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Environmental and genetic risk factors for susceptibility and progression in Parkinson's disease

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Environmental and genetic risk factors for susceptibility and progression in Parkinson's disease

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in Epidemiology

by

Kimberly Carol Paul

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ABSTRACT OF THE DISSERTATION

Environmental and genetic risk factors for susceptibility and progression in Parkinson's disease

by

Kimberly Carol Paul

Doctor of Philosophy in Epidemiology

University of California, Los Angeles, 2016

Professor Beate R. Ritz, Chair

Parkinson's disease (PD) is one of the most common neurodegenerative disorders and has a complex multifactorial etiology, likely involving not only exposure to environmental toxins but also an underlying genetic susceptibility. Several major molecular pathways are implicated in PD pathogenesis; many, including impaired ubiquitin-proteasome system, mitochondrial dysfunction, and neuroinflammation, involve oxidative stress as an underlying mechanism. Further, the course and severity of symptom progression is highly variable, and oxidative stress related pathways may be involved in symptom progression.

Widely used organophosphate (OP) pesticides can induce oxidative stress and are reported to increase PD risk, and may be involved in symptom progression. Additionally, two single nucleotide polymorphisms (SNPs) from the *PON1* gene influence the ability to metabolize OPs. Nitric oxide synthase (*NOS*) genes are candidates for PD because NOS enzymes produce nitric oxide (NO), a pro-oxidant that can damage neurons. The *NFE2L2* and *PPARGC1α* genes

encode for important transcription factors that activate multiple antioxidant defense mechanisms in response to oxidative stress.

In the Parkinson's Environment and Gene (PEG) case control study, we investigated 8 *NOS* SNPs and interactions with both household and ambient agricultural OP exposures assessed with geographic information system (GIS) and PD susceptibility with logistic regression models. In the patient only cohort, we employed repeated-measures regression to assess associations between ambient OP exposure and/or *PON1* L55M genotypes and symptom progression. And finally, we investigated the influence of haplotypes for *NFE2L2* and *PPARGC1α* and their interactions with exposures to the pesticides maneb and paraquat (MB/PQ) on PD occurrence (using logistic regression models) and also on progression of motor symptoms and cognitive decline in patients followed prospectively (repeated measures models).

In comparing PD in homozygous variant carriers of *NOS2A* rs1060826 versus homozygous wildtype or heterozygotes, we estimate an adjusted OR of 1.51 (95% CI=0.95, 2.41). When considering interactions between *NOS1* rs2682826 and OP exposure from household use, the OR for frequent OP use alone 1.30 (95% CI=0.72, 2.34) and for the CT+TT genotype alone 0.89 (95% CI= 0.58, 1.39), and frequent OP use combined with the CT+TT genotype was 2.84 (95% CI=1.49, 5.40) (interaction p-value 0.04). Similar results were seen for ambient OP exposure. Interactions between OP exposure and 3 other *NOS1* SNPs and a genetic risk score combining all *NOS1* SNPs reached statistical significance.

High OP exposures were associated with faster progression of both motor (UPDRS=0.002) and cognitive scores (MMSE p=0.008). The *PON1* 55MM genotype was associated with worse cognitive scores and faster progression of motor (UPDRS=0.01) and depressive symptoms (GDS p=0.008). We also found the *PON1* L55M variant to interact with OP exposures in influencing

MMSE cognitive scores (p=0.02).

Two *NFE2L2* haplotypes were associated with significant increases in the risk of developing PD ($p<0.04$) and with faster cognitive decline (MMSE $p<0.0006$). None of the *PPARGC1α* haplotypes were marginally associated with PD risk. However, one haplotype interacted with MB/PQ exposure (p=0.03), such that highly exposed haplotype carriers showed no increased risk of PD while these pesticides increased PD risk in wildtype haplotype carriers. Additionally, three *PPARGC1α* haplotypes were associated with differing rates of motor symptom progression.

The observations are consistent with the hypothesis that oxidative stress-inducing mechanisms influence PD risk and progression.

The dissertation of Kimberly Carol Paul is approved.

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1. Background and Introduction

1.1 Dissertation Objectives

The aim of this dissertation is to investigate the influence of environmental and genetic factors believed to play a role in oxidative stress and Parkinson's disease (PD) susceptibility and progression. Briefly, the first project examines the risk of PD associated with ambient organophosphate (OP) pesticide exposure and how this risk is mediated by genetic variation in the nitric oxide synthase (NOS) genes. The second project examines whether ambient OP exposure and the *PON1* L55M genotype, which is involved in detoxifying OP pesticides, act together to influence the rate of motor, cognitive, and mood symptom progression in PD. The third project investigates the influence of common variants in the genes *NFE2L2* and *PPARGC1α,* genes which encode transcription factors involved in regulating endogenous antioxidant enzymes, on PD susceptibility and progression of symptoms, and assesses gene variant-pesticide interactions specifically with oxidative stress inducing co-exposures to the pesticides maneb and paraquat (MB/PQ).

1.2 Parkinson's disease background

PD is the second most common neurodegenerative disorder, characterized by the progressive depletion of dopaminergic neurons in the substantia nigra of the brain and the development of aberrant protein aggregates, Lewy bodies. PD affects approximately 1% of individuals over the age of 60, with around 1 million prevalent cases in the USA and 7 to 10 million worldwide, and 60,000 new cases identified every year (PDF.org 2014). With more people living longer and age as a risk factor for PD, the number of people with PD is expected to increase in the coming years. PD diagnosis is based on clinical assessment, with the presence of at least two of the four cardinal features, resting tremor, rigidity, bradykinesia, and postural

instability. Non-motor symptoms associated with the disease include cognitive impairment and depression. The progressive nature of the disease leads to eventual decline in both motor and non-motor symptoms; as symptoms become more pronounced, they result in disability and loss in quality of life, such as difficulty walking, talking, or completing other simple tasks (Global PD Survey 2002).

Genetic and environmental factors alone can cause Parkinsonism, as seen with rare mutations in several genes linked to familial PD (Schiesling et al. 2008) and exposure to the toxic metabolite of MPTP (Langston 1985). However, the majority of PD cases are idiopathic, with a complex multifactorial etiology, likely involving not only exposure to environmental toxins but also genetic susceptibility. Several major molecular pathways are implicated in PD pathogenesis; many, including impaired ubiquitin-proteasome system (UPS), mitochondrial dysfunction, and neuroinflammation, involve oxidative stress as an underlying mechanism (Jenner 2003; Dauer and Przedborski 2003; Hwang et al 2013), inspiring investigators to focus on factors related to reactive oxygen or nitrogen species (ROS/RNS), such as nitric oxide (NO), antioxidants, or certain pesticide metabolites (Ryan et al. 2013).

Many environmental risk factors for PD have been proposed and investigated. Pesticide exposure has been associated with an increase risk in PD in many studies, with a meta-analysis of 19 studies reporting a pooled odds ratio of 1.94 (95% CI=1.49, 2.53) (Priyadarshi et al, 2000). Coffee consumption and smoking have also been widely associated with a decreased risk of PD, with some studies showing dietary factors and estrogen are also associated with PD risk (Lau and Breteler, 2006). Mutations in a number of genes have been linked to monogenic PD, including *SNCA, Parkin, PINK1, DJ-1*, and *LRRK2,* however, these are rare and make up only a small percent of PD cases (Lau and Breteler, 2006). In idiopathic PD, many candidate gene studies

have been done, investigating genes involved in dopamine metabolism, mitochondrial function, toxin metabolism, as well as genes linked to other neurodegenerative diseases (Lau and Breteler, 2006). Genome-wide association studies have also identified variants in *SNCA, GBA*, the HLA region, among others (Nalls et al 2014).

2. Organophosphate Pesticide Exposures, Nitric Oxide Synthase Gene Variants, and Gene–Pesticide Interactions in a Case-Control Study of Parkinson's Disease

2.1 Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive depletion of dopaminergic neurons in the substantia nigra of the brain. Both genetic and environmental factors alone can cause Parkinsonism, as seen with rare mutations in several genes linked to familial PD (Schiesling et al. 2008) and exposure to the toxic metabolite of MPTP (Langston 1985). Idiopathic PD however is believed to result from multiple etiologies most of which likely require not only exposure to environmental toxins but also an underlying genetic susceptibility (Schapira 2006). Several major molecular pathways are implicated in PD pathogenesis, including mitochondrial dysfunction resulting from or in oxidative/nitrosative stress (Dauer and Przedborski 2003), inspiring investigators to focus on reactive oxygen or nitrogen species (ROS/RNS) such as nitric oxide or certain pesticide metabolites (Ryan et al. 2013). Although a number of genetic variants and environmental factors have been consistently implicated in PD etiology, rarely have reported gene-environment interactions been replicated, including those for nitric oxide synthase gene variants and pesticide exposures.

Nitric oxide (NO), a chemical messenger and free radical by-product of reactions catalyzed by nitric oxide synthase (NOS) enzymes, is essential for numerous physiologic

processes, including neurotransmission, but is also a pro-oxidant capable of contributing to oxidative/nitrosative stress and damaging an array of cell types, including dopaminergic neurons (Kavya et al. 2006). Three genes encode NOS enzymes: *NOS1* on chr 12 encodes neuronal NOS (nNOS), *NOS2A* on chr 17 encodes inducible NOS (iNOS), and *NOS3* on chr 5 encodes endothelial NOS (eNOS). *NOS1* and *NOS2A* are of particular interest in PD due to their expression in the brain (Licinio et al. 1999). A number of single nucleotide polymorphisms (SNPs) in the *NOS1* and *NOS2A* genes have previously been linked to PD risk, but few reports implicated the same SNPs (Hague et al. 2004; Hancock et al. 2008; Huerta et al. 2007; Levecque et al. 2003; Schulte et al. 2006). While the functionality of these SNPs is still unknown and the epidemiologic evidence inconclusive, much stronger support for an involvement of *NOS* in neurotoxicity is provided by laboratory studies. In animal models inhibition of nNOS prevents MPTP-induced Parkinsonism in both baboons and mice (Hantraye et al. 1996; Schulz et al. 1995), and MPTP-induced neuronal damage is diminished in mice lacking either the *NOS1* or the *NOS2A* gene (Liberatore et al. 1999; Przedborski et al. 1996). Post mortem studies also found higher levels of NO in the nigrostriatal region in PD brains (Hunot et al. 1996).

Organophosphates (OP), pesticides commonly used agriculturally and until recently in households, have long been investigated in relation to PD, not only due to neurotoxicity through action on acetylcholinesterase, their primary target, but also the ability to induce oxidative stress through increased production of reactive oxygen species (Bagchi et al. 1995; Lukaszewicz-Hussain 2010). With evidence that both NO and pesticide exposures are contributing to neuronal damage through the same pathways, we speculate that they may act synergistically to increase PD risk. For example, OP induced oxidative stress alone has the potential to lead to mitochondrial complex I dysfunction and as a result further generation of superoxides; however

superoxides readily react with nitric oxide to form peroxynitrite (NO_3^-), a more potent toxicant able to irreversibly inhibit mitochondrial respiration (Dauer and Przedborski 2003; Kavya et al. 2006; Lukaszewicz-Hussain 2010). Adding more complexity, variations in two functional SNPs of the *PON1* gene are known to influence the ability to metabolize and detoxify OPs and influence PD risk (Lee et al. 2013). Statistical interactions between *NOS1* SNPs and home pesticide use in PD were first seen in a North American family study (Hancock et al. 2008). Here, we will attempt to replicate this reported finding for household pesticide use, examining interactions with household pesticide exposures, and also to contribute new information about the interaction with OP pesticides specifically, both from household use and ambient exposure to agricultural pesticides, and with other *NOS1* genetic variants, while also taking into account increased susceptibility due to *PON1* status.

2.2 Materials and Methods

All procedures described were approved by the University of California at Los Angeles (UCLA) Human Subjects Committee and informed consent was obtained from all participants.

Participant recruitment

We enrolled incident PD patients along with population-based controls between January 2001 and December 2010 from three highly agricultural central California counties (Kern, Tulare, Fresno) known for the high use of agricultural pesticides. Detailed participant recruitment (Costello et al. 2009; Wang et al. 2011) and case definition criteria (Jacob et al. 2010; Kang et al. 2005) have been previously described and published.

Briefly, of the 1,167 PD patients initially identified through large medical groups, neurologists, and public service announcements, 604 did not meet eligibility criteria for the

following reasons: 397 were not diagnosed with PD within 3 years prior to recruitment, 134 lived outside the tri-counties, and 73 did not have PD. From the 563 potential cases, 90 could not be examined by our movement disorder specialist (JB), 56 declined or moved away, 34 became too ill or died prior to the scheduled appointment; of 473 examined by us (JB), 94 did not meet published criteria for idiopathic PD (Hughes et al. 1992), an additional 13 were reclassified as not having PD during follow-up (Ritz et al. 2012), and 6 participants withdrew between examination and interview. Of the remaining 360 cases, 357 provided information and biologic samples necessary for inclusion in at least one of our analyses.

To be eligible as population-based controls, participants must have been over the age of 35, having lived within one of the three counties for at least 5 years prior to enrolment, and not have a diagnosis of PD. We identified potentially eligible population-based controls from the same tri-county area initially through both Medicare enrollee lists (2001) and publicly available residential tax-collector records (2001-2010) (Kern, Fresno, and Tulare County Tax Assessor), and after 2001 only through residential tax-collector records. We used two sampling strategies to increase enrolment success and representativeness of the source population: 1) random selection from the Medicare enrollee lists and of residential parcels (identified from the tax-collector records) followed by mail or phone enrolment and 2) random selection of clustered households (five per cluster, identified through the tax-collector records) we visited in person to enroll eligible controls; these enrolment methods have been described in detail in more detail previously (Costello et al. 2009; Wang et al. 2011).

From the first sampling method, we contacted 1,212 potentially eligible controls. Of these individuals, 457 were ineligible: 409 were younger than 35 years of age, 44 too ill to participate, and 4 primarily resided outside the study area. Of the 755 eligible population controls, 409

declined participation, were too ill or moved before an interview was possible; resulting in the enrolment of 346 population controls. From the second sampling strategy, 4,756 individuals were screened, of which 3,515 were ineligible (88% of these were out of the age range) and 634 of the eligible controls declined participation; 607 population controls were enrolled, but 183 of them completed only an abbreviated interview and did not contribute all data needed for this analysis. Additionally, an early mailing (for which the number of eligible participants who declined was not known) produced 62 controls. Of the 832 recruited controls, 337 were excluded because they lacked *NOS* genotyping data. Thus, in total only 495 controls provided information and biologic samples necessary for inclusion in at least one of the analyses, 333 originating from the first control recruitment effort.

Pesticide exposure assessment

Cases and controls were interviewed by telephone to obtain information on demographic characteristics, risk factors, and included detailed questions on home pesticide use and lifetime occupational and residential histories. During the interview, participants provided information on chemical use in the home, lawn, or garden. More detail on this exposure assessment has been published (Narayan et al. 2013). Briefly, participants were asked to recall names of chemicals or products if possible, or partial product names, manufacturer names (e.g. Raid), targets (e.g. weed control, plant disease, ants, spiders, etc.), or formulation of products (e.g. liquid, granules, bait, etc.). This interview data was supplemented with information about ingredients from the California Department of Pesticide Regulation (CDPR) product label database (CDPR 2013b). The active ingredient (chemical contributing the largest percentage to a product's composition) was then categorized into chemical classes, again using the CDPR product label database. Interviewers additionally asked about frequency of use (none or rarely (once a year or less),

sometimes (2-11 times a year), or regularly (more than once a month)) during four different periods: young adult (16-24), adult (25-<45), middle age (45-<65) and senior (≥65). Only use by the participant themselves was considered.

We assessed lifetime home pesticide use by calculating a weighted average frequency of use (Narayan et al. 2013). For each pesticide class we multiplied the midpoint of the frequency category by years in each age period up to 10 years before index date (date of diagnosis or interview), summed across age periods, and divided by the total number of years between ages 16 to 10 years prior to index date. Those with an average frequency of use of any reported pesticide class above or equal to the pesticide class specific median found in exposed controls were considered "frequent users" of any pesticide and those with an average frequency of use below the median for all pesticides as "occasional users" for the 'any household pesticide use' exposure assessment. For household use of OPs, those with an average frequency above or equal to the OP use median found in exposed controls were considered "frequent users" of OPs and those with an average use below the median to all pesticides as "occasional users". We then classified participants in mutually exclusive groups, as "frequent users" of OP pesticides, as described above, "frequent users" of other non-OP pesticides, those who did not frequently use OPs but did use other pesticides frequently, and "occasional users" of pesticides; in primary analysis for household OP use, we excluded "frequent users" of other non-OP pesticides, only comparing "frequent users" of OP pesticides to "occasional users", comparisons using "frequent users" of non-OPs were included in secondary analyses.

