Supplementary Information

* + - 1. **Introduction**

While a comprehensive evaluation of interactions of PD susceptibility has yet to be performed, several studies have investigated the interactions between various genes of interest1-4. Although the results of most gene-gene interactions studies in PD to date have pointed toward independent effects for PD susceptibility variants, an exception to this has been assessments of functional-genetic interaction between *LRRK2* and *PARK16* locus5-7, with a previous study demonstrating through protein-protein interaction arrays that *RAB7L1* is a binding partner for *LRRK2*5 More recently, Kuwahara et al also demonstrated that orthologues of *LRRK2* and *RAB7L1* in C. elegans neurons work together concomitantly in an ordered pathway to determine axonal length6. One recent study also found function evidence that overexpression of *RAB7L1,* a candidate gene for *PARK16* locus, reversed the effects of the *LRRK2* mutation and rescued the phenotype8. Furthermore, effect of the *LRRK2* risk variant (rs1176052) was negated in individuals with a copy of the protective allele for the *PARK16* variant (rs823114)8. This initial identification of an interaction between *LRRK2* and *PARK16* regarding susceptibility to PD leads to several important follow-up questions, such as whether the interaction is still evident when even larger series are examined, whether it is consistent for subjects of differing ethnicities and from different geographic regions, and whether it remains apparent for other variants in the two loci. Therefore, the aim of this study was to evaluate the interaction between several different *LRRK2* and *PARK16* variants in determining PD risk using a Caucasian series consisting of more than 10,000 subjects from 14 different centers, and an Asian series comprised of more than 5,000 subjects from 5 different centers.

* + - 1. **Methods**

2.1 Participants

Diagnosis of PD was made according to standard criteria9,10. Controls were individuals free of any extra-pyramidal disorder. All subjects were unrelated, and carriers of pathogenic *LRRK2* mutations were excluded. Demographic information of PD patients and controls for each GEoPD site is provided in Supplementary Table 1. We selected *LRRK2* rs1491942 and *LRRK2* rs7133914 in concordance with previously demonstrated associations11,12. Of note, *LRRK2* rs7133914 (*LRRK2* p.R1398H) is part of a 3-SNP haplotype (p.N551K-R1398H-K1423K) that has been shown to be protective for PD, and was selected for inclusion for this study as it has been shown to be the most likely functional variant on the haplotype13,14. The Japanese site from our Asian series utilized genotype data from a previous GWAS15. If the *LRRK2* and *PARK16* SNPs of interest in this study were not directly genotyped in Japanese GWAS cohort, we used proxy SNPs with r2 values >0.8 to fully capture genetic information. Using these proxy criteria, we were able to match exact SNPs from the Japanese GWAS cohort for four SNPs (rs708725, rs823139, rs823156, rs11240572) within the *PARK16* locus, and a single proxy SNP, rs2201144, was identified for *LRRK2* rs1491942. *LRRK2* rs7133914 was not genotyped in the Asian series and was genotyped for only a subset of the Caucasian series (3622 patients, 3042 controls). Furthermore, Haploview was used to measure the LD coefficient between SNPs used in our study and with that of MacLeod et al5. All variants followed Hardy Weinberg Equilibrium (HWE) in controls. All genotype call rates were >97%. We found limited linkage disequilibrium between the variants within the same gene or locus (r2<0.50 in all cases).

The Department of Human Genetics of the Helmholtz Center served as the genotyping core and performed all genotyping (Munich). Each site sent 100–200 ng of DNA to the laboratory core. Case-control status was blinded at the genotyping code for each site. We performed the genotyping on a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry on a MassArray system (Sequenom, San Diego, CA). A mass spectrometer analyzed the cleaned extension products (Bruker Daltronik, USA), and the MassArray Typer 4.0.2.5 software was used for peak identification (Sequenom). The AssayDesigner software 4.0 (Sequenom) was used for assay design, with the default parameters for the iPLEX Gold chemistry and the Human GenoTyping Tools ProxSNP and PreXTEND (Sequenom). One multiplex assay was used to genotype all of the variants. To check genotype clustering, an experienced investigator blinded to patient affection status visually reviewed the sample results.

