerks); R Nobel, D Baker, D LaDonna, ME Rothfuss, L Doerr (deceased), L Rumfola, and B Kavanaugh (secretarial staff); and J Wendel (CLINFO manager).

Biostatistics Center—Department of Biostatistics, University of Rochester Medical Center: C Odoroff (deceased) and D Oakes (chief biostatisticians); M McDermott and S Eberly (biostatisticians); S Plumb (lead programmer); and A Watts, L Yorkey, A Choi, and K Gerwitz (analyst-programmers).

Pharmacy Center—Strong Memorial Hospital: P Evans (chief pharmacist); and L Dellapena and V Singletary (pharmacy technicians).

Safety Monitoring Committee—R Herndon (chair, January 1, 1987, to June 30, 1988), Portland, Oreg; P T-ariot (chair, July 1, 1988, to present), Rochester, NY; and E Bell, RC Griggs, and WJ Hall, Rochester, NY.

Scientific Advisory Committee—CD Marsden (chair), London; TN Chase, Bethesda; G Cohen, J Fleiss, and R Mayeux, New York; L Jacobs and AJ Moss, Rochester, NY; and E Melamed, Tel Aviv, Israel.

Assay Standards Committee—R Roth (chair), New Haven CT; M Galloway, Detroit; I Irwin, San Jose CA; P LeWitt, Detroit; and G Vatassery, Minneapolis.

Neuropsychological Testing Committee—P Como (chair), Rochester, NY; J St Cyr, Toronto; Y Stern and J Williams, New York; and R Wilson, Chicago.

Monitoring Committee, National Institute of Neurological Disorders and Stroke—EM Stadlan (chair), Bethesda; M Alter, Philadelphia; K Bergmann, New Hyde Park, NY; J Cedarbaum, White Plains, NY; J Ellenberg, Bethesda; and R Kibler, Atlanta.

CSF Assay Center—Lafayette Clinic, Wayne State University: M.P. Galloway (director); M. Kaplan (deceased), R. Lodhi, M.J. Keegan, B. Matthews, E.A. Novak.

Deprenyl Metabolites Assay Center—Institute for Medical Research, San Jose CA: I. Irwin (director).

Tocopherol Assay Center—Our Lady of Mercy Medical Center, Bronx, NY: E. Norkus (director).

Specimen Repository—Department of Neurology, University of Rochester: D Flood (director), T McNeill, N Harary, L Koek.

Laboratory Surveillance Testing—SciCor Laboratories, Indianapolis: RL Creveling (director).

### **Massachusetts General Hospital Investigators and Coordinators**

JR Mazzulli, G Bwala.

### **Harvard NeuroDiscovery Center Biomarker Study (HBS)**

Investigators and study coordinators—Harvard NeuroDiscovery Center: CR Scherzer, BT Hyman, AJ Ivinson, NE Maher, AK Sarokhan, KC Lockhart, A Santarlasci; Brigham and Women's Hospital: LR Sudarsky, MT Hayes, E Hart; Massachusetts General Hospital: JH Growdon, MA Schwarzschild, AY Hung, AW Flaherty, D Blacker, A-M Wills, US Sohur, VK Unni, NI Mejia, A Viswanathan, SN Gomperts, MW Albers, KE Swords, RK Rudel, JT Hirschberger; OA Padilla; University of Ottawa: M Schlossmacher; Harvard School of Public Health: A Ascherio; Biogen Idec: BM Ravina.

Data coordination staff—Harvard NeuroDiscovery Center: B Khadka, OA Padilla, B Zheng, JJ Locascio.

Biobank management staff—Harvard NeuroDiscovery Center: SS Roderick, CG Kan, Z Liao.

### **SEARCH Steering Committee Members**

CM Tanner, GW Ross, RD Abbott, JW Langston.

Enrolling Institutions, Investigators and Coordinators (listed by number of exams contributed)—The Parkinson's Institute, Sunnyvale, CA: CM Tanner, D Roucoux, S Yerabati, A Smith; University of South Florida, Tampa, FL: RA Hauser, D Delaney, SC Wolfrath, J Nemeth, T Mclain; Baylor College of Medicine, Houston, TX: J Jankovic, C Hunter, L Shinawi, KR Flores; Emory University School of Medicine, Atlanta, GA: SA Factor, C Ingram; Beth Israel Medical Center, New York, NY: S Bressman, A Deligtisch, K Boyar, A Wolff; Toronto Western Hospital, Toronto, Ontario, Canada: C Marras, M Enjati; University of Kansas Medical Center, Kansas City, KA: K Lyons, C Mack, A Langhammer, T Gales; Veterans Affairs Pacific Islands Health Care System, Honolulu, HI: GW Ross, FK Wakashige.

Parkinson's Institute Coordination, Interviewer and Analytic Staff—M Lu, K Comyns, M Korell, L Sterling, R VanVeghten, M Padua, J Wright, B Allred, L Bushman, M Bynum, C Cage, A Carroll, M Chang, R Cook, E Ferriter, M Franco, K Gorman, M Kemp, S Knutson, S Lavoie, E Mills, C Nguyen, P Niedle, J Pia, B Priestley, V Sims, JA Soetmelk, B Song, B Stang, M Staver, A Stover.

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