Ambient pesticide exposure resulting from commercial applications to agricultural crops was estimated using a geographic information system (GIS) based computer model, which links geocoded lifetime residential and occupational address histories of participants, California state

mandated pesticide use report (CA-PUR) data (CDPR 2013a), which include information on all agricultural pesticide applications and the date, location, and amount applied, and land use surveys from California's Department of Water Resources (CDWR 2013), which provides the exact location of specific crops. We provide a brief description here and a more detailed and technical discussion of the GIS method has been published (Cockburn et al. 2011). For all pesticides, we summed the pounds of chemical applied per year per acre within a 500-m radius buffer of each address. For each participant, we then calculated a study period average for each chemical from 1974 to 10 years prior to the participant's index year by summing the year-specific averages and dividing that sum by the total number of years in the relevant time period. If a participant was missing geocode location information for any given year, we used simple imputation, substituting the individual's average value from their recorded years. CA-PUR data indicated that the study population was exposed to 36 different chemicals classified as OPs based on information from CDPR and the pesticide action network (PAN) pesticide database (Kegley et al. 2014) (see supplemental material, table 2-S1, for a complete list). Exposures over the same time period at both residential and occupational addresses were included, and each participant could have been exposed at both locations, only one, or neither. We dichotomized exposure to each of the individual OP chemicals based on each chemical's median level in exposed controls, and then summed the number of OP chemicals that each participant was exposed to above the median, counting chemical exposures from both residence and occupation; we then classified OP exposure based on the exposure distribution of the OP sum from the controls in the following manner: high exposure, exposed to > 11 OP chemicals (top quartile in exposed controls), none/low exposure: 0-11 OP chemicals.

In addition to using CA-PUR data to estimate ambient exposure at each occupational

location, direct occupational exposure was derived from a job exposure matrix (JEM), where participants' level of exposure was estimated for each reported occupation (Liew et al. 2014). However, JEM based occupational exposure was not used for our primary analysis, as exposures to the specific pesticides of interest here, OPs, could not be estimated, but was included as a covariate to control for other sources of pesticide exposure.

SNP selection and genotyping methods

Altogether 8 SNPs from *NOS1* and *NOS2A* were selected, *NOS1* rs2682826 and rs1047735 and *NOS2A* rs1060826 based on previous PD research (Hague et al. 2004; Hancock et al. 2008; Levecque et al. 2003) and *NOS1* rs3741475, rs3741480, and rs816353 and *NOS2A* rs2297518, and rs3730013 to optimize gene coverage.

Participants provided blood or saliva samples for genetic analyses, which were stored and processed at the UCLA Biologic Specimen Core Facility. Several collaborative research projects performed genotyping of our samples for these SNPs; this led to a different number of participants with data available for each SNP. *NOS1* SNPs rs1047735, rs2682826, and rs3741475 and *NOS2A* SNPs rs1060826, rs2297518, and rs3730013 genotyping was conducted at Stanford Human Genome Center; PCR assays were conducted with TaqMan Universal Master Mix (Applied Biosystems), primers and probes were designed based on the NCBI DNA sequence and purchased from ABI (Applied Biosystems, Foster City, CA, USA). Fluorescence data files from each plate were analyzed by automated allele calling software (ABI Prism 7900 HT Sequence Detection System 2.1). Fill in genotyping for additional cases and controls recruited later in the study for each of these SNPs except rs3741475 was performed at the UCLA Genomics Core Facility using the Applied Biosystems SNPlex array (Tobler et al. 2005). *NOS1*

rs3741480 and rs816353 genotyping was performed at the University of Washington's SF Functional Genomics and Bioinformatics Core Laboratory using the Fluidigm BioMark HD system (Fluidigm Corporation, South San Francisco, CA). All SNPs had a call rate over 98.5% except for rs1047735 (97%), rs3741480 (93%), and rs816353 (93%). Additionally, *PON1* genotyping was conducted at the UCLA Genomics Core using pyrosequencing for L55M (rs854560), and for Q192R (rs662) using the Fluidigm BioMark HD system at the University of Washington. *PON1* metabolizing status was based on published report (O'Leary et al. 2005); briefly, "slower" metabolizers are considered those with a MM genotype at L55M and QQ or QR at Q192R, other genotypes are considered "faster" metabolizers.

To safeguard against systematic genotype errors due to using different genotyping centers for fill-in genotyping of *NOS1* SNPs rs1047735, rs2682826, and *NOS2A* SNPs rs1060826, rs2297518, and rs3730013, 97 participants were included in both genotyping experiments; they provided an interlaboratory genotype call rate concordance of 99.8% (1 discordant call at rs2682826 for 1 participant). This is in addition to the 5-10% duplicate samples included in each individual experiment used to confirm quality genotyping. Additionally, for each of the 5 SNPs, we used logistic regression to examine whether genotype could predict the centers where genotyping was performed, assuming an allelic model (comparing the minor allele to the major allele), and using the control population only; we found no statistically significant associations by center suggesting no systemic error by center (data not shown).

NOS1 Genetic Risk Score

We created a genetic risk score (GRS) based on the 5 *NOS1* SNPs genotyped. The score counts the minor alleles, such that participants homozygous for the minor allele received a 2,

heterozygous participants a 1, and those homozygous wild type a 0, at each locus, for a total range of 0 to 10. In sensitivity analyses, we examined an alternate GRS based on 3 *NOS1* SNPs only (rs2682826, rs1047735, and rs3741480, total range 0 to 6), excluding rs816353, as it is in moderate LD (=0.6) with rs1047735 and was not in Hardy-Weinberg equilibrium, and rs3741475, as it is in moderate LD (=0.6) with rs2682826.

Statistical methods

We examined Hardy-Weinberg equilibrium in control participants for all polymorphisms using a chi-square test, and checked LD between each SNP. We used unconditional logistic regression to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for SNP marginal effects assuming a recessive genetic model (homozygous for the minor allele versus any major allele) for *NOS2A* rs1060826 and a dominant model (any minor allele versus homozygous for the major allele) for *NOS1* rs1047735 and rs2682826 to compare with prior report (Levecque et al. 2003). As previous model selection was not based on functional significance, for these SNPs and all other SNPs for which we had no *a-priori* genetic hypotheses, we additionally assumed an additive genetic model (where each copy of the variant allele increases the risk by the same amount). We also adjusted for potential confounders including sex, age (continuous), cigarette smoking status (ever/never), European ancestry (yes, exclusively European ancestry / no, any non-European ancestry), education (<12 years, 12 years, >12 years), and *PON1* metabolizing status ("faster" or "slower" metabolizers; (O'Leary et al. 2005)).

Gene-environment interactions were assessed with *NOS1* rs2682826 and pesticide use due to previous report (Hancock et al. 2008) during primary analysis. Statistical interactions were assessed by introducing a multiplicative interaction term (e.g. product term: gene x

pesticide) into a logistic model that relied on a dominant genetic model. We also conducted secondary, exploratory analyses, assessing interactions between all other *NOS* SNPs and OP exposure, assuming a dominant genetic model, and between the genetic risk score and OP exposure.

A multiple test correction was not implemented, as the SNPs under analyses were selected based on previous research reports that supported associations with PD. Gene-gene interaction analyses between *NOS* SNPs and *PON1* status were not performed, as we did not have an *a-priori* hypothesis supporting this relationship and concerns about sufficient power given both the lack of marginal genetic effects and our sample size (≥80% power to detect interaction OR of 3.2 or above).

We conducted sensitivity analyses for SNP marginal effects restricting to participants with European ancestry only and adjusting for PD family history (PD in a first degree relative: yes/no). For gene-environment analyses, in sensitivity analysis we mutually adjusted for household OP pesticide use, ambient OP exposure and occupational exposures derived from our JEM. We also assessed the interaction between household use of non-OP pesticides and NOS rs2682826.

Power calculations were performed using Quanto version 1.2.4 (Gauderman and Morrison 2006), LD using Haploview (Barrett et al. 2005) and all other analyses using SAS 9.3 (SAS Institute Inc., Cary, NC).

2.3 Results

Study participants were predominantly of European ancestry, over the age of 65, and did not report a family history of PD (table 2-1). Cases had a higher proportion of males, never

smokers, and slower PON1 metabolizer's (table 2-1); note, 121 participants (48 cases (13%) and 73 controls (15%)) were missing *PON1* genotyping and thus PON1 metabolizer status. Cases were more likely to have frequently used household pesticides (OR = 1.69, 95% CI: 1.21, 2.36 for any pesticide use, and OR = 2.05, 95% CI: 1.30, 3.24 for OP use specifically), and to have had high ambient exposure to agricultural OP pesticides (OR = 2.99, 95% CI: 1.92, 4.65; see supplemental material, table 2-S1). All models controlled for age, sex, smoking status, European ancestry, education, and PON1 metabolizer status.

The population was in Hardy-Weinberg equilibrium for all SNPs evaluated ($p > 0.05$), except for rs816353 ($p=0.02$) (table 2-2), and the SNPs in each gene were in low to moderate LD with each other (*NOS1* values ranging from 0.27 to 0.59, for *NOS2A* from less than 0.10 to 0.30) (data not shown). For *NOS2A* rs1060826, cases were more likely to have a homozygous variant (OR=1.56, 95% CI 1.10, 2.38 without PON1 adjustment and OR = 1.51; 95% CI: 0.95, 2.41 with PON1 adjustment) (table 2-2). We did not find any other SNPs to be significantly associated with PD, aside from *NOS1* rs1047735 based on an additive model without adjustment for PON1; however, we did not detect an association with the *a-priori* selected recessive model (Leveqcue et al. 2003) (table 2-2). When restricting analyses to participants of European ancestry only, results did not change (data not shown).

Investigating *NOS1* rs2682826 and any household pesticide, we estimated a non-significant interaction based on the p-value of the product term (p-value=0.18; supplemental material, table 2-S2). When we limited to household OP use specifically (excluding those with frequent use on non-OP pesticides), the product term reached statistical significance (p-value=0.04); the genetic variant in occasional users of household pesticides did not contribute to an increased risk of PD, in contrast to frequent OP users, where OP exposed variant T allele

carriers were at increased risk compared to the wildtype ($OR_{CC+OP\ use}$=1.30, 95% CI=0.72, 2.34 vs. $OR_{CT/TT+OP\ use}$=2.84, 95% CI=1.49, 5.40; table 2-3, supplemental material, figure 2-S1). When we limited household pesticide use to only non-OP pesticides (excluding those with frequent use of OP pesticides), we did not see a significant interaction (interaction p-value=0.66; supplemental material table 2-S2). Results for ambient OP exposures were similar to those seen with household OP use; again the genetic variant in those with no/low ambient OP exposure did not influence PD risk (OR_{CC} =0.99, 95% CI=0.70, 1.40), while high ambient OP exposure in the wildtype population was associated with an increased PD risk ($OR_{CC+OP\ exp}$ =2.42, 95% CI=1.27, 4.61), and those with a variant allele highly exposed to ambient OPs were at the highest risk ($OR_{CT/TT+OP\ exp}$=4.83, 95% CI=2.39, 9.73; table 2-3) (interaction p-value=0.15).

In secondary, exploratory analysis, we detected 3 other significant statistical interactions between *NOS1* SNPs, rs1047735, rs816353, and 3741480, and ambient OP exposure (table 2-3). For each SNP, we detected a moderate pesticide association in homozygous wildtype carriers, comparing those highly exposed to ambient OPs with a wildtype genotype to those with no/low exposure and a wildtype genotype (ORs range from 1.43 (95% CI=0.69, 2.96) to 2.07 (95% CI=1.09, 3.91); table 2-3); while highly, exposed variant allele carriers were at the highest risk when compared with those with no/low exposure and a wildtype genotype (ORs range from 3.78 (95% CI=2.04, 6.99) to 5.42 (95% CI=2.54, 11.52); table 2-3). Similar trends are seen also with the household OP use; though only the product term with rs3741480 reached statistical significance; we detected no increase to moderate non-significant increases in risk when comparing frequent use of OP pesticides to occasional use in wildtype carriers (ORs range from 0.93 (95% CI=0.44, 1.99) to 1.62 (95% CI=0.88, 2.98); table 2-3) and the highest risk was found in variant allele carriers who frequently used OP pesticides compared to wildtype occasional

users (ORs range from 1.90 (95% CI=1.06, 3.41) to 2.31 (95% CI=1.22, 4.37); table 2-3). We also detected interactions using the genetic risk scores (GRS), again based on the product term between the GRS, which we treated as a linear variable, and the pesticide exposure indicators (p-values for interaction ranged from 0.01 to 0.09; see supplemental material, table 2-S3). For example, for the 5 SNP *NOS1* GRS (range 0-10 variant alleles) and ambient OP exposure, no significant risk increase was detected per additional variant allele copies in those with none or low exposure ($OR_{\text{per 1 variant allele}}$=1.04, 95% CI=0.97, 1.12), as seen with each individual SNP, while in those highly exposed to ambient OP exposure, there was a significant increase in risk per each additional variant allele copy ($OR_{\text{per 1 variant allele+OP exp}}$=1.90, 95% CI=1.04, 3.43).

Investigating *NOS2* SNPs, we did not detect any marginal associations except for *NOS2A* rs1060826 (table 2-2) or significant interactions with pesticide exposure, based on the p-value of the product term (data not shown). Mutually adjusting for household OP pesticide use, ambient OP exposures, ambient maneb and paraquat exposures, occupational exposures to pesticides (JEM), and other *NOS* SNPs and limiting to participants of European ancestry only changed the estimates minimally (<10%; data not shown).

2.4 Discussion

In this investigation, we identified a positive marginal association with *NOS2A* SNP rs1060826 and PD. Importantly, we also identified multiple *NOS1* -pesticide interactions, providing support for the involvement of OP pesticides in PD, especially in genetically susceptible subpopulations.

Animal models of PD suggest that environmental factors and aging together induce oxidative stress, and depending on genetic background and a biological system's antioxidant

capacities this can lead to cell death or survival (Varçin et al. 2012). Some PD related genes might induce oxidative/nitrosative stress, such as *NOS* via regulating NO, while others modulate cell survival following exposure to oxidative stressors, such as *PON1* and other metabolic or antioxidant gene products. In our population, while controlling for *PON1*, OP exposure was positively associated with PD, and variation in multiple regions throughout the *NOS1* gene further modified this association. This is consistent with the hypothesis that NO and pesticides act synergistically to influence PD risk, with reactive oxygen or nitrogen species (ROS/RNS) from multiple sources acting in a potentiating manner, overwhelming the balance between pro-oxidants and the antioxidant capability of dopamine neurons.

Our population-based case-control study provided a unique opportunity to investigate *NOS* genes while adjusting for the contributions of *PON1* on OP metabolism and assess their role in modifying the effect of OP pesticide exposures in PD. Consistent with the NCI-NHGRI Working Group on Replication in Association Studies criteria for high quality replications of association results (Group 2007), our study provides an independent population, similarity and improvement in exposure assessment, and adequate sample size (≥80% power to detect previously reported marginal effect sizes, and OP interaction ORs ≥2.3, given summary parameters based on previous report (Hancock et al. 2008; Levecque et al. 2003)). Additionally, we estimated pesticide exposure from multiple sources – household and agricultural uses – and the observed associations mutually corroborated each other.

PD is a commonly misdiagnosed disease (Meara et al. 1999; Wermuth et al. 2012). Different from most epidemiology studies, our PD cases were all seen and well characterized by UCLA movement disorder specialists at least once and 70% were followed many years for disease progression (Ritz et al. 2012), minimizing bias from disease misclassification.

Additionally, population controls were drawn from the same region as the cases, likely providing adequate representativeness of the source population.

The vast majority of previous epidemiologic studies investigating pesticides have relied solely on self-reported information for home pesticide use, a method prone to differential recall error, as the degree to which study participants may forget details, or misreport their past pesticide use may differ between cases and controls. We improved and enriched our self-reported measure of gardening, yard, and indoor uses with the information about active ingredients provided in the California Department of Pesticide Regulation's (CDPR) product label database that lists all household products registered in CA for use. Thus, we only partially depended on recall for home pesticide exposure assessment, as participants did not need to report specific chemicals but products or types of products. In addition, we assessed ambient exposures with a geographic information system approach that integrates state mandated pesticide use reports (PUR), land use data, and address information. This GIS-driven and pesticide record based ambient exposure assessment approach does not rely on participant recall. However, our ambient pesticide exposure method does not account for factors such as wind patterns at the time of application, geographic features which may influence pesticide drift, and the assumption that the participant was at the recorded location during the relevant time period; thus we did not eliminate the possibility of exposure misclassification. Our two exposure measures are unrelated, as household use was not influenced by nearby agricultural applications; nevertheless we saw similar patterns when assessing gene-environment interactions.

A comparison of the previously reported *NOS* SNP marginal associations is presented in table 2-4 (those SNPs not included were not investigated previously). There are inconsistencies in the reported marginal associations of the *NOS* SNPs. Additionally, none of these SNP regions

have emerged from PD GWA studies (Nalls et al. 2014). Although evidence for an involvement of *NOS2A* rs1060826 in PD susceptibility was relatively consistent in many candidate gene studies, there is no clear direction in the association, though a positive association as seen in our population has been published before (Hancock et al. 2008). We also did not replicate previous positive marginal associations reported for *NOS1* rs1047735 or rs2682826. Gene-pesticide interactions with *NOS1* rs2682826 were first described in a study of 169 families, the authors report a positive association between ever pesticide use (in the home, garden, or work) among those with the homozygous wildtype genotype (OR=3.52, 95% CI=1.87, 6.95), but no association between pesticide use and PD in those with a variant allele (Hancock et al. 2008). This is in contrast to the associations we report for *NOS1* rs2682826, where we found positive associations among the OP exposed variant carriers, with smaller or no pesticide associations in the homozygous wildtype carriers (table 2-3). There may be a number of explanations for these discrepancies. For instance, marginal genetic associations ignore environmental exposures. If an environmental factor is necessary for a genetic variant to influence disease risk, populations with genetic variant carriers who are also exposed to the environmental factor are better able to detect gene-disease associations; on the other hand not accounting for such environmental risk factors can result in varying consistency for reports of marginal associations (Ott 2004). This issue seems particularly important for the *NOS1* rs2682826, for which gene-environment interactions have been hypothesized and reported for both cigarette smoking (Levecque et al. 2003) and pesticide exposure (Hancock et al. 2008). Additionally, an inadequate reference population, disease misclassification, insufficient power, study population heterogeneity, and population stratification may also result in between-study inconsistencies (Ioannidis 2007).