2.4 Statistical analysis

Sensitivity of the results of gene-gene interaction analysis to the use of random effects models was also considered16. Not all of the sites had complete information on age and gender, and thus we performed secondary analyses with adjustment for age and gender. Cochran’s Q test of homogeneity was used to evaluate between-site heterogeneity, along with the I2 metric, which is interpreted as the proportion of variation in between-site interaction ORs that is due to heterogeneity beyond chance17,18.

To aid in the interpretation of tests of interaction, we also combined the two variants involved in the given interaction into one variable, allowing for a different category for each different genotype combination. The association between this genotype combination variable and PD was then evaluated using a fixed effects logistic regression model adjusted for GEO-PD site, where the most common genotype combination was the reference category, and ORs and 95% CIs were estimated in relation to this reference category. P-values were also calculated for comparison of the reference category, though the ORs and 95% CIs are of most interest in interpreting the interaction (or lack thereof) between the given two variants and p-values are presented mostly for completeness. We refer to these analyses where the joint effect of the given two SNPs on PD risk is being examined as “tests of association”. To be clear, these tests of association are presented only to assist in the interpretation of the aforementioned gene-gene interactions, and do not represent tests of interaction themselves19.

Variants with a minor allele frequency (MAF) less than 10% in either one of the series (Caucasian or Asian) were evaluated under a dominant model (i.e. presence vs. absence of the minor allele) in all analyses to maintain consistency of statistical models and allow for comparison of results between series. Subjects were coded as either 0 (absence of the minor allele) or 1 (presence of the minor allele) for each variant. Variants with a MAF of 10% or greater in both the Asian and Caucasian series were examined under an additive model (i.e. effect of each addition allele), with the subject coded as (0,1,2), depending on the number of copies of the minor allele. Under this rule, *LRRK2* variant rs7133914 and *PARK16* rs11240572 were coded under the dominant scheme, and *LRRK2* 1491942 and *PARK16* rs823139 [*RAB7L1*], rs70875 [*RAB7L1*], rs823156 [*SLC41A1*], and rs708723 [*RAB7L1*] were coded under the additive scheme. All statistical tests were two-sided, with multiple testing correction set at 0.005 for 2-sided p-value, due to the 10 different combinations of SNPs. All statistical analyses were performed using R Statistical Software (version 3.0.2, R Foundation for Statistical Computing, Vienna, Austria).

**3. Results**

A total of 19 sites contributed 7627 PD patients and 8261 neurologically normal patients (Supplementary Table 1). Out of the 19 sites, 14 sites contributed data of Caucasian ancestry and five sites contributed data of Asian ancestry. The proportion of men ranged from 41.6% to 67.8% in PD patients and 35.1% to 62.3% in controls (Supplementary Table 1). The mean age at onset was 68.1 years for PD patients, and comparable to the mean age of study of healthy controls was 67.5 years (Supplementary Table 1). Of note, given the homogenous nature of the Belgium population, we combined the data from the two Belgium sites (Garraux and von Broeckhoven) in all analyses due to the smaller sample size of the Garraux site (Supplementary Table 1).

Associations of each of the eight individual *PARK16* and *LRRK2* variants with risk of PD are shown in Supplementary Table 2. Although some of these associations have largely been shown previously, it is helpful to first understand single-variant associations before examining interactions. *LRRK2* rs1491942 was associated with an increased risk of PD in the Caucasian series (OR: 1.16, P=2.2E-05) but not the Asian series (OR: 1.10, P=0.03) (Supplementary Table 2). For *PARK16*, a risk effect was observed for rs708723 in the Asian series (OR: 1.76, P=0.00011) but not Caucasian series (OR: 1.03, P=0.32), while protective effects were noted for both rs708725 in the Caucasian series and for rs708725, rs823139, rs823156, and rs11240572 in the Asian series (Supplementary Table 2). Supplementary Tables 3-5 contain the results for the multiplicative interaction analysis between *LRRK2* rs1491942 and *PARK16* SNPs, for both the combined and the individual Caucasian and Asian series. Supplementary Table 6 shows the results for the stratified analysis between *LRRK2* rs1491942 and *PARK16* rs11240572, where we observe a trend in the stratified analysis; however the multiplicative interaction is not significant after multiple testing correction. Supplementary Table 7 displays the results of the multiplicative interaction between *LRRK2* rs7133914 and the *PARK16* SNPs, no interaction effects of note were observed.

**4. Discussion**

The identification of genetic mutations in genes linked to familial forms of PD (e.g. *LRRK2*, *VPS35*, *DNAJC13*) and genetic variability within the *PARK16* locus in GWAS strongly implicates the role of retromer and lysosomal pathway in PD pathogenesis20,21. Mutant forms of *LRRK2* have been shown to affect *RABL71* dependent lysosomal clustering and thus linking endosomal pathway to PD. Additionally, previous functional studies have demonstrated that *LRRK2* and *RAB7L1* work together in regulating axonal elongation6. Interestingly, deficiency of the *PARK16* locus candidate gene, *RAB7L1*, in mammalian or Drosophila dopamine neurons causes neurodegeneration; in contrast, overexpression of the *RAB7L1* rescued the *LRRK2* mutant phenotype suggesting that both *RAB7L1* and *LRRK2*genes bound together and functionally interact with each other in regulating neurite length process in vitro and in-vivo7. Therefore, to understand the impact of interaction in world-wide populations, we performed a large-multi-center study to assess the genetic evidence of interaction between *LRRK2* and *PARK16* locus.

The results of our study do not provide evidence of a genetic interaction between *PARK16* and *LRRK2* variants with regard to risk of PD. Heterogeneity in between-site interaction OR estimates was minimal, thus the lack of interaction was consistently observed. It is unlikely that our results are influenced by analyzing different SNPs as compared to MacLeod study. Indeed, we observed high D’ prime (range 0.5 -1.0) between our *LRRK2* SNPs and SNPs analyzed in a previously published study, and similarly for our *PARK16* SNPs (range 0.15-1) indicating that our SNPs fully captured the genetic information covered by MacLeod et al.

Of note, the directionality of effect estimates, albeit with a much weaker effect size observed in the present study, involving the specific *LRRK2* rs1491942/*PARK16* rs11240572 interaction are in agreement with previously published findings8. Specifically, while both studies observed ORs very close to 1 for the *LRRK2* variant that was examined in the given study in carriers of the rare *PARK16* allele, ORs associated with the *LRRK2* variant were greater than 1 in our study (ORs of 1.13 and 1.17 in the Asian and Caucasian series) and in the MacLeod study (ORs ranging from 1.31 to 2.49 in the 4 different series examined) for non-carriers of the *PARK16* minor allele.

Several limitations of our study should be noted. Genetic interaction studies are limited by sample size and power due to the fact that variable of focus in an interaction study is the presence of the genotype of interest for both variants, and this occurs much less frequently than the individual variant genotypes. As a result, even with our large sample size, power is still limited to detect moderate to small gene-gene interaction effects. Therefore, the possibility of a false-negative finding is important to bear in mind, and 95% confidence limits for interaction OR estimates should be considered when interpreting results. Finally, we cannot exclude the possibility that population stratification could have had an impact on our results, however our analysis adjusting for GEO-PD site would have accounted for that to a large extent.

In conclusion, our study does not provide strong evidence to support previous findings that *LRRK2* and *PARK16* variants may interact in determining risk of PD for a given individual. However, there was some degree of concordance between our interaction findings and those that were previously reported, with the caveat that our results were much weaker than the strong *LRRK2*-*PARK16* interaction that was previously reported7. Larger series will be needed to resolve whether a true *LRRK2*-*PARK16* interaction occurs. Of note, even with the large GEoPD sample size, which we have accrued to perform current study, we are likely underpowered to detect weaker interaction effects; however such studies will be critical if we are to understand the role of gene-gene interaction in disease susceptibility.