We found strong associations for PD in participants with certain *NOS1* genotypes

exposed to commonly used OP pesticides through two independent sources – home and agricultural use - consistent with the importance of oxidative stress-inducing mechanisms in combination with increased vulnerability due to low *PON1* OP metabolizer capacity. Our findings support a role for *NOS2A* genetic variants in PD susceptibility and *NOS1* as a modifier of associations with PD in OP pesticide exposed populations.

2.5 Tables

Table 2-1. General Characteristics of the study population, n=852.

Characteristic	Cases (n=357)	Controls (n=495)
Age of PD diagnosis, median (range)	70 (34-88)	
Age at interview, median (range)	72 (37-90)	68 (35-94)
Male sex, n (%)	204 (0.57)	243 (0.49)
First degree relative with PD, n (%)		
No	304 (0.85)	450 (0.91)
Yes	53 (0.15)	45 (0.09)
Cigarette smoking, n (%)		
Never	188 (0.53)	227 (0.46)
Ex	150 (0.42)	221 (0.45)
Current	19 (0.05)	47 (0.09)
European ancestry, n (%)		
Yes	288 (0.81)	441 (0.89)
No	69 (0.19)	54 (0.11)
Education, n (%)		
0 to <12 years	65 (0.18)	43 (0.09)
12 years	95 (0.27)	101 (0.20)
>12 years	197 (0.55)	351 (0.71)
PON1 metabolizer status, n (%)[a]		
Faster	264 (0.85)	376 (0.89)
Slower	45 (0.15)	46 (0.11)

[a]121 participants missing PON1 genotypes, 48 cases (13%) and 73 controls (15%)

Table 2-2. Marginal estimates (ORs and 95% CIs) for genetic variation in *NOS1* and *NOS2A* SNPs in association with PD; assuming an additive genetic model unless otherwise specified.

SNP	Genotype	Cases n (%)	Controls n (%)	Model 1: No *PON1* Adjustment		Model 2[a]: *PON1* Adjustment		
				Adjusted OR[b] (95% CI)	p value	Adjusted OR[b] (95% CI)	p value	SNP HWE p value[c]
NOS1 rs1047735[d]	CC	155 (0.45)	211 (0.51)	1.00		1.00		
	CT	143 (0.41)	176 (0.42)	1.28 (1.02, 1.60)		1.18 (0.92, 1.52)		
	TT	49 (0.14)	30 (0.07)	1.63 (1.04, 2.55)	0.04	1.39 (0.84, 2.29)	0.20	
	CT/TT vs CC			1.20 (0.90, 1.61)	0.22	1.07 (0.78, 1.47)	0.68	0.41
NOS1 rs2682826[d]	CC	178 (0.50)	231 (0.51)	1.00		1.00		
	CT	154 (0.43)	198 (0.44)	1.08 (0.85, 1.36)		1.13 (0.87, 1.46)		
	TT	24 (0.07)	26 (0.06)	1.16 (0.72, 1.85)	0.54	1.28 (0.76, 2.14)	0.36	
	CT/TT vs CC			1.05 (0.79, 1.39)	0.74	1.11 (0.81, 1.52)	0.51	0.06
NOS1 rs3741475	CC	167 (0.64)	165 (0.64)	1.00		1.00		
	CT	84 (0.32)	84 (0.33)	1.07 (0.78, 1.48)		0.99 (0.69, 1.41)		
	TT	11 (0.04)	7 (0.03)	1.15 (0.61, 2.18)	0.66	0.97 (0.47, 2.00)	0.94	0.34
NOS1 rs3741480	TT	93 (0.33)	138 (0.33)	1.00		1.00		
	TC	130 (0.46)	215 (0.51)	0.90 (0.72, 1.12)		0.90 (0.72, 1.12)		
	CC	58 (0.21)	69 (0.16)	0.81 (0.52, 1.27)	0.36	0.81 (0.52, 1.27)	0.36	0.33
NOS1 rs816353	GG	89 (0.32)	143 (0.34)	1.00		1.00		
	TG	141 (0.50)	225 (0.53)	1.21 (0.96, 1.53)		1.21 (0.96, 1.53)		
	TT	51 (0.18)	54 (0.13)	1.47 (0.93, 2.33)	0.10	1.47 (0.92, 2.33)	0.10	0.02
NOS2A rs1060826[e]	GG	129 (0.36)	179 (0.42)	1.00		1.00		

	AG	170 (0.48)	204 (0.48)	1.28 (1.03, 1.59)		1.26 (0.99, 1.59)		
	AA	57 (0.16)	46 (0.11)	1.63 (1.06, 2.52)	0.03	1.58 (0.99, 2.53)	0.06	
	AA vs GG/AG			1.56 (1.01, 2.38)	0.04	1.51 (0.95, 2.41)	0.08	0.28
NOS2A rs2297518	GG	238 (0.67)	268 (0.62)	1.00		1.00		
	AG	108 (0.30)	138 (0.32)	0.84 (0.65, 1.08)		0.78 (0.59, 1.03)		
	AA	9 (0.03)	25 (0.06)	0.70 (0.42, 1.17)	0.18	0.61 (0.35, 1.06)	0.08	0.20
NOS2A rs3730013	CC	156 (0.44)	193 (0.46)	1.00		1.00		
	CT	161 (0.45)	190 (0.45)	0.99 (0.79, 1.24)		1.02 (0.80, 1.30)		
	TT	39 (0.11)	39 (0.09)	0.99 (0.63, 1.55)	0.96	1.04 (0.64, 1.69)	0.88	0.43

[a]121 participants missing *PON1* genotype, 48 cases (13%) and 73 controls (15%)
[b]Additionally adjusted for age (continuous), sex, ever smoked, education, and European ancestry indicator
[c]HWE p-value based on control population only
[d]Additionally assumed dominant genetic model due to prior report
[e]Additionally assumed recessive genetic model due to prior report

Table 2-3. Interaction, main, and joint effect estimates for NOS1 SNPs and OP exposure in association with PD.

SNP	Major/ Minor allele	Exposure Category	*Homozygous wildtype* Cases / Controls	*Homozygous wildtype* Adj OR[a] (95% CL)	*Homozygous wildtype* p value	*Variant allele carrier* Cases / Controls	*Variant allele carrier* Adj OR[a] (95% CL)	*Variant allele carrier* p value	p for interaction
		Household OP Use[b]							
NOS1 rs2682826	C/T	Occasional Use	81/109	1.00 (ref)		70/104	0.89 (0.58, 1.39)	0.62	
		Frequent Use	32/39	1.30 (0.72, 2.34)	0.39	41/22	2.84 (1.49, 5.40)	0.002	0.04
NOS1 rs1047735	C/T	Occasional Use	82/107	1.00 (ref)		64/96	0.82 (0.52, 1.28)	0.39	
		Frequent Use	33/33	1.62 (0.88, 2.98)	0.12	38/23	2.31 (1.22, 4.37)	0.01	0.21
NOS1 rs816353	G/T	Occasional Use	51/80	1.00 (ref)		85/154	0.86 (0.55, 1.34)	0.5	
		Frequent Use	19/27	1.22 (0.60, 2.51)	0.58	49/40	2.01 (1.12, 3.59)	0.02	0.14
NOS1 rs3741480	T/C	Occasional Use	83/161	1.00 (ref)		53/74	0.73 (0.46, 1.14)	0.16	
		Frequent Use	53/40	0.93 (0.44, 1.99)	0.85	15/26	1.90 (1.06, 3.41)	0.03	0.02
NOS1 rs3741475	C/T	Occasional Use	86/84	1.00 (ref)		33/43	0.70 (0.39, 1.25)	0.23	
		Frequent Use	32/25	1.59 (0.83, 3.07)	0.17	21/6	3.46 (1.28, 9.37)	0.01	0.07
		Ambient OP Exposure[c]							
NOS1 rs2682826	C/T	None/Low	117/169	1.00 (ref)		116/162	0.99 (0.70, 1.40)	0.96	

		High	33/18	2.42 (1.27, 4.61)	0.01	43/12	4.83 (2.39, 9.73)	<.0001	0.15
NOS1 rs1047735	C/T	None/Low	114/155	1.00 (ref)		112/156	0.93 (0.65, 1.32)	0.67	
		High	32/19	2.07 (1.09, 3.91)	0.03	42/10	5.42 (2.54, 11.52)	<.0001	0.04
NOS1 rs816353	G/T	None/Low	72/118	1.00 (ref)		142/245	0.95 (0.66, 1.36)	0.77	
		High	17/17	1.59 (0.76, 3.32)	0.22	50/19	4.24 (2.30, 7.83)	<.0001	0.03
NOS1 rs3741480	T/C	None/Low	139/250	1.00 (ref)		75/112	0.83 (0.57, 1.19)	0.3	
		High	49/19	1.43 (0.69, 2.96)	0.33	18/18	3.78 (2.04, 6.99)	<.0001	0.01
NOS1 rs3741475	C/T	None/Low	111/114	1.00 (ref)		56/64	0.86 (0.54, 1.36)	0.52	
		High	38/14	2.93 (1.48, 5.80)	0.002	25/5	4.52 (1.61, 12.64)	0.004	0.36

[a]Adjusted for age (continuous), sex, ever-smoked, European ancestry, education, and PON1 status.
[b]Participants with an average frequency of household OP use per year during ages 16-<10 years prior to index age that was at or above the median average use in exposed controls were assigned to the "Frequent Use" category. Those in the "Occasional Use" category had an average frequency of use per year during ages 16-<10 years prior to index age that was below the median for any household pesticide (excluded subjects who did not frequently use OPs but frequently used other pesticides).
[c]Ambient pesticide exposure, counting total number of OPs exposed to (above the median level seen in exposed controls) at both occupation and residence, from 1974 (year of CA-PUR implementation) to 10 years before diagnosis or interview. Cut point based on top quartile in exposed controls

Table 2-4. Comparison of SNP marginal effects from previous investigation.

Study	Population	Cases	Controls	*NOS1* rs1047735 OR (95% CI)	*NOS1* rs2682826 OR (95% CI)	*NOS2A* rs1060826 OR (95% CI)
Levecque et al. (2003)	French	209	488	1.20 (0.85, 1.69)[a]	1.53 (1.08, 2.16)[a]	0.50 (0.29, 0.86)[b]
Hague et al. (2004)	Finnish	147	137	No association (p=0.63)[c]	No association (p=0.25)[c]	0.50 (0.27, 0.93)[b]
Schulte et al. (2006)	German	340	680	N/A	N/A	0.89 (0.61, 1.30)[b]
Huerta et al. (2007)	Asturias	450	200	N/A	No association[c]	No association[c]
Hancock et al. (2008)	US Caucasians	169 families		Positive association, minor allele (A) over-transmitted[d]	Positive association, minor allele (T) over-transmitted[d]	Positive association, minor allele (A) over-transmitted[d]

Abbreviations: N/A Not applicable because not investigated
[a]Dominant genetic model, CT+TT vs CC
[b]Recessive genetic model, AA vs GG+GA
[c]OR and 95% CI not provided
[d]Family based transmission-disequilibrium tests were used to examine association between SNPs with PD, comparing the distributions of alleles transmitted to affected offspring to alleles not transmitted (Hancock et al. 2008). Over-transmission of the minor allele indicates the minor allele at a given locus was transmitted to those with PD more than expected, and represents a positive or "risk" association between the allele and PD.

2.6 Supplement

Supplemental Material, Table 2-S1. Pesticide list and adjusted odds ratios.					
Pesticide	**CDPR Chem Code / Category**	**Cases (n=306) [n (%)]**	**Controls (n=397) [n (%)]**	**Adj OR[a] (95% CI)**	**P value**
Household Pesticides					
Any Pesticide[b]	Occasional Use	151 (0.49)	207 (0.60)	1.00 (ref)	--
	Frequent Use	155 (0.51)	138 (0.40)	1.69 (1.21, 2.36)	0.002
OP Pesticides[b]	Occasional Use	151 (0.67)	207 (0.78)	1.00 (ref)	--
	Frequent Use	73 (0.33)	57 (0.22)	2.05 (1.30, 3.24)	0.002
Ambient Organophosphates					
OP Exposure Indicator[c]	>11 OPs	76 (0.25)	37 (0.09)	2.99 (1.92, 4.65)	<.0001
Monocrotophos	52	64 (0.21)	44 (0.11)	2.00 (1.29, 3.09)	0.002
Bensulide	70	5 (0.02)	5 (0.01)	1.56 (0.44, 5.49)	0.49
Dicrotophos[d]	72	5 (0.02)	2 (0.01)	--	
Trichlorfon	88	35 (0.11)	15 (0.04)	3.01 (1.58, 5.73)	0.0008
Carbophenothion	110	25 (0.08)	15 (0.04)	2.36 (1.20, 4.64)	0.01
Ddvp	187	6 (0.02)	5 (0.01)	1.81 (0.54, 6.01)	0.34
S,S,S-Tributyl Phosphorotrithioate	190	57 (0.19)	49 (0.12)	1.53 (0.99, 2.35)	0.06
Dioxathion	192	18 (0.06)	8 (0.02)	3.00 (1.26, 7.12)	0.01
Diazinon	198	97 (0.32)	106 (0.27)	1.18 (0.84, 1.67)	0.34
Dimethoate	216	137 (0.45)	99 (0.25)	2.38 (1.71, 3.33)	<.0001
Disulfoton	230	50 (0.16)	35 (0.09)	1.91 (1.19, 3.09)	0.008
Chlorpyrifos	253	62 (0.2)	71 (0.18)	1.17 (0.79, 1.73)	0.44
Ethion	268	46 (0.15)	20 (0.05)	2.94 (1.66, 5.21)	0.0002
Merphos	293	51 (0.17)	28 (0.07)	2.51 (1.52, 4.17)	0.0003
Azinphos-Methyl	314	69 (0.23)	69 (0.17)	1.34 (0.91, 1.97)	0.14
Phosmet	335	70 (0.23)	71 (0.18)	1.29 (0.88, 1.90)	0.19
Malathion	367	90 (0.29)	63 (0.16)	1.99 (1.36, 2.90)	0.0004
Oxydemeton-Methyl	382	71 (0.23)	40 (0.1)	2.58 (1.67, 3.99)	<.0001
Methyl Parathion	394	25 (0.08)	19 (0.05)	1.43 (0.74, 2.77)	0.28

Naled	418	81 (0.26)	57 (0.14)	2.08 (1.41, 3.08)	0.0002
Parathion	459	102 (0.33)	83 (0.21)	1.82 (1.28, 2.59)	0.0009
Phorate	478	58 (0.19)	43 (0.11)	1.82 (1.16, 2.84)	0.009
Phosalone	479	25 (0.08)	11 (0.03)	3.15 (1.51, 6.58)	0.002
Mevinphos	480	56 (0.18)	37 (0.09)	2.11 (1.34, 3.34)	0.001
Phosphamidon	482	11 (0.04)	5 (0.01)	3.16 (1.05, 9.44)	0.04
Sulfotep	558	5 (0.02)	9 (0.02)	0.87 (0.29, 2.64)	0.8
Demeton	566	32 (0.10)	22 (0.06)	1.90 (1.06, 3.40)	0.03
Tepp[d]	577	3 (0.01)	1 (0.003)	--	
Ethephon	1626	64 (0.21)	52 (0.13)	1.64 (1.08, 2.50)	0.02
Leptophos	1676	9 (0.03)	7 (0.02)	1.95 (0.71, 5.36)	0.19
Acephate	1685	88 (0.29)	51 (0.13)	2.57 (1.72, 3.82)	<.0001
Methidathion	1689	76 (0.25)	64 (0.16)	1.65 (1.12, 2.42)	0.01
Methamidophos	1697	36 (0.12)	28 (0.07)	1.59 (0.93, 2.73)	0.09
Dialifor	1799	20 (0.07)	7 (0.02)	3.63 (1.48, 8.94)	0.005
Fenamiphos	1857	21 (0.07)	15 (0.04)	1.65 (0.81, 3.34)	0.17
Profenofos	2042	35 (0.11)	19 (0.05)	2.37 (1.30, 4.31)	0.005

[a]Adjusted for age (continuous), sex, ever-smoked, European ancestry indicator, education, and *PON1* status (O'Leary et al. 2005) .

[b]Participants with an average frequency of household OP use per year during ages 16-<10 years prior to index age that was at or above the median average use in exposed controls were assigned to the "Frequent Use" category. Those in the "Occasional Use" category had an average frequency of use per year during ages 16-<10 years prior to index age that was below the median for any household pesticide (excluded subjects who did not frequently use OPs but frequently used other pesticides, 82 cases and 81 controls). 52 controls missing household pesticide use information.

[c]Ambient pesticide exposure, counting total number of OPs exposed to (above the median level seen in exposed controls) at both occupation and residence, from 1974 (year of CA-PUR implementation) to 10 years before diagnosis or interview. Cut point based on top quartile in exposed controls. No cases (n=306) or controls (n=397) missing ambient pesticide exposure information.

[d]No OR calculated due to small numbers

Supplemental Material, Table 2-S2. Interaction and joint effect estimates between *NOS1* rs2682826 and household pesticide exposure in association with PD.

Pesticide Exposure	*Homozygous Wild Type (CC)*				*Variant Carrier (CT+TT)*			
	Cases n (%)	**Controls n (%)**	**Adj OR[a] (95% CI)**	**p value**	**Cases n (%)**	**Controls n (%)**	**Adj OR[a] (95% CI)**	**p value**
Any Household Pesticide Use[b]								
Occasional Use	81 (0.54)	109 (0.59)	1.00		70 (0.45)	104 (0.60)	0.88 (0.57-1.36)	0.56
Frequent Use	68 (0.46)	76 (0.41)	1.32 (0.83-2.11)	0.24	87 (0.55)	69 (0.40)	1.80 (0.15-2.84)	0.01
p value for interaction								*0.18*
Household non-OP Pesticide Use[c]								
Occasional Use	81 (0.69)	109 (0.75)	1.00		70 (0.60)	104 (0.69)	0.87 (0.56-1.35)	0.53
Frequent Use (non-OPs)	36 (0.31)	37 (0.25)	1.33 (0.75-2.36)	0.33	46 (0.40)	47 (0.31)	1.38 (0.82, 2.32)	0.23
p value for interaction								*0.66*

[a]Adjusted for age (continuous), sex, ever-smoked, minority status, and *PON1* status (O'Leary et al. 2005) .

[b]Participants with an average frequency of household OP use per year during ages 16-<10 years prior to index age that was at or above the median average use in exposed controls were assigned to the "Frequent Use" category. Those in the "Occasional Use" category had an average frequency of use per year during ages 16-<10 years prior to index age that was below the median for any household pesticide (excluded subjects who did not frequently use OPs but frequently used other pesticides).

[c]"Frequent Use (non-OPs)" and "Occasional Use" same as described above except those in the "Frequent Use (non-OPs)" had an average frequency use per year to any non-OP pesticides at or above the median in exposed controls (e.g excluding frequent users of OPs).

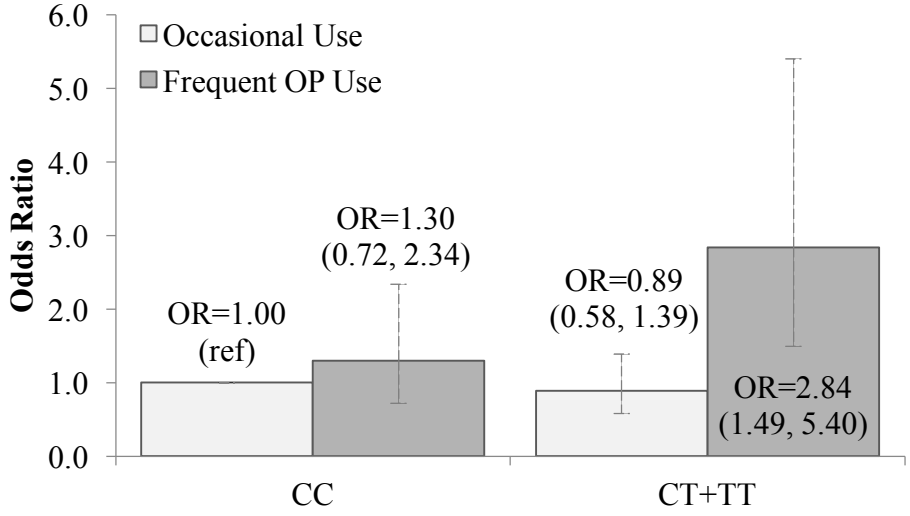
Supplemental Material, Table 2-S3. Effect estimates of *NOS1* genetic risk score (per allele) and OP exposure in association with PD.

	Adj OR[a] (95% CL)	p value	Adj OR[a] (95% CL)	p value	p for interaction
5 SNP *NOS1* Genetic Risk Score (GRS)					
Ambient OP Exposure	None/Low		High		
GRS (per 1 variant allele)[b]	1.04 (0.97, 1.12)	0.26	1.90 (1.04, 3.43)	0.03	0.01
Household OP Use	Occasional Use		Frequent Use		
GRS (per 1 variant allele)[b]	1.00 (0.91, 1.10)	0.98	1.45 (0.80, 2.63)	0.22	0.07
3 SNP *NOS1* Genetic Risk Score (GRS)					
Ambient OP Exposure	None/Low		High		
GRS (per 1 variant allele)[b]	1.06 (0.95, 1.18)	0.33	2.20 (1.27, 3.80)	0.42	0.01
Household OP Use	Occasional Use		Frequent Use		
GRS (per 1 variant allele)[b]	1.00 (0.87, 1.16)	0.95	1.63 (0.93, 2.87)	0.09	0.09

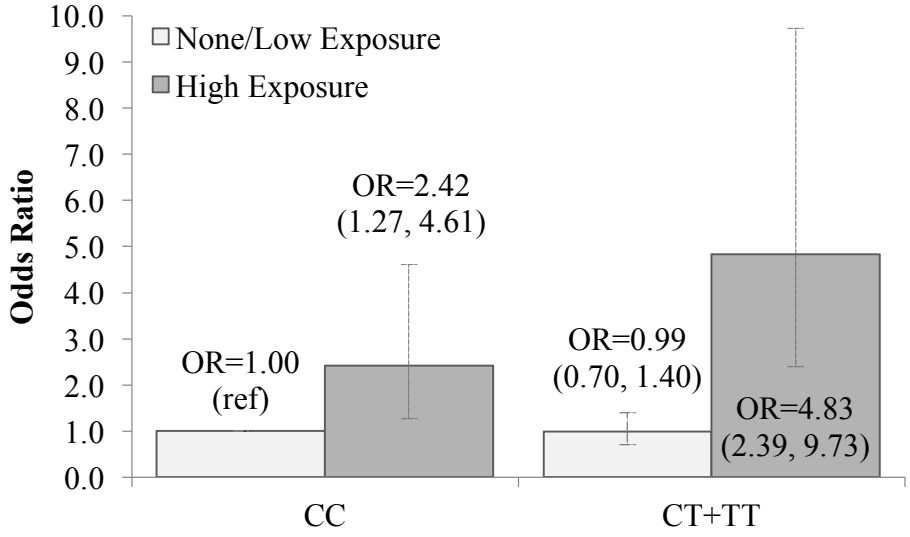
[a]Adjusted for age (continuous), sex, ever-smoked, European ancestry, education, and *PON1* status (O'Leary, 2005).

[b]Range for 5 SNP GRS 0-10 variant alleles, for 3 SNP GRS 0-6 variant alleles; treated as linear variables

1-A *NOS1* rs2682826 and household OP use



1-B *NOS1* rs2682826 and ambient OP exposure



Supplemental Material, Figure 2-S1. Interaction between *NOS1* rs2682826 and (**A**) household OP use (excluding frequent users of non-OP pesticides) and (**B**) ambient OP exposure. The figure displays the adjusted odds ratio (OR) and 95% confidence intervals for the joint and main effect estimates of OPs and rs2682826. P-value for interaction between (**A**) household OP use and rs2682826, 0.04.and (**B**) and ambient OP exposure and rs2682826, 0.15. Adjusted for age, sex, smoking status, European ancestry, education and *PON1* metabolizing status.

3. Organophosphate pesticide exposure and PON1 L55M in Parkinson's disease progression

3.1 Introduction

Parkinson's disease (PD), a progressive neurodegenerative disorder with selective degeneration of dopaminergic neurons and related motor symptoms, has many important non-motor features that contribute to its phenotype and functional decline, most prominently cognitive impairment and neuropsychiatric symptoms (Post et al. 2007). Dementia in PD patients is estimated to be as much as 2-6-fold more common than in unaffected individuals; up to 75% of PD patients who live more than 10 years after diagnosis are expected to develop dementia, while depression affects up to half of all PD patients (Aarsland and Kurz 2010). Over the course of disease, the severity and/or frequency of motor and non-motor symptoms increase and health related quality of life becomes a major concern for patients and caregivers (Santos-García and de la Fuente-Fernández 2013). Yet, very little is known about factors contributing to the course and progression of these disease features.

Pesticide exposures have consistently been associated with the development of PD (Freire and Koifman 2012), but to date no epidemiologic studies have investigated the influence of pesticides on PD symptom progression. Pesticide exposures can induce oxidative stress and mitochondrial dysfunction and impair the ubiquitin-proteasome system, mechanisms that have been related to neuronal cell death in PD (Rhodes et al. 2013; Terry 2012). For these same reasons, it is possible that these exposures may also contribute to faster symptom progression. Organophosphate (OP) insecticides are among the most commonly used pesticides agriculturally. The National Health and Nutrition Examination Survey (NHANES), 1999-2000, found that more

than 50% of participants in this national population sample had measurable levels of OP pesticide metabolites in their urine (NHANES 2011). OPs have long been investigated in relation to PD susceptibility both due to neurotoxic action and their ability to induce oxidative stress among other mechanisms (Bagchi et al. 1995; Lukaszewicz-Hussain 2010; Terry 2012).

Additionally, OPs have been associated with other PD related non-motor symptoms. In general populations, OP pesticides have been reported as contributing to cognitive impairment, inducing deficits in signal detection, information processing, attention, and memory among others, and been linked to depression and suicide (Jaga and Dharmani 2007; London et al. 2005; Terry 2012; Zaganas et al. 2013). Animal studies have provided some support for these observations, finding that chronic, low level OP exposure (1) is associated with sensorimotor gating, spatial learning, recognition memory, cognitive flexibility and sustained attention (Terry 2012), and (2) influences serotonin levels, possibly explaining how OP exposures may influence mood (London et al. 2005).

Many OP pesticides are activated to a toxic analog (oxon) by cytochrome P450 (Costa et al. 2003), and the oxon is subsequently detoxified by the paraoxonase activity of the PON1 hydrolyzing enzyme (Costa, Lucio G., Furlong 2007). Activity of PON1 is influenced by common single nucleotide polymorphisms (SNPs) in the *PON1* gene, including *PON1* L55M (rs854560). *PON1* L55M has been shown to directly influence PON1 levels and activity (Brophy et al. 2001; Garin et al. 1997; Mackness et al. 1993). We have previously reported that this variant modifies PD risk related to OP exposures (Lee et al. 2013), and there is evidence for a role of PON1 in Alzheimer's and vascular dementia, potentially through its anti-atherosclerotic function (Wehr et al. 2009; Zhub et al. 2015). PON1 also is an arylesterase, responsible for metabolism of aromatic esters (Cervellati et al. 2014). Both paraoxonase and arylesterase

activities of the protein are responsible for the anti-inflammatory and antioxidant activities of high density lipoprotein (HDL) and PON1 has been shown to prevent LDL oxidation in-vitro (Cervellati et al. 2014).

Here, we will investigate whether ambient agricultural OP exposure assessed with a geographic information system (GIS) that employed pesticide use reports and land use information, and *PON1* L55M genetic variation act together to influence the rate of motor, cognitive, and mood symptom progression in PD. We will rely on a prospectively followed population-based cohort of Parkinson's patients from three highly agricultural Central California counties, followed on average for more than seven years into their disease course.

3.2 Methods

All procedures described were approved by the University of California at Los Angeles (UCLA) Human Subjects Committee and informed consent was obtained from all participants.

Study Population

This longitudinal cohort includes 246 PD patients recruited as part of the Parkinson's Environment and Gene (PEG) population-based case-control study in Central California. More detail on recruitment methods (Costello et al. 2009; Gatto et al. 2010) and case definition criteria (Kang et al. 2005) for the case-control study and the longitudinal cohort (Ritz et al. 2012) have been published previously. Briefly, 373 incident, idiopathic PD patients, diagnosed within 3 years of recruitment, compose the base population for this longitudinal cohort. All patients were seen by movement disorder specialists (JB, YB) at least once at baseline, many on multiple occasions, and confirmed as having probable idiopathic PD based on published criteria (Hughes et al. 1992). At the first follow-up after baseline, 108 patients (29%) were lost to follow-up (64

were deceased, 6 too ill, 17 withdrew, and 21 could not be re-contacted). We successfully re-examined 265 (71%) patients during follow-up, and 13 of these participants were re-classified as not having idiopathic PD upon examination. Of the remaining 252 PD patients, 246 provided the data necessary for this investigation. Of these patients, 65 (26%) participated in 2 exams (3.6 years of mean follow-up, 5.9 years into disease), 174 (71%) in 3 exams (5.7 years of mean follow-up, 7.6 years into disease), and 7 (3%) participants in 4 exams (6.3 years of mean follow-up, 8.0 years into disease).

Assessment of PD Progression

Trained interviewers collected detailed information on demographic and risk factors and for each participant UCLA movement disorder specialists conducted physical examinations at baseline and during each follow-up to assess progression. Specifically, motor symptoms were assessed with the Unified Parkinson's Disease Rating Scale (UPDRS) part III, which assesses speech, facial expression, tremor, rigidity, hand, arm, and leg movements, posture, gait, postural stability, and bradykinesia. If possible, patients were examined off PD medications (82% of the baseline exams and 80% of follow-up exams). For patients who we could only examine on medication, we estimated an off-score by adding the difference of the whole study population's mean off- and mean on- scores at the time of exam to the patient's on-score (Ritz et al. 2012). Cognitive function was assessed at each exam with the Mini-Mental State Exam (MMSE), a widely used 30-point instrument that tests for orientation, attention, memory, language, and visual-spatial skills. For 3 patients at baseline and 6 during the first follow-up exam, we had to substitute the in-person MMSE with a 26-point telephone version of the MMSE and applied validated weights to make these scores comparable as recommended (Newkirk et al. 2004). Finally, we used the Short Form Geriatric Depression Scale (GDS) to measure depression

symptoms with 15 questions it has been widely used and validated in older populations (Burke et al. 2015). We previously validated the GDS in our PD population, finding high sensitivity and positive predictive value compared with the Structured Clinical Interview for DSM Disorders (SCID) and Patient Health Questionnaire (PHQ-9) instruments (Thompson et al. 2011).

Organophosphate Exposure Assessment

We estimated ambient exposure to OP pesticides, primarily from commercial agricultural application, using a geographic information system (GIS) based computer model which links California state mandated pesticide use reports (CA-PUR) for all commercial pesticide application since 1974 that contain information on date, location, type and amount of pesticide applied (CDPR 2013), with land use surveys providing the location of specific crops (CDWR 2013), and with geocoded lifetime address histories for each of our participants (both residential and occupational addresses). A total of 36 pesticides considered OPs in the pesticide action network (PAN) pesticide database (Kegley et al. 2014) contributed to our OP exposure measure; for a more detailed description see (Paul et al. 2015). Briefly, for each pesticide, we summed the pounds of pesticide applied per year and per acre within a 500-m buffer of each address to create a study-period (1974-baseline interview) specific exposure summing pounds applied each year and dividing by the number of years in the time period. Both residential and occupational addresses were included, and participants could have been exposed at both locations, one, or neither. We dichotomized exposure to each chemical according to the chemical-specific median pounds per acre in the exposed and counted the number of OP chemicals each participant was exposed to; we used this count to characterize participants as highly exposed (top quartile of count) or low/moderately exposed.

PON1 L55M Genotyping

Participants provided blood or saliva samples for genetic analyses, which were stored and processed at the UCLA Biologic Specimen Core Facility. *PON1* L55M (rs854560) genotyping was conducted at the UCLA Genotyping and Sequencing Core Facility using pyrosequencing, which achieved a 100% call rate. The 55MM genotype (TT, homozygous variant) was used to designate "slower" PON1 metabolizing as observed in human serum analyses of PON1 that showed that metabolizing activity is slowest in homozygous variants (O'Leary et al. 2005); also, we previously reported a positive interaction between the SNP and OP exposures for PD in our study (Lee et al. 2013; Manthripragada et al. 2010).

Statistical Methods

To evaluate differences in baseline demographics and symptom characteristics between OP exposure groups and PON1 metabolizing status we used either chi-square or student's two-tailed t-tests. We used repeated-measures regression analyses (Proc MIXED; SAS 9.4, SAS Institute, Cary, NC) to investigate between-subject and within-subject (time-dependent/progression) associations between OP exposure and PON1 and progression scores (MMSE, UPDRS, GDS) over follow-up. We selected a variance components correlation structure for within-subject associations. Including interaction terms between exposures and age (in lieu of follow-up time due to collinearity) allows us to estimate the difference in annual change in score for the three different outcome measures according to exposure; age refers to the age at each exam, centered at the mean age at time of baseline exam (68.9 years). In order to explore the influence of OP exposure and *PON1* L55M genotypes, we relied on longitudinal models containing OP exposure alone, L55M alone, OP exposure with the OP*PON1 interaction, and finally a model with OP

and PON1 and the OP*PON1 interaction. The regression coefficient (β) for the interaction terms with age represents the difference in annual change in outcome score (UPDRS, MMSE, or GDS), for example the yearly difference in score between OP exposed and unexposed subjects. In each model we also adjusted for sex, European ancestry (yes/no), years of education, smoking status (ever/never), and PD duration prior to baseline interview (0-3 years). We used SAS 9.4 (SAS Institute Inc., Cary, NC) for all analysis.

3.3 Results

Both demographic characteristics and baseline health indicators were similar by OP exposure and PON1 metabolizing status, although those most highly OP exposed were more likely to be male and have lower baseline MMSE scores (Table 3-1). Patients enrolled at baseline who were too ill or died before a second exam was conducted were significantly older, had less years of education, worse baseline exam scores (MMSE, UPDRS, GDS), and a lower proportion of PON1 slower metabolizers than patients we re-enrolled for at least one follow-up exam; however, they were similar in terms of PD duration, sex, smoking status, European ancestry, and OP exposure status. Participants not ill or deceased but lost to follow-up for other reasons were not statistically different from enrolled patients in terms of demographic factors, baseline exam scores, OP exposure, or PON1 status.

Using repeated measures linear regression models and controlling for age, sex, smoking, European ancestry, and education, we found that the highly OP exposed group of patients exhibited a significantly faster annual decline in MMSE, this was true across all models (OP*age β=-0.08 to -0.07, p=0.007 to 0.01). This estimated effect size for OP exposure across time was greater than that associated with aging alone (age β=-0.06 to -0.07). Slower PON1 metabolizer

status was associated with a lower MMSE score in model 2, containing only PON1 associations (PON1 β=-0.80, p=0.0003) and model 3, including OP exposure over time (PON1 β=-0.53, p=0.06), the interaction term with OP exposure in this model also indicates that slower metabolism and OP exposure together contributed to lower MMSE scores (PON1*OP β=-1.42, p=0.02), this interaction remained significant in model 4, in which we included the PON1*age term (PON1*OP β=-1.45, p=0.02); see table 3-2. The predicted decline in MMSE score across age by high OP exposure and PON1 status is visualized in figure 3-1a.

For the UPDRS-III, higher scores represent worse motor symptoms; again, across all models, high OP exposure was associated with significantly faster motor decline, (OP*age β=0.41 to 0.42, p=0.004 to 0.003), three times the effect size of the aging term (age β=0.09 to 0.15, p=0.09 to 0.003). Additionally, across all models, slower PON1 metabolizing status was associated with a higher UPDRS score, with faster progression for models allowing for variation over time (PON1*age β=0.29, p=0.01, models 2 and 4), and a lower between subject UPDRS score in model 3 that did not allow the score to vary over time by PON1 status (β=2.46, p=0.08); we did not see evidence for an interaction between PON1 metabolizing status and OP exposure and UPDRS; see table 3-2 and figure 3-1b. OP exposure was not associated with changes in GDS measured depressive symptom scores in our population (table 3-2 and figure 3-1c); however, in both models allowing for variation in score over time by PON1, slower PON1 metabolizers experienced a faster increase in GDS score (PON1*age β=0.09, p=0.008, models 2 and 4). When treating age as a random effect, to account for the differences in follow-up time between exams and age at time of exams, and the intercept in each model as a random effect, to account for differences in baseline exam scores, effect estimates were similar (for example, Model 3 and MMSE: age*OP β=-0.08, p=0.03, age β=-0.06, p=<.0001, OP β=0.08, p=0.80,

PON1 β=-0.36, p=0.33, PON1*OP β=-1.72, p=0.02).

3.4 Discussion

The rate and patterns of PD symptoms during disease progression are highly variable. Considerable motor and non-motor symptoms may accumulate over time and contribute strongly to disability and diminished quality of life (Global Parkinson's Disease Survey Steering Committee 2002; Poewe and Mahlknecht 2009); within a few years of diagnosis, some patients become wheelchair bound, cognitively impaired, severely depressed, and/or experience many other non-motor symptoms. In contrast other patients are spared major disabilities until later in disease progression. Although there is a notable knowledge gap regarding factors that contribute to or modify this heterogeneity of phenotype and severity, there are few longitudinal population-based PD cohorts, and investigators are just beginning to examine what may have an influence. Here, for the first time, we present evidence that long-term organophosphate pesticide exposure and/or *PON1* L55M slow metabolizer status are associated with PD symptom progression in three major domains – motor, cognitive, and mood-related symptom decline.

We found that high cumulative OP exposure, PON1 slower metabolizer status (55MM), and the interactions between these two factors were associated with faster decline or lower MMSE scores (PON1) over follow-up. Further, the estimated effect size for OP exposure across time was greater than the one associated with aging alone, meaning that those highly exposed to OP pesticides are expected to experience a decline in MMSE score more than twice as fast as in the comparison group (visualized in figure 3-1a). For PON1 metabolizer status, slower metabolism alone also predicted lower MMSE scores, and, moreover, appeared to interact with OP exposure (p=0.02), such that slow PON1 metabolizer patients with higher OP exposures had

lower MMSE scores. We did not associate PON1 with faster cognitive decline during follow-up, possibly indicating that any PON1 effect on cognition had already occurred by the time of our baseline exam. We saw similar results for the UPDRS-III, where the highly OP exposed patients showed a faster increase in UPDRS over time, nearly three times or more that of aging. Further, slower PON1 metabolizing status alone predicted higher UPDRS scores, with faster motor function decline during follow-up (models allowing the PON1 effect to vary with time). We did not find PON1 to modify the effect of OP exposure on motor score ($p=0.72$), which might suggest that OP exposure and PON1 influence motor progression independently or that we did not have enough sample size to estimate such an interaction. In our population, aging was associated with a decline in depressive symptoms as measured by the GDS, a trajectory consistent with prior PD research showing that younger patients experience more depression, likely resulting from a greater perceived negative quality of life (Cummings 1992; Giladi et al. 2000). Although OP exposure does not appear to play a role in the depressive symptoms score in our PD population, again, slow PON1 metabolizer status was associated with a faster rate of developing depressive symptoms, suggesting PON1 influences depression independent of OP metabolism.

Organophosphate pesticides are designed to inhibit acetylcholinesterase enzyme activity, resulting in an excess of cholinergic stimulation acutely affecting the motor and central nervous system of targeted insects (Terry 2012). Additionally, cell toxicity may also result from the induction of mitochondrial dysfunction and oxidative stress, with some evidence that low-level chronic exposures may have lasting toxic effects (Terry 2012; Zaganas et al. 2013). That PD patients chronically exposed to OP pesticides at low ambient levels experience faster cognitive decline is supported by a growing body of evidence that links long-term pesticide exposure to

memory, learning, and attention deficits, as well as dementia and Alzheimer's disease (AD) among others (Hayden et al. 2010; Terry 2012; Zaganas et al. 2013). A few reports associated pesticide exposure with AD. Including a large cohort investigation (n=3,084, with 500 dementia cases and 344 AD cases) in Cache County, UT, which found occupational pesticide exposure to be associated with an increased risk of dementia (hazard ratio (HR) =1.38, 95% CI=1.09, 1.76) and AD (HR=1.53, 95% CI=1.05, 2.23)(Hayden et al. 2010). This study replicated a French study of 1,507 elderly whose cognitive performance was worse among those occupationally exposed to pesticides (insecticides, herbicides, or fungicides); analysis in men showed a significant association between AD and occupational exposure (relative risk (RR) =2.39, 95% CI=1.02, 5.63)(Baldi et al. 2003). Although OP exposure has not been investigated relative to motor symptom decline, OP exposure has widely been associated with PD susceptibility (Alavanja et al. 2004; Wirdefeldt et al. 2011). It is possible that the same biologic pathways play a role for PD progression and susceptibility, namely oxidative stress and mitochondrial dysfunction (Bagchi et al. 1995; Terry 2012).

PON1 is important for OP metabolism and specifically detoxification. The L55M SNP modified the risk of developing PD after OP exposure in our study (Lee et al. 2013; Manthripragada et al. 2010). Here, we newly followed these PD patients from baseline (early after their diagnosis) to document decline over the course of disease. We found that this same variant seems to modify OP exposure associations related to cognitive decline. Furthermore, the 55MM genotype, which results in lower PON1 activity, alone also predicts lower MMSE, higher UPDRS, and higher GDS scores. Beyond its function for OP metabolism, PON1, as a component of high density lipoproteins (HDL), acts in an anti-oxidant and anti-atherosclerotic fashion preventing low density lipoproteins (LDL) from being oxidized (Zhao et al. 2012).

Which of these functions contribute to cognitive decline in PD or both remains unclear; though multiple independent investigations associate PON1 with AD and vascular dementia, both by examining genomic variation and with enzyme activity (Alam et al. 2014). This suggests that PON1 influences cognitive outcomes not solely via its OP metabolism function. A recent meta-analysis of 69 studies associated L55M, as one of four polymorphisms apart from APOE, with vascular dementia (Zhub et al. 2015), numerous studies found serum PON1 activity decreased in dementia or AD patients, and one reported that MMSE scores were dependent on PON1 activity (Bednarska-Makaruk et al. 2013; Dantoine et al. 2002; Erlich et al. 2006; Helbecque et al. 2004; Sato and Morishita 2015; Wehr et al. 2009). Although this provides biological plausibility for PON1's contributions to cognitive decline in PD aside from OP metabolism, based on current epidemiologic research, PON1 alone may not be related to PD development in the absence of OP exposure (Liu et al. 2012).

Mechanisms for motor symptom decline in PD are not well understood, and further research is needed to establish any role for PON1. Yet, several findings suggest a role of lipid and cholesterol metabolism in not only vascular dementia and AD - as discussed - but also PD pathogenesis (De Lau et al. 2006; Reiss et al. 2004). Multiple case-control and cohort studies have implicated lower levels of cholesterol with increased PD risk (De Lau et al. 2006; Huang et al. 2007; Simon et al. 2007), yet again by what mechanism is unknown. Interestingly, in vitro studies show alpha-synuclein, a primary component of the aberrant protein aggregations in Lewy bodies of PD patients, is closely associated with cholesterol-enriched lipid rafts in the cell membranes, and alpha-synuclein oligomerization may be regulated by fatty acids (Welch and Yuan 2003). Further, the concentration of coenzyme Q10, an electron acceptor in the mitochondrial respiratory chain and a powerful antioxidant, is highly dependent on cholesterol

(Johansen et al. 1991; Kaikkonen et al. 1999). Given the importance of oxidative stress and mitochondrial dysfunction in PD pathogenesis, it is possible that cholesterol, and in turn PON1, is influencing motor symptom progression through coenzyme Q10 or alpha-synuclein oligomers. Finally, multiple studies associated lower PON1 activity with an increased risk of depression (Barim et al. 2009; Bortolasci et al. 2014; Rice et al. 2009), including a large cohort of British women in which slower PON1 metabolism as measured by the Q192R SNP was associated with increased depression risk (OR=1.22, 95% CI=1.05, 1.41) (Lawlor et al. 2007). This has also been attributed to the antioxidant and anti-inflammatory mechanisms of PON1.

As expected in a cohort of very elderly subjects, we were unable to follow all PD patients enrolled at baseline. There was loss to follow-up due to death and illness, these patients at baseline were older and had worse MMSE, UPDRS, and GDS exam scores, consequently selection bias is possible. Additionally, with our ambient pesticide exposure assessment, we did not account for meteorological factors that may influence pesticide drift and we had to assume that study participants were at their residential or occupational location during relevant periods; thus, exposure misclassification cannot be excluded. Lastly, although we do not have follow-up data for a non-PD population, and thus we cannot tell whether the longitudinal findings are specific to PD or whether the same type of symptom progression would be observed in a non PD affected population with similar risk factors to ours, this investigation still provides important potential insights into PD symptom and cognitive decline.

Our study is one of less than a handful of population-based prospective PD patient cohorts worldwide and the only one to date to collect environmental and occupational exposure data. All of our patients were seen in person and examined by UCLA movement disorder specialists (mainly JB, YB) to confirm diagnosis and assess progression; follow-up began early

in disease course (within 3 years of diagnosis); and due to our population-based design, our results are more generalizable to PD populations than patient cohorts assembled at tertiary care centers. In terms of exposure assessment, the majority of epidemiologic studies to date rely on self-reported pesticide exposure, a method prone to recall error, as participants may forget or be unaware of pesticide use. Our pesticide exposure assessment relied on a GIS tool and pesticide use and land use records that do not rely on participant recall, and also allow us to investigate specific pesticides or chemical classes of interest, like OPs, providing a good population and opportunity to investigate environmental exposures.

Although our findings need to be re-examined and replicated in future studies, this study provides support for the involvement of both OP pesticides and PON1 in PD motor and non-motor progression. Given the importance of symptom progression for patients' health related quality of life and for predicting mortality (Forsaa et al. 2010), addressing this knowledge gap and identifying modifiable predictors for rate or severity of symptoms during disease course is important for both patients and for developing preventive measures.

3.5 Tables

Table 3-1. Baseline demographic characteristics and follow-up information by ambient OP exposure, PON1 metabolizing status, and enrollment status.

Characteristic Mean ± SD or n (%)	Cohort (N=246)	OP Exposure[a]		PON1 Metabolizing Status[b]		Lost to Follow-up[c]	
		None/ Low (N=197)	High (N=49)	Fast/ Average (N=204)	Slower (N=39)	Died/ Too Ill (n=70)	Withdrew/ Unavailable (n=38)
Demographics							
Age at interview	68.9 ± 9.8	68.8 ± 10.1	69.1 ± 8.2	68.7 ± 9.5	69.5 ± 11.4	76.4 ± 6.0*	68.2 ± 13.4
Age at Diagnosis	67.0 ± 9.9	67.0 ± 10.3	67.0 ± 8.1	66.8 ± 9.6	67.7 ± 11.5	74.3 ± 5.9*	66.0 ± 13.5
PD Duration (y)							
Prior to baseline	2.0 ± 1.5	2.0 ± 1.4	2.2 ± 2.0	2.1 ± 1.6	1.9 ± 1.4	2.3 ± 1.6	2.2 ± 1.4
Last follow-up	7.5 ± 2.6	7.1 ± 2.7	7.1 ± 3.3	7.0 ± 2.7	7.5 ± 3.0	--	--
Follow-up (y)	5.2 ± 2.1	5.2 ± 2.1	5.2 ± 2.4	5.0 ± 2.1	5.8 ± 2.3	--	--
PD Family History	38 (0.15)	32 (0.16)	6 (0.12)	30 (0.15)	8 (0.21)	9 (0.13)	5 (0.13)
European Ancestry	197 (0.81)	161 (0.82)	36 (0.73)	161 (0.79)	34 (0.87)	58 (0.83)	29 (0.76)
Male	140 (0.57)	104 (0.53)	36 (0.73)*	116 (0.57)	22 (0.56)	40 (0.57)	23 (0.61)
Ever Smoker	111 (0.45)	92 (0.47)	19 (0.39)	95 (0.47)	14 (0.36)	35 (0.50)	21 (0.55)
Years of School	13.7 ± 4.4	13.9 ± 4.3	12.9 ± 4.8	13.8 ± 4.4	13.3 ± 3.6	12.4 ± 3.2*	13.6 ± 2.9
Baseline Health Indicators							
MMSE	28.1 ± 2.3	28.3 ± 2.1	27.5 ± 2.7*	28.2 ± 2.4	27.8 ± 1.8	26.4 ± 3.1*	27.8 ± 2.6
GDS	3.2 ± 3.3	3.2 ± 3.3	3.2 ± 3.3	3.3 ± 3.4	2.6 ± 2.4	4.4 ± 3.0*	3.6 ± 2.8

UPDRS III,	19.6 ± 9.6	19.3 ± 9.2	21.0 ± 10.8	19.6 ± 9.3	19.0 ± 10.6	27.8 ± 14.0*	21.8 ± 10.7
Levodopa Use	158 (0.68)	132 (0.71)	26 (0.58)	133 (0.69)	24 (0.63)	57 (0.84)*	23 (0.61)
LD (mg/day)	283.4 ± 297.6	302.7 ± 289.3	221.7 ± 236.5	295.3 ± 288.0	240.1 ± 244.9	323.9 ± 211.4	250.7 ± 277.1
LED (mg/day)	351.1 ± 264.8	372.8 ± 299.6	304.8 ± 234.4	363.7 ± 299.8	327.6 ± 221.6	370.4 ± 243.6	316.4 ± 297.8
PD Subtype							
Tremor Dominant	56 (0.23)	44 (0.22)	12 (0.24)	45 (0.22)	11 (0.28)	3 (0.04)	7 (0.18)
PIGD Dominant	159 (0.65)	130 (0.66)	29 (0.59)	135 (0.66)	22 (0.56)	60 (0.86)*	27 (0.71)
Intermediate	31 (0.13)	23 (0.12)	8 (0.16)	24 (0.12)	6 (0.15)	7 (0.10)	4 (0.11)
Exposures of Interest							
High OP Exposure	49 (0.20)	--	--	40 (0.20)	9 (0.23)	16 (0.23)	12 (0.32)
Slower PON1 Metabolizer[b]	39 (0.16)	30 (0.15)	9 (0.18)	--	--	3 (0.05)*	3 (0.08)

*p-value<0.05
[a]Based on PON1 rs854560 (Lee et al. 2013; O'Leary et al. 2005)
[b]Comparison group is the enrolled cohort (n=252)

Table 3-2. Repeated measures linear model results for three separate models, predicting change in 1) Mini-mental State Exam, 2) Unified Parkinson's Disease Rating Scale, and 3) Geriatric Depression Scale.

Characteristic	Outcome 1: MMSE		Outcome 2: UPDRS		Outcome 3: GDS	
	β Coefficient	P value	β Coefficient	P value	β Coefficient	P value
Model 1: OP Only						
Age	-0.06	<.0001	0.15	0.003	-0.04	0.009
High OP Exposure	-0.32	0.21	-0.77	0.54	0.11	0.74
High OP Exposure*Age	-0.07	0.0095	0.41	0.004	0.04	0.33
Model 2: PON1 Only						
Age	-0.07	<.0001	0.14	0.006	-0.05	0.0007
Slower PON1 Metabolizer	-0.80	0.003	1.68	0.20	-0.28	0.45
Slower PON1 Metabolizer*Age	-0.01	0.63	0.29	0.01	0.09	0.008
Model 3: OP*age + PON1*OP						
Age	-0.06	<.0001	0.15	0.002	-0.04	0.009
High OP Exposure	-0.02	0.93	-1.00	0.47	0.17	0.65
High OP Exposure*Age	-0.08	0.007	0.42	0.003	0.04	0.33
Slower PON1 Metabolizer	-0.53	0.06	2.46	0.08	0.06	0.87
High OP Exposure*Slower PON1 Metabolizer	-1.42	0.02	0.59	0.85	-0.31	0.72
Model 4: OP*age +PON1*age + PON1*OP						
Age	-0.06	<.0001	0.09	0.09	-0.05	0.0004
High OP Exposure	-0.03	0.93	-0.96	0.49	0.18	0.63
High OP Exposure*Age	-0.08	0.007	0.42	0.003	0.04	0.32
Slower PON1 Metabolizer	-0.46	0.12	1.49	0.31	-0.25	0.54
Slower PON1 Metabolizer*Age	-0.02	0.45	0.29	0.01	0.09	0.008
High OP Exposure*Slower PON1 Metabolizer	-1.45	0.02	1.10	0.72	-0.13	0.88

Abbreviations: MMSE = Mini-mental State Exam; UPDRS=Unified Parkinson's Disease Rating Scale; GDS= Geriatric Depression Scale

Models also controlled for PD duration prior to baseline, sex, European ancestry, years of schooling, and smoking status

Results shown as regression coefficients (β); interaction term between OP exposure and age represents the difference in annual change in score between the high and low/no exposure groups for the three different exams, UPDRS, MMSE, and GDS.

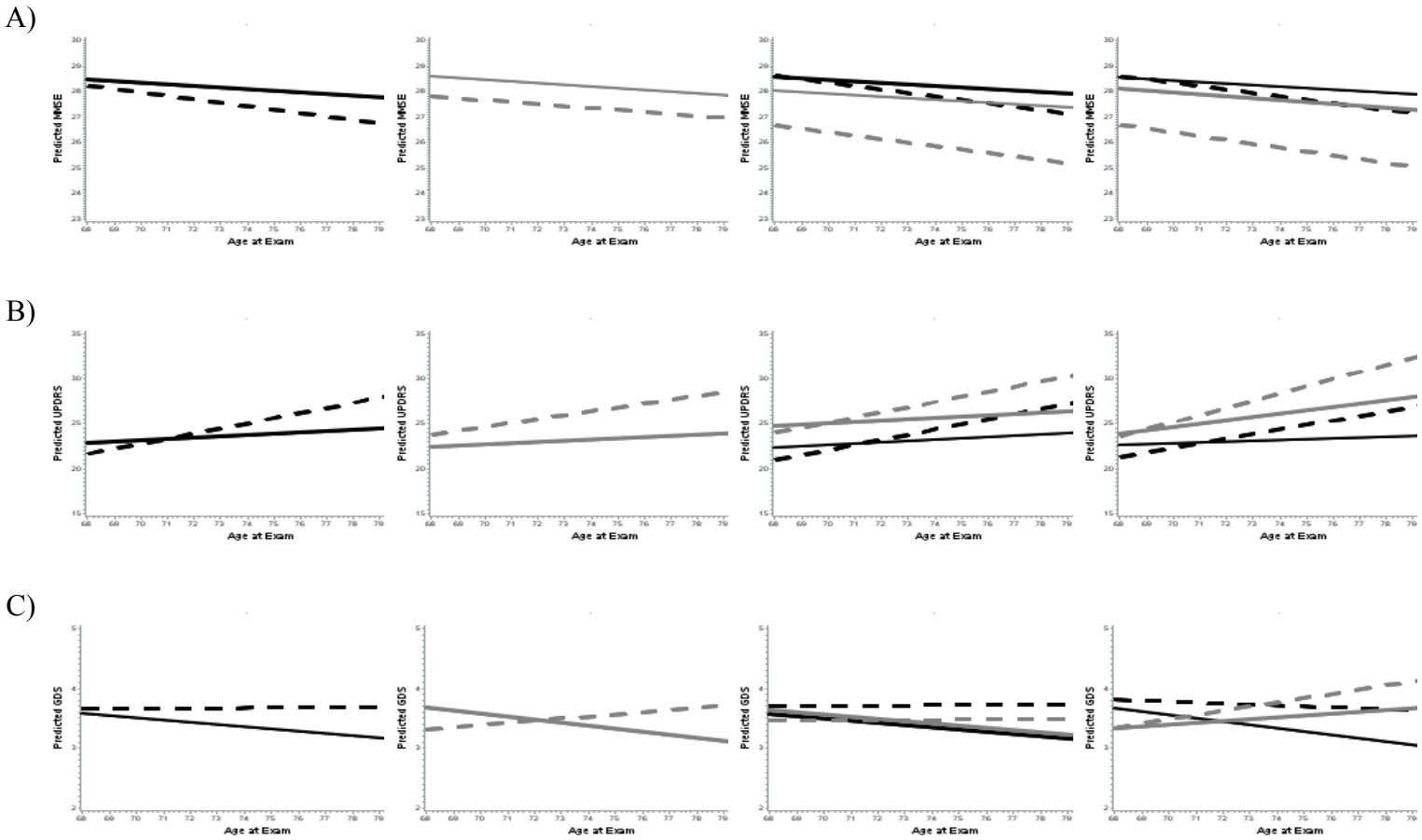

Model 1: OP*age
Model 2: PON1*age
Model 3: OP*age + OP*PON1
Model 4: OP*age + PON1*age + OP*PON1
A)
B)
C)
Predicted MMSE
Predicted UPDRS
Predicted GDS
Age at Exam
Legend Models 1 and 2
Low OP Exposure
High OP Exposure
Legend Models 3 and 4
Low OP Exposure, Average PON1 metabolizer
High OP Exposure, Average PON1 metabolizer

Figure 3-1. Predicted progression of A) MMSE, B) UPDRS-III, and C) GDS scores in cohort by OP exposure and PON1 metabolizing status by models. Given the mean years of schooling and PD duration prior to baseline, female, non-smoker, and European ancestry.

4. *NFE2L2*, *PPARGC1α*, and Oxidative Stress in Parkinson's disease susceptibility and progression

4.1 Introduction

Parkinson's disease (PD), the second most common neurodegenerative disease, is characterized by the progressive loss of dopaminergic neurons in substantia nigra region of the brain. Several major molecular pathways are implicated in cellular dysfunction and neuronal death in PD; many, including impaired ubiquitin-proteasome system (UPS), mitochondrial dysfunction, and neuroinflammation, involve oxidative stress as an underlying mechanism (Jenner 2003; Dauer and Przedborski 2003; Hwang et al 2013). While it is not well understood whether and how oxidative stress contributes also to motor or non-motor symptom progression, a long history of post-mortem studies indicates increases in oxidative stress at the end stage of the illness when neuronal loss was marked, showing excess free radicals, increased iron levels, and decreased glutathione (GSH) among other markers (Jenner 2003). Furthermore, the influence that oxidative stress has may accelerate as cellular dysfunction (e.g. mitochondrial dysfunction, etc) accelerates and disease progresses. Similarly, a genetically determined inability to cope with oxidative stress may contribute and enhance the effects of exposure to toxicants that increase oxidative stress such as pesticides.

Cells have many antioxidant mechanisms to counteract reactive oxygen species (ROS)/oxidative stress, including a battery of endogenous antioxidant enzymes (Clark and Simon 2009). Nuclear factor-erythroid 2 related factor 2 (Nrf2), encoded by the *NFE2L2* gene, and peroxisome proliferator activator receptor γ coactivator 1α (PGC-1α), encoded by the *PPARGC1α*, are transcription factors involved in the regulation of many antioxidant enzymes in

response to oxidative stress and targets for neurodegenerative disease therapy (Clark and Simon 2009).

Once activated by oxidative stress, Nrf2 binds to specific promoter regions of multiple cytoprotective genes to upregulate the transcription of antioxidant enzymes, including glutathione (GSH), one of the factors which may determine vulnerability of dopaminergic (DA) neurons to oxidative stress, and NAD(P)H quinone oxidoreductase-1 (NQO1), which exerts a protective effect against toxic DA metabolites implicated in PD pathogenesis (Hwang et al 2013). A functional *NFE2L2* 3-SNP promoter haplotype, associated with transcriptional activity, has been linked with both decreased risk of developing PD and older age of PD onset in a European case-control study (von Otter et al. 2010; von Otter et al. 2014). Additionally, animal models provide support for *NFE2L2* involvement in PD, Nrf2 deficient mice and neuronal cultures showed increased MPTP toxicity, the toxic metabolite known to acutely induce Parkinsonism in humans (Chen et al. 2009; Lee et al. 2003). On the other hand, over expression of Nrf2 protected against locomotor activity loss in Drosophila modeled Parkinsonism (Barone et al. 2011).

PGC-1α is believed to mediate the protective responses of many antioxidant enzymes located primarily in the mitochondria, of great interest for PD given that mitochondrial dysfunction has been strongly implicated in PD etiology (Clark and Simon 2009). Further, PGC-1α, a multifunctional protein, is also a critical regulator of metabolism, including the adipogenesis and gluconeogenesis pathways (Clark and Simon 2009); this again is compelling for PD as some research associated type 2-diabetes and glucose sensitivity with PD risk and symptom severity (Yang et al 2011). In a population based genome-wide expression study, genes expressed in response to PGC-1α were less strongly expressed in PD patients (Zheng et al.

2010). Moreover, a recent candidate gene study suggested the *PPARGC1α* SNPs rs6821591 and rs2970848 are associated with age at PD onset while rs8192678 and rs6821591 were linked to longevity (Clark et al. 2011). Interestingly, *PPARGC1α* knockout mice experienced over 5 times the loss of DA neurons in response to MPTP exposure than wild-type mice (St-Pierre et al. 2006).

Here we investigate the influence of variants in the *NFE2L2* and PPARGC1α genes on PD onset and symptom progression, using haplotypes constructed from multiple SNPs, in our population-based study of PD patients and community controls. And further, we explore how genetic variants in these genes mediate the risk of PD associated with exposure to oxidative stress inducing pesticides, maneb and paraquat (MB/PQ), which have previously been linked to PD in our studies (Costello et al 2009).

4.2 Methods

Study Population

To investigate PD onset, we used 472 PD patients and 532 controls of European ancestry from the Parkinson's Environment and Gene (PEG) population-based case-control study living in three agricultural Central California counties (Kern, Fresno, and Tulare) at time of enrollment between 2001-2015. Participants were considered eligible if they were 35 or older, had lived in California for at least 5 years, and were residing in one of the three counties at the time of enrollment. All patients were seen by study movement disorder specialists (JB, YB) at least once at baseline, many on multiple occasions, and confirmed as having probable idiopathic PD according to published criteria (Hughes et al, 1992).

Patients were recruited initially (from 2001-2007) through large medical groups,

neurologists, and public service announcements and from 2010-2015 through the state-mandated pilot California Parkinson's Disease Registry (CAPDR), see figure 1. During the first round (2001-2007) of patient recruitment, we identified 1,167 potential PD patients; 604 were not eligible (397 not diagnosed with PD within 3 years of recruitment, 134 did not live in the tri-county study area, and 73 did not have PD). Of 563 potential cases, our movement disorder specialists were able to examine 473 patients, 94 did not meet criteria for idiopathic PD, 13 were reclassified as not having idiopathic PD during follow-up, and 6 subjects withdrew; 360 incident (diagnosed within 3 years) PD patients were enrolled. For the second round of case recruitment (2010-2015), there were 4,672 registry recorded potential PD patients with an address in the tri-county study area; we were able to contact and assess case reporting accuracy for 2,363. Overall 1,648 were found not to be eligible for our study (158 were diagnosed with PD more than 3 years before recruitment, 327 did not have PD, 935 were deceased, 156 were too ill, institutionalized, or unable to communicate/contact, and 92 lived outside the tri-county area), and 247 potential patients refused to participate. Out of 581 potential cases, 472 were seen by our movement disorder specialists at the time of this analysis; 69 participants did not have idiopathic PD, and for 10 a PD diagnosis could not be established reliably, 13 were too ill, and 1 withdrew. Thus during the second round, we enrolled 376 confirmed PD patients. However, genotyping (2014) took place before the enrollment of 114 PD patients from this strategy, thus they are not included in analysis. Additionally, to avoid issues of population stratification in genetic analyses, we excluded 150 patients of non-European ancestry, leaving 472 PD patients of European ancestry for analysis.

A total of 879 controls were enrolled from 2001-2011 in the same tri-county area and included in genotyping. Initially, we identified potential eligible controls through Medicare

enrollee lists (2001) but switched to publicly available residential tax-collector records after the Health Insurance Portability and Accountability Act (HIPPA) was implemented. We employed two sampling strategies: 1) random selection of residential parcels and mail or phone enrollment and 2) clustered random selection of five households we visited in person (Wang et al, 2011). Using the first sampling strategy, we contacted 755 eligible control participants, 409 declined participation and 346 population controls were enrolled. Additionally, from an early mailing with an unknown number of eligible subjects who declined, we enrolled 62 controls. We identified 1,241 eligible population controls from the second sampling strategy, 634 declined participation and 607 individuals were enrolled, though 183 only completed an abbreviated interview and 77 participants were not genotyped. Of the 755 controls with the necessary data, we excluded 221 participants of non-European ancestry, leaving 534 controls for analysis.

For our PD progression analyses, we relied on a prospective patient only cohort, which attempted to follow patients from the first round of recruitment (n=360) (for more detail see Ritz et al 2012). Briefly, at first attempted re-contact, 108 patients (29%) could not be re-examined (64 were deceased, 6 too ill, 17 withdrew, and 21 could not be contacted). Of the remaining 252 patients, 11 did not provide the data necessary for the progression analyses, and we excluded 49 participants of non-European ancestry. Of the 192 patients included in the longitudinal analyses, 37 participated in only two exams and 155 in three, for a mean follow-up of 5.3 years (SD=2.1) and an average of 7.3 years of PD duration (SD=2.8). Figure 4-1 details the flow of recruitment for both the case-control and cohort studies.

Data Collection

Trained interviewers recorded information on demographic and risk factors for all patients

and controls; physical exams for patients were performed by movement disorder specialists (JB, YB) at baseline and at each follow-up, confirming PD diagnosis and assessing disease progression. Motor symptoms were assessed by the physicians with the Unified Parkinson's Disease Rating Scale (UPDRS) part III. A higher score indicates worse motor symptoms. If possible, patients were examined off PD medications (82% of the baseline exams and 80% of follow-up exams). For patients we could only examine on medication, we estimated an off-score by adding the difference of the whole study population's mean off- and mean on- scores at the time of exam to the patient's on-score (Ritz et al. 2012). Cognitive function was assessed at each exam with the Mini-Mental State Exam (MMSE), a common 30-point test, including tests of orientation, attention, memory, language, and visual-spatial skills. A lower score indicates worse cognitive performance. A 26-point telephone version of the MMSE exam, validated to estimate the in-person MMSE, was administered in lieu of an in-person exam for 3 patients at baseline exams and 6 at the first follow-up; for these participants, validated weights were applied create scores comparable with the 30-point in-person interview (Newkirk et al, 2004).

Maneb/Paraquat Pesticide Exposure

We estimated ambient exposure to pesticides, primarily from commercial agricultural application, using a geographic information system (GIS) based computer model. More information on this method has been published (Cockburn et al. 2011). The model links California pesticide use reports (CA-PUR), which are state mandated for all commercial pesticide application since 1974 and contain information on date, location, type and amount of pesticide applied (CDPR 2013), with land use surveys providing the location of specific crops (CDWR 2013), and with geocoded lifetime address histories for each of our participants (both residential and occupational addresses). For both maneb and paraquat, we summed the pounds

of each pesticide applied per year and per acre within a 500-m buffer of each address to create a study-period (1974- diagnosis or baseline interview (controls)) average exposure by summing the pounds applied each year and dividing by the number of years in the time period. We dichotomized exposure to both maneb and paraquat according to the pesticide-specific median average exposure in the exposed control population. We considered participants highly exposed to MB/PQ if they were exposed to the chemicals based on the dichotomized measure.

SNP Selection and Genotyping

We selected three SNPs from the *NFE2L2* gene, rs35652124, rs6706649, rs6721961 that make up a functional haplotype in the promoter region of the gene that has been associated with transcriptional activity (Marzec et al 2007; von Otter et al. 2010). We inferred haplotype frequencies from the three SNPs (ordered rs35652124, rs6706649, rs6721961 based on genome location) using the PHASE haplotype software, which employs a Bayesian method, imputing a-priori predictions about the patterns of haplotypes expected in natural populations (Stephens et al. 2001; Stephens and Donnelly 2003). We further selected four *PPARGC1α* SNPs, three tag SNPs based on previous marginal genetic associations, rs6821591, rs2970848, and rs4235308, and one functional SNP, rs8192678 (Gly482Ser), where variants cause altered the protein sequence and structure for PGC-1α (Clark et al. 2011). Again we used PHASE to impute *PPARGC1α* haplotypes for analysis (haplotype order rs6821591, rs8192678, rs2970848, rs4235308). DNA was extracted from blood or saliva samples at the UCLA Biologic Specimen Core Facility. Genotyping was done using the Fluidigm BioMark system (Fluidigm Corporation, South San Francisco, CA).

Statistical Methods

We examined Hardy-Weinberg equilibrium in control participants for all polymorphisms using a chi-square test. To evaluate differences between cases and controls and those followed versus those lost to follow-up in the cohort we used either chi-square (categorical variables) or student's two-tailed t-tests (continuous variables). For haplotype genetic analyses related to PD onset and progression, we relied on an allelic genetic model to examine the influence of haplotypes, that is we compared each haplotype to a single reference haplotype, as each individual has two haplotypes, one on each paired chromosome, the sample size is 2n. For the *NFE2L2* haplotype we set the reference level to the "TCT" haplotype, in order to assess the influence of the "TCG" haplotype, the most frequent in our population, which was previously reported as being related to PD risk (Von Otter et al, 2010). As there were no a-priori hypotheses for the *PPARGC1α* haplotype, we used the most frequent haplotype "TAAT" as the reference. For single SNP associations, we relied on an additive genetic model, where each copy of the variant allele increases the risk by the same amount, except for NFE2L2 rs6721961, where only two patients and four controls were homozygous for the variant, and we relied on a dominant genetic model (any minor allele versus homozygous for the major allele).

For PD susceptibility analyses, we used unconditional logistic regression to calculate odds ratios (ORs) and 95% confidence intervals (CIs). Statistical interactions were assessed by introducing a multiplicative interaction term (e.g. product term: haplotype x pesticide) into the logistic model. For PD progression, we used repeated-measures regression analyses (Proc MIXED; SAS 9.4, SAS Institute, Cary, NC) to investigate within-subject (time-dependent/progression) associations between haplotypes and progression scores (MMSE, UPDRS) over follow-up. We report the interaction term between haplotypes and age (in lieu of follow-up time due to collinearity), which allows us to estimate the difference in annual change

in score for the outcome measures according to haplotype groups; age refers to the age at each exam, centered at the mean age at time of baseline exam (68.9 years).

In all models, we adjusted for age (at interview for controls and diagnosis for patients), sex, smoking status (ever/never), and education (<12 years, 12 years, >12 years). All analysis was performed with SAS 9.4 (SAS Institute Inc., Cary, NC).

4.3 Results

Demographic characteristics of the case/control and patient cohort population can be found in table 4-1. The PD patients were older, had less years of education, a lower proportion of smokers, and a higher proportion of males and maneb/paraquat pesticide exposure relative to the control population. In the longitudinally followed population, the patients we followed were younger, had more years of education, and scored better on baseline PD symptom scores (UPDRS and MMSE) than patients lost to follow-up.

All SNPs investigated were in HWE in the control population. The individual SNP genotype frequencies and PD associations can be found in supplemental table 4-1; *NFE2L2* rs6721961 was the only SNP marginally associated with PD in our population (OR=0.67, 95% CI=0.49, 0.91). Haplotype frequencies and associations with PD are displayed in table 4-2. The *NFE2L2* "CCG" haplotype was associated with a significant increase in risk of developing PD (OR=1.39, 95% CI=1.01, 1.91), and the "TCG" haplotype was associated with an even stronger risk increase (OR=1.50, 95% CI=1.11, 2.03) relative to the "TCT" haplotype in the case/control population (table 4-2). The same two haplotypes, "TCG" and "CCG", were also associated with significantly faster annual cognitive decline as measured with the MMSE in the PD patient only cohort ("TCG": β=-0.101, SE=0.03, p=0.0003; "CCG": β=-0.099, SE=0.02, p=0.0006) relative

to the "TCT" genotype (table 4-3). These associations will remain significant after a strict Bonferroni multiple testing correction. Neither haplotype was associated with annual change in the UPDRS score. Individual SNP associations with progression are found in supplemental table 4-2, the *NFE2L2* rs6721961 GT/TT genotype was associated with slower cognitive decline (β=0.104, SE=0.03, p=0.0003) relative to the GG genotype and *NFE2L2* rs6706649 CT/TT with slower motor symptom decline (β=-0.294, SE=0.12, p=0.018) relative to the CC genotype.

None of the *PPARGC1α* haplotypes were marginally associated with PD risk (table 4-2). Three *PPARGC1α* haplotypes however changed the rate of motor symptom progression, "TGAC", "CGAC", and "TAAC", with patients carrying the "TGAC" and "TAAC" haplotypes showing worse annual motor symptom scores during follow-up ("TGAC": β=0.376, SE=0.15, p=0.01; "TAAC": β=0.337, SE=0.17, p=0.05) and "CGAC" retaining better scores (β=-0.352, SE=0.14, p=0.01) relative to the wildtype "TAAT" haplotype (table 3). The "CGAT" haplotype was also suggestively associated with lower annual cognitive scores during follow-up (β=-0.051, SE=0.03, p=0.05) relative to the "TAAT" reference haplotype (table 3). In single SNP analysis, the *PPARGC1α* rs6821591 CT and TT genotypes were associated with significantly worse annual motor relative to the CC genotype (TT: β=0.461, SE=0.14, p=0.001). None of the other *PPARGC1α* SNPs were individually associated with progression.

Assessing the effects of *PPARGC1α* haplotypes in combination with MB/PQ exposure, we found statistical interactions with the "CGGT" (p=0.02) and possibly "CGAT" (p=0.08) haplotypes. Consistent with the marginal analyses, we saw no associations for the *PPARGC1α* haplotypes and PD without pesticide exposure (table 4), and PD risk was increased for those MB/PQ exposed with the wildtype haplotype "TAAT" (OR=1.55, 95% CI=1.10, 2.20). Across haplotypes, PD risk for high MB/PQ exposure is increased (OR=1.29, 95% CI=0.86, 1.94 to

OR=1.68, 95% CI=0.95, 2.98; table 4-4), except among carriers of the "CGGT" and "CGAT" haplotypes, which appeared to protect against PD risk from MB/PQ exposure i.e. there was no risk increase observed in highly MB/PQ exposed carriers of these haplotypes (OR=1.02, 95% CI=0.67, 1.55 and OR=1.05, 95% CI=0.69, 1.59; table 4-4). The interactions between individual SNPs and MB/PQ are found in supplemental table 4-S3, where rs6821591 and rs8192678 also showed statistical interactions ($p \leq 0.05$), with the CC genotype of rs6821591 and GG genotype of rs8192678 similarly seeming to protect against PD risk from MB/PQ exposure.

We did not find significant statistical interactions between the *NFE2L2* haplotypes and MB/PQ exposure, though again consistent with the marginal analyses, we estimated PD risk increases for the *NFE2L2* haplotypes relative to reference "TCT" haplotype in those unexposed to MB/PQ (OR=1.71, 95% CI=0.98, 2.99 to OR=1.84, 95% CI=1.18, 2.87; supplemental table 4-S4) and an increase of PD risk in MB/PQ exposed carriers with the "TCT" reference haplotype (OR=1.83, 95% CI=1.06, 3.17). We also estimated increased risks of PD in those with both MB/PQ exposure and a risk haplotype ("TCG" and "CCG"), for example, participants with both the "TCG" haplotype and MB/PQ exposure were at the highest risk for PD (OR=2.36, 95% CI=1.51, 3.69) relative to the "TCT"-no/low exposure reference group. Supplemental table 4-S4 also shows the individual SNP and MB/PQ interactions, no individual SNP showed statistical interactions.

4.4 Discussion

Oxidative stress and pathways associated with cellular oxidative stress like mitochondrial dysfunction are generally thought to contribute to PD etiology. Nrf2 and PGC-1α play a complementary and overlapping role in regulating the endogenous cellular antioxidant defense

system (Clark and Simon 2009). Here, we provide support for the involvement of genetic haplotypes ("TCG" and "CCG") in *NFE2L2* with PD susceptibility based on our case/control data and importantly also found that they may contribute to faster cognitive decline in patients we were able to follow longitudinally. We also identified *PPARGC1α* as a mediator of MB/PQ pesticide exposure risk in PD and our longitudinal data of patients suggested a possible involvement in motor symptom progression as well for variants in this gene.

The three SNPs of our *NFE2L2* promoter haplotype have been shown to influence functional activity, with rs6721961 affecting basal *NFE2L2* expression (Marzec et al, 2007). Interestingly, in single SNP investigations in our data, rs6721961 was the only SNP associated with PD (supplemental table 4-S1), and showed a strong association with cognitive symptom progression (supplemental table 4-S2).

Although epidemiologic evidence for the role of *NFE2L2* in PD is limited, our findings contradict those from a previous Polish case/control population that associated the "TCG" haplotype with protection against PD risk (OR=0.6, 95% CI=0.4, 0.9) (von Otter 2010); interestingly, this association lost magnitude and significance when combined with other European populations (OR=0.92, 95% CI=0.81, 1.04) (von Otter 2014). Our results remained in the opposite direction of those previously reported when we compared the "TCG" to all other genotypes (OR=1.16, 95% CI=0.97, 1.38), as done in the European study (von Otter 2010; von Otter 2014). Study population heterogeneity or disease misclassification may explain between-study inconsistencies (Ioannidis 2007). But it is also plausible that the differences in findings may have resulted from unaccounted for environmental factors which mediated the genetic response, given that Nrf2 is regulated and activated through oxidative stress related pathways. Our population was recruited from highly agricultural communities, and we have found a

number of specific pesticides to be positively associated with PD risk in our studies, many which may induce oxidative stress (Wang et al 2011; Ritz et al, 2016; Abdollahi et al. 2004). In our population, *NFE2L2* haplotypes did not appear to mediate exposure to MB/PQ (supplemental table 4-S3), pesticides previously associated with PD in our population and reactive oxygen species (ROS) production (Costello et al 2009; Uversky 2004). While we did not estimate any statistical interactions, we did still associate the "TCG" and "CCG" haplotypes with an increased risk of PD in those without exposure to MB/PQ.

In our patient cohort, we detected strong associations between the *NFE2L2* haplotypes, "TCG" and "CCG", and faster cognitive symptom progression. Nrf2 is not only a therapy target for PD, but for several neurodegenerative diseases, including Alzheimer's disease (AD) (Calkins et al 2009); cognitive decline and dementia in PD and AD are hypothesized to have overlapping etiologies, supported by observations that PD patients with dementia have a higher cortical amyloid-β (Aβ) plaques burden (a feature of AD) than PD patients without dementia (Gearing et al, 1995; Ross and Poirer, 2004). Interestingly, the European researchers who reported on *NFE2L2* and PD also found that an *NFE2L2* haplotype was associated with faster progression of AD (von Otter et al, 2010), supporting the involvement of *NFE2L2* in cognition decline.

We did not detect any marginal associations between the *PPARGC1α* haplotype, individual SNPs, and PD risk. However, four of the eight haplotypes analyzed were associated with PD symptom progression, "CGAT" with faster cognitive decline and "TGAC", "CGAC", and "TAAC" with motor symptom progression relative the "TAAT" (wild-type) reference group. PGC-1α, like Nrf2, binds to antioxidant response elements (ARE), promoter regions of genes coding for antioxidant enzymes, to upregulate their transcription (Clark and Simon 2009). PGC-1α regulated antioxidant enzymes, however, exert their influence primarily on mitochondria

(Clark and Simon 2009). Our results, therefore, seems to implicate mitochondrial related oxidative stress as relevant for PD progression. Interestingly, the *PPARGC1α* haplotype "CGGT" also seems to mediate the risk of MB/PQ pesticide exposure in our case/control population, with the "CGGT" haplotype mitigating PD risk associated with high MB/PQ exposure relative to carriers of the most common "TAAT" haplotype who were not exposed to pesticides. We have previously reported in our population that ambient agricultural MB/PQ exposure increases the risk of PD (Costello et al, 2009). Paraquat has been widely used to create animal models of PD, and its cytyotoxicity is heavily related to ROS production (Uversky 2004); combination exposures (maneb and paraquat) have been shown to result in even greater PD-related pathology in the animal models (Uversky 2004). Of particular note, MB/PQ pesticides have also been related to mitochondrial dysfunction (Czerniczyniec et al, 2011). Again the involvement of *PPARGC1α* in PD risk related to MB/PQ exposure further implicates mitochondrial dysfunction and related ROS generation as an important mechanism for neurodegeneration in the dopaminergic system, in support of a growing body of evidence that *PPARGC1α* contributes to neurodegenerative disorders, including PD. As mentioned earlier, two of the SNPs in our haplotype have previously been associated with age of PD onset and the other two with longevity; *PPARGC1α* is also associated with AD, Huntington's disease, and amytrophic lateral sclerosis (ALS) (Clark et al, 2011; Tsunemi et al 2012; Rona-Voros et al 2010; Cui et al 2006; Liang et al 2011).

The PEG case/control population provides many advantages that allow us to pose and investigate mechanistic hypotheses. Most epidemiologic studies rely of self-reported information for pesticide exposure assessment, a method prone to differential recall error, and which generally does not allow for investigation of specific pesticides. We assessed ambient

MB/PQ exposure with a geographic information system (GIS) approach utilizing state mandated pesticide use reports. Thus we do not rely on participant recall for pesticide use, and are able to investigate specific chemicals. Additionally, PD is a commonly misdiagnosed disease (Meara et al. 1999; Wermuth et al. 2012); our PD cases were all seen and well characterized by UCLA movement disorder specialists at least once and many multiple times as part of our patient cohort, minimizing bias from disease misclassification. Additionally, the population controls were drawn from the same region as the cases, likely providing adequate representativeness of the source population.

Further, our prospectively followed patient cohort is one of less than a handful of population-based PD patient cohorts worldwide, and the first to our knowledge to investigate these genes. We were able to follow patients on average more than seven years into disease. Although, as expected in a cohort of elderly patients, we were not able to follow-up all patients enrolled at baseline, mostly because the patient was too ill or deceased (n=70). Those lost to follow-up were older and scored worse on baseline health indicators UPDRS and MMSE (table 1); consequently, selection bias is possible, though there were no differences in haplotype frequencies by follow-up status (supplemental table 4-S5). Additionally, given that we do not have follow-up data on a non-PD population, we cannot tell whether the longitudinal findings are specific to cognitive decline in PD or whether the same type of decline would be observed among a matched control population. However, our study provides an independent population of adequate sample size (≥80% power to detect previously reported marginal effect sizes), and we were able to restrict to Caucasian participants of European ancestry to limit confounding by population stratification.

While, our findings need to be re-examined and replicated in future studies with larger

sample sizes and longer follow-up, our study provides support for the involvement of *NFE2L2* in both PD susceptibility and cognitive symptom progression, and for *PPARGC1α* modifying PD risk in MB/PQ exposed subjects and possibly contributing to motor symptom progression, consistent with the importance of oxidative stress-inducing mechanisms in PD onset and progression.

4.5 Tables

Table 4-1. Population exposure and characteristics of PEG study population with genotyping and European Ancestry only.

Variable (Mean ± SD / n (%))	PD Case/Control Study			PD Progression Cohort		
	PD Patients	Controls	P value	PD Patients w/ follow-up	PD Patients lost to follow-up	P value
	n=472	n=532		n=192	n=94	
Age (y)	69.40 ± 10.0	67.7 ± 11.7	0.01	67.5 ± 9.9	72.4 ± 10.6	0.0002
Male	290 (0.61)	265 (0.50)	0.0002	107 (0.56)	54 (0.57)	0.78
Ever Smoker	213 (0.45)	285 (0.54)	0.008	81 (0.42)	48 (0.51)	0.16
Schooling						
<12	40 (0.08)	34 (0.06)		16 (0.08)	16 (0.17)	
12	122 (0.26)	107 (0.20)	0.03	56 (0.29)	28 (0.30)	0.07
>12	310 (0.66)	391 (0.74)		120 (0.63)	50 (0.53)	
UPDRS[a]	21.04 ± 10.2	--	--	18.8 ± 9.0	24.1 ± 11.0	<.0001
MMSE[a]	27.77 ± 2.6	--	--	28.3 ± 2.1	26.8 ± 3.0	<.0001
High MB/PQ Exposure	252 (0.53)	249 (0.47)	0.04	102 (0.53)	47 (0.50)	0.62

Abbreviations: OS=Oxidative Stress inducing pesticide; MB/PQ=Maneb/Paraquat pesticide

[a]Scores at Baseline (case/control study) interview

Table 4-2. *NFE2L2* and *PPARGC1A* haplotypes and PD susceptibility marginal analysis in 472 PD cases and 532 controls, assumed allelic genetic model (2n).

Haplotype		Case	Control	OR (95% CI)	P Value
***NFE2L2* Promoter Haplotype**	TCT	91 (0.10)	138 (0.13)	1.00 (ref)	--
	TCG	467 (0.49)	477 (0.45)	1.50 (1.11, 2.03)	0.008
	CCG	279 (0.30)	319 (0.30)	1.39 (1.01, 1.91)	0.04
	TTG	107 (0.11)	130 (0.12)	1.29 (0.88, 1.87)	0.19
***PPARGC1A* Haplotype**	TAAT	259 (0.27)	278 (0.26)	1.00 (ref)	--
	CGGC	148 (0.16)	156 (0.15)	0.99 (0.74, 1.32)	0.95
	CGGT	137 (0.15)	156 (0.15)	0.94 (0.70, 1.26)	0.68
	CGAT	122 (0.13)	154 (0.14)	0.85 (0.63, 1.14)	0.28
	TGAC	62 (0.07)	79 (0.08)	0.86 (0.59, 1.25)	0.43
	CGAC	78 (0.08)	78 (0.07)	1.03 (0.72, 1.48)	0.87
	TAAC	66 (0.07)	75 (0.07)	0.90 (0.62, 1.32)	0.60
	Other	72 (0.08)	88 (0.08)	0.89 (0.62, 1.27)	0.51

Models also control for age, sex, smoking status, and education

Table 4-3. *NFE2L2* and *PPARGC1A* haplotype and PD progression outcomes, UPDRS (motor score) and MMSE (cognitive score), using linear repeated measures model, assuming allelic genetic model (2n). European ancestry only, n=192.

Haplotype		n (%)	UPDRS β[a]	UPDRS SE	UPDRS P Value	MMSE β[a]	MMSE SE	MMSE P Value
***NFE2L2* Promotor Haplotype**	TCT	41 (0.11)	ref	--	--	ref	--	--
	TCG	180 (0.47)	-0.069	0.14	0.62	-0.101	0.03	0.0003
	CCG	118 (0.31)	0.024	0.15	0.87	-0.099	0.03	0.0006
	TTG	45 (0.12)	-0.289	0.17	0.09	-0.056	0.03	0.09
***PPARGC1A* Haplotype**	TAAT	95 (0.25)	ref	--	--	ref	--	--
	CGGC	61 (0.16)	-0.077	0.12	0.52	-0.017	0.02	0.48
	CGGT	61 (0.16)	-0.149	0.11	0.19	0.003	0.02	0.91
	CGAT	49 (0.13)	0.088	0.13	0.50	-0.051	0.03	0.05
	TGAC	28 (0.07)	0.376	0.15	0.01	-0.015	0.03	0.61
	CGAC	32 (0.08)	-0.352	0.14	0.01	0.005	0.03	0.87
	TAAC	24 (0.06)	0.337	0.17	0.05	-0.027	0.03	0.42
	Other	34 (0.09)	0.061	0.15	0.69	0.019	0.03	0.52

Models control for age, sex, smoke, education, and PD duration prior to baseline (0-3 years)

[a]β represents interaction with age (centered)

Table 4-4. Maneb/Paraquat pesticide exposure-*PPARGC1A* haplotype/SNP interactions and PD susceptibility, assuming an additive genetic model for rs8192678 (n) and an allelic genetic model for the haplotype analysis (2n).

Haplotype/ Genotype	Main Effects			Joint Effects			Interaction P Value
	Haplotype effect with no/low pesticide exposure			High MB/PQ Exposure Across Haplotype levels			
	Case / Control	OR (95% CI)	P Value	Case / Control	OR (95% CI)	P Value	
***PPARGC1A* Haplotype**							
TAAT	114/153	1.00 (ref)	--	154/125	1.55 (1.10, 2.20)	0.01	--
CGGC	71/80	1.18 (0.78, 1.77)	0.44	77/76	1.29 (0.86, 1.94)	0.22	0.24
CGGT	70/73	1.32 (0.87, 2.00)	0.19	67/83	1.05 (0.69, 1.59)	0.82	0.02
CGAT	61/74	1.11 (0.73, 1.70)	0.63	61/80	1.02 (0.67, 1.55)	0.94	0.08
TGAC	26/45	0.84 (0.49, 1.46)	0.55	35/34	1.32 (0.77, 2.26)	0.30	0.98
CGAC	32/40	0.99 (0.58, 1.69)	0.97	46/38	1.63 (0.98, 2.70)	0.06	0.88
TAAC	31/48	0.83 (0.49, 1.40)	0.48	35/27	1.68 (0.95, 2.98)	0.08	0.50

Models control for age, sex, smoke, and education

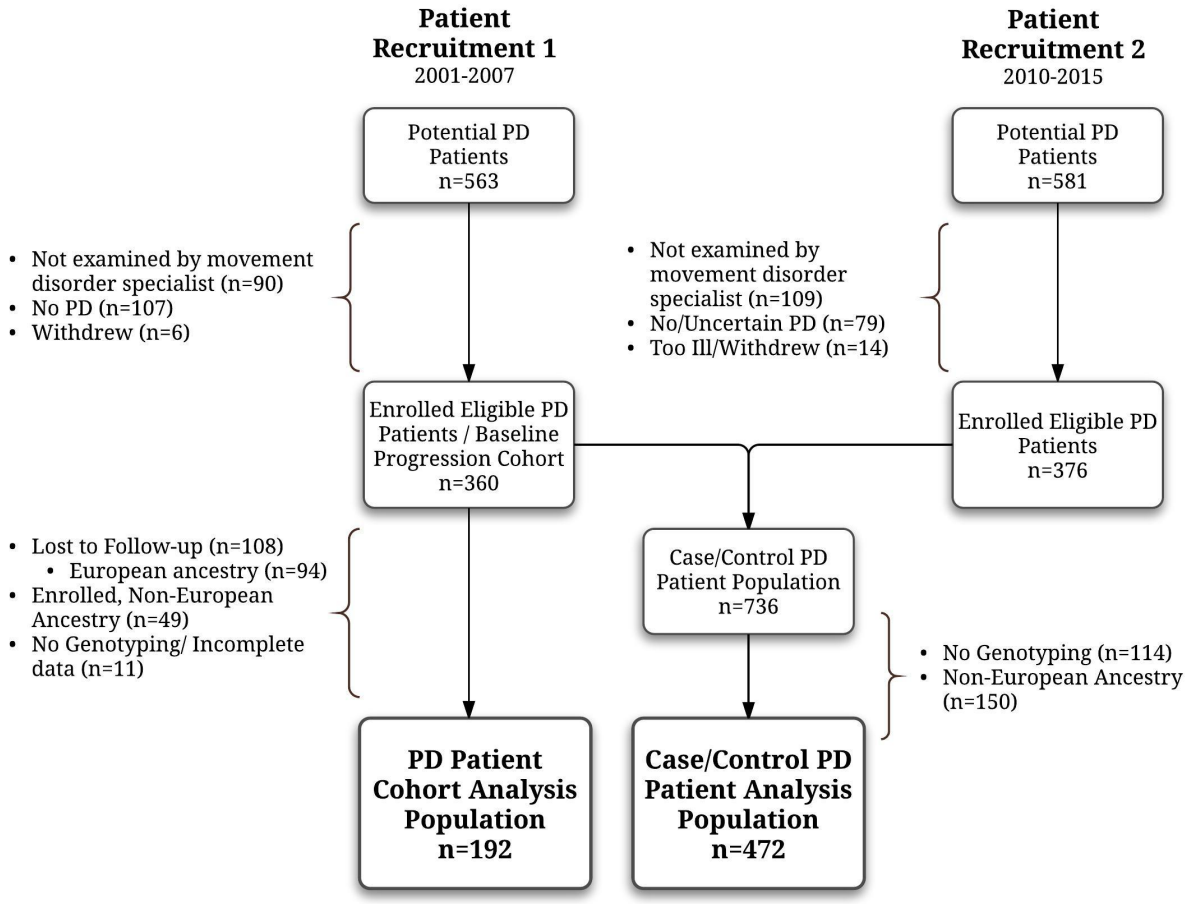


Figure 4-1. PD patient recruitment flow diagram for both the case/control and cohort studies.

4.6 Supplement

Supplemental Table 4-S1. Marginal *NFE2L2* and *PPARGC1A* SNP associations with PD, assuming an additive genetic model unless otherwise specified. Caucasian only.

SNP	Haplo-type Position	Major / Minor Allele	Case (%) / Control (%)	Ref Homozygous wildtype	Case (%) / Control (%)	OR (95% CI) Heterozygotes	Case (%) / Control (%)	OR (95% CI) Homozyogous variant	P-value
NFE2 rs35652124	1	T / C	226 (0.49) / 258 (0.49)	1.00 (ref)	197 (0.43) / 222 (0.42)	1.00 (0.82, 1.23)	35 (0.08) / 46 (0.09)	1.01 (0.67, 1.51)	0.97
NFE2 rs6706649	2	C / T	365 (0.78) / 409 (0.77)	1.00 (ref)	95 (0.20) / 110 (0.21)	0.94 (0.71, 1.23)	6 (0.01) / 10 (0.02)	0.88 (0.50, 1.52)	0.64
NFE2 rs6721961*	3	G / T	379 (0.81) / 397 (0.75)	1.00 (ref)	87 (0.19) / 130 (0.19)	0.67 (0.49, 0.91)	2 (0.004) / 4 (0.01)	--	0.01
PPARGC1A rs6821591	1	C/T	127 (0.27) / 137 (0.26)	1.00 (ref)	227 (0.49) / 263 (0.50)	1.01 (0.87, 1.18)	112 (0.24) / 124 (0.24)	1.02 (0.76, 1.38)	0.88
PPARGC1A rs8192678	2	G/A	205 (0.44) / 235 (0.45)	1.00 (ref)	200 (0.43) / 233 (0.44)	1.04 (0.89, 1.22)	60 (0.13) / 58 (0.11)	1.09 (0.79, 1.5)	0.60
PPARGC1A rs2970848	3	A/G	201 (0.43) / 230 (0.43)	1.00 (ref)	215 (0.46) / 250 (0.47)	0.96 (0.82, 1.13)	51 (0.11) / 49 (0.09)	0.92 (0.67, 1.27)	0.61
PPARGC1A rs4235308	4	T/C	175 (0.37) / 202 (0.38)	1.00 (ref)	214 (0.46) / 248 (0.47)	0.97 (0.83, 1.13)	78 (0.17) / 77 (0.15)	0.94 (0.69, 1.28)	0.69

Models control for age, sex, smoking status, and education

*Assumed dominant genetic model given sample size of homozygous variant genotype

Supplemental Table 4-S2. *NFE2L2* and *PPARGC1A* SNPs and PD progression outcomes, UPDRS (motor score) and MMSE (cognitive score), using linear repeated measures model, assuming an additive genetic model. European ancestry only, n=192.

		UPDRS			MMSE		
Genotype	**n (%)**	**β[a]**	**SE**	**P Value**	**β[a]**	**SE**	**P Value**
***PPARGC1A* SNPs**							
rs6821591							
CC	55 (0.29)	ref	--	--	ref	--	--
CT	94 (0.49)	0.230	0.07	0.001	0.010	0.01	0.45
TT	43 (0.22)	0.461	0.142		0.021	0.028	
rs8192678							
GG	94 (0.49)	ref	--	--	ref	--	--
GA	76 (0.40)	0.092	0.08	0.26	0.003	0.02	0.83
AA	22 (0.11)	0.185	0.163		0.007	0.032	
rs2970848							
AA	79 (0.41)	ref	--	--	ref	--	--
AG	86 (0.45)	-0.112	0.07	0.13	0.013	0.01	0.35
GG	27 (0.14)	-0.223	0.148		0.027	0.029	
rs4235308							
TT	77 (0.40)	ref	--	--	ref	--	--
TC	79 (0.41)	0.034	0.06	0.59	-0.003	0.01	0.82
CC	36 (0.19)	0.068	0.128		-0.006	0.025	
***NFE2L2* SNPs**							
rs35652124							
TT	77 (0.40)	ref	--	--	ref	--	--
TC	79 (0.41)	0.170	0.09	0.07	-0.019	0.02	0.24
CC	36 (0.19)	0.340	0.18		-0.038	0.04	
rs6706649*							
CC	150 (0.78)	ref	--	--	ref	--	--
CT	39 (0.20)	-0.294	0.12	0.02	-0.300	0.21	0.14
TT	3 (0.02)						
rs6721961*							
GG	152 (0.79)	ref	--	--	ref	--	--
GT	39 (0.20)	0.082	0.14	0.23	0.104	0.03	0.0003
TT	1 (0.01)						

Models control for age, sex, smoke, education, and PD duration prior to baseline (0-3 years)

*Assumed dominant genetic model given sample size of homozygous variant genotype

[a]β represents interaction with age (centered)

Supplemental Table 4-S3. Maneb/Paraquat pesticide exposure-*PPARGC1A* SNP interactions and PD susceptibility, assuming an additive genetic model.

PPARGC1A Genotype	Main Effects: Haplotype effect with no/low pesticide exposure			Joint Effects: High MB/PQ Exposure Across Haplotype levels			Interaction P Value
	Case / Control	OR (95% CI)	P Value	Case / Control	OR (95% CI)	P Value	
rs6821591							
CC	59/64	1.00 (ref)	--	68/73	0.82 (0.53, 1.28)	0.37	--
CT	114/135	0.78 (0.60, 1.02)	0.07	113/128	1.03 (0.72, 1.48)	0.87	0.01
TT	43/80	0.61 (0.37, 1.03)		69/44	1.30 (0.83, 2.04)	0.26	
rs8192678							
GG	95/116	1.00 (ref)	--	110/119	1.01 (0.70, 1.45)	0.98	--
GA	98/130	0.87 (0.66, 1.15)	0.32	102/103	1.28 (0.92, 1.79)	0.14	0.05
AA	22/34	0.76 (0.44, 1.31)		38/24	1.64 (1.02, 2.63)	0.04	
rs2970848							
AA	84/129	1.00 (ref)	--	117/101	1.62 (1.12, 2.34)	0.01	--
AG	110/126	1.23 (0.93, 1.63)	0.15	105/124	1.40 (1.00, 1.96)	0.05	0.08
GG	23/26	1.52 (0.87, 2.65)		28/23	1.21 (0.75, 1.98)	0.44	
rs4235308							
TT	88/106	1.00 (ref)	--	87/96	1.11 (0.75, 1.62)	0.61	--
TC	90/128	0.95 (0.74, 1.22)	0.69	124/120	1.28 (0.92, 1.77)	0.15	0.30
CC	47/39	0.90 (0.55, 1.49)		39/30	1.47 (0.93, 2.34)	0.10	

Models control for age, sex, smoke, and education

Supplemental Table 4-S4. Maneb/Paraquat pesticide exposure-*NFE2L2* haplotype/SNP interactions and PD susceptibility, assuming an allelic model for the haplotype analysis (2n) and an additive genetic model (n) for SNPs unless otherwise specified.

Haplotype/ Genotype	Main Effect			Joint Effects			Interaction P Value
	Haplotype effect with no/low pesticide exposure			High MB/PQ Exposure Across Haplotype levels			
	Case / Control	OR (95% CI)	P Value	Case / Control	OR (95% CI)	P Value	
NFE2L2 Promotor Haplotype, allelic genetic model (2n)							
TCT	36/74	1.00 (ref)	--	55/64	1.83 (1.06, 3.17)	0.03	--
TCG	228/267	1.84 (1.18, 2.87)	0.007	239/210	2.36 (1.51, 3.69)	0.0002	0.25
CCG	129/163	1.76 (1.10, 2.81)	0.02	150/156	2.09 (1.31, 3.32)	0.002	0.18
TTG	47/62	1.71 (0.98, 2.99)	0.06	60/68	1.83 (1.07, 3.14)	0.028	0.17
Individual SNP analysis, additive genetic model unless specified (n)							
rs35652124							
TT	102/143	1.00 (ref)	--	124/115	1.36 (0.96, 1.94)	0.08	--
TC	97/114	1.05 (0.78, 1.40)	0.75	100/108	1.30 (0.94, 1.94)	0.11	0.65
CC	13/23	1.10 (0.61, 1.97)		22/23	1.24 (0.76, 2.04)	0.39	
rs6706649							
CC	173/227	1.00 (ref)	--	192/182	1.33 (1.00, 1.78)	0.05	--
CT	43/48	1.02 (0.69, 1.52)	0.91	52/62	1.12 (0.76, 1.67)	0.56	0.49
TT	2/7	1.05 (0.47, 2.32)		4/3	0.95 (0.46, 1.95)	0.88	
rs6721961*							
GG	184/210	1.00 (ref)	--	195/187	1.14 (0.85, 1.52)	0.38	--
GT/TT	34/72	0.50 (0.31, 0.79)	0.003	55/62	0.98 (0.64, 1.49)	0.91	0.09

Models control for age, sex, smoke, and education

*Assumed dominant genetic model given sample size of homozygous variant genotype, see supplemental table 1

Supplemental Table 4-S5. Distribution of haplotypes among PD patients cohort

Haplotype		PD Patients w/ follow-up n (%)	PD Patients lost to follow-up n (%)
NFE2L2 **Promoter Haplotype**	TCT	41 (0.11)	19 (0.10)
	TCG	180 (0.47)	98 (0.52)
	CCG	118 (0.31)	48 (0.26)
	TTG	45 (0.12)	23 (0.12)
PPARGC1A **Haplotype**	TAAT	95 (0.25)	54 (0.29)
	CGGC	61 (0.16)	30 (0.16)
	CGGT	61 (0.16)	23 (0.12)
	CGAT	49 (0.13)	24 (0.13)
	TGAC	28 (0.07)	8 (0.04)
	CGAC	32 (0.08)	20 (0.11)
	TAAC	24 (0.06)	13 (0.07)
	Other	34 (0.09)	16 (0.08)

5 Conclusion and Public Health Implications

This dissertation examines environmental and genetic factors related to oxidative stress and both the risk of Parkinson's disease and symptom progression in patients. We found strong associations for PD in participants with certain *NOS1* genotypes exposed to commonly used OP pesticides through two independent sources – home and agricultural use, consistent with *NOS1* as a modifier of associations with PD in OP pesticide exposed populations. Among PD patients, we provide support for the involvement of both OP pesticides and PON1 in PD motor and non-motor symptom progression. And finally, we provide evidence for the involvement of *NFE2L2* in both PD susceptibility and cognitive symptom progression, and for *PPARGC1α* modifying PD risk in MB/PQ exposed subjects and possibly contributing to motor symptom progression.

PD is a major health concern for older adults, associated with significant morbidity and mortality, and threatens to become even more prominent with increasing life expectancy and the aging of populations. Primary prevention is imperative for reducing this burden. Further, given the importance of symptom progression for patients' health related