References

1. Elbaz A, Ross OA, Ioannidis JP, et al. Independent and joint effects of the *MAPT* and *SNCA* genes in Parkinson disease. Annals of neurology 2011; 69:778-792.

2. Goris A, Williams-Gray CH, Clark GR, et al. Tau and alpha-synuclein in susceptibility to, and dementia in, Parkinson's disease. Annals of neurology 2007; 62:145-153.

3. Trotta L, Guella I, Solda G, et al. *SNCA* and *MAPT* genes: Independent and joint effects in Parkinson disease in the Italian population. Parkinsonism & related disorders 2012; 18:257-262.

4. Wider C, Vilarino-Guell C, Heckman MG, et al. *SNCA*, *MAPT*, and *GSK3B* in Parkinson disease: a gene-gene interaction study. European journal of neurology 2011; 18:876-881.

5. Beilina A, Rudenko IN, Kaganovich A, et al. Unbiased screen for interactors of leucine-rich repeat kinase 2 supports a common pathway for sporadic and familial Parkinson disease. PNAS 2014; 7: 2626-2631.

6. Kuwahara T, Inoue K, D’Agati VD, et al. *LRRK2* and *RAB7L1* coordinately regulate axonal morphology and lysosome integrity in diverse cellular contexts. Nature 2016, 6: 29945.

7. MacLeod DA, Rhinn H, Kuwahara T, et al. RAB7L1 interacts with *LRRK2* to modify intraneuronal protein sorting and Parkinson's disease risk. Neuron 2013; 77:425-439.

8. Soto-Ortolaza AI, Heckman MG, Labbe C, et al. GWAS risk factors in Parkinson's disease: *LRRK2* coding variation and genetic interaction with *PARK16*. American journal of neurodegenerative disease 2013; 2:287-299.

9. Gelb DJ, Oliver E, Gilman S. Diagnostic criteria for Parkinson disease. Archives of neurology 1999; 56:33-39.

10. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry. 1992 Mar; 55(3): 181-4.

11. Trinh J, Vilarino-Guell C, Ross OA. A commentary on fine mapping and resequencing of the *PARK16* locus in Parkinson's disease. Journal of human genetics 2015; 60:405-406.

12. Ross OA, Soto-Ortolaza AI, Heckman MG, et al. Association of *LRRK2* exonic variants with susceptibility to Parkinson's disease: a case-control study. Lancet Neurol 2011; 10:898-908.

13. Lill CM, Roehr JT, McQueen MB, et al. Comprehensive research synopsis and systematic meta-analyses in Parkinson's disease genetics: The PDGene database. PLoS genetics 2012; 8: e1002548.

14. International Parkinson Disease Genomics C, Nalls MA, Plagnol V, et al. Imputation of sequence variants for identification of genetic risks for Parkinson's disease: a meta-analysis of genome-wide association studies. Lancet 2011; 377: 641-649.

15. Tan EK, Skipper LM. Pathogenic mutations in Parkinson disease. Human mutation 2007; 28:641-653.

16. Satake W, Nakabayashi Y, Mizuta I, et al. Genome-wide association study identifies commn variants at four loci as genetic risk factors for Parkinson's disease. Nature Genetics 2009; 41:1303-1307.

17. DerSimonian R, Laird N. Meta-analysis in clinical trials. Controlled clinical trials 1986; 7: 177-188.

18. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Statistics in medicine 2002; 21:1539-1558.

19. Higgins JP, Thompson S, Deeks J, Altman D. Statistical heterogeneity in systematic reviews of clinical trials: a critical appraisal of guidelines and practice. Journal of health services research & policy 2002; 7:51-61.

20. VanderWeele TJ, Tchetgen Tchetgen EJ. Attributing effects to interactions. Epidemiology 2014; 25:711-722.

21. Tang FL, Erion JR, Tain Y, et al. *VPS35* in dopamine neurons is required for endosome-to-golgi retrieval of Lamp2a, a receptor of chaperone-mediated autophagy that is critical for alpha-synuclein degration and prevention of pathogenesis of Parkinson‘s Disease. Journal of Neuroscience 2015; 29:10613-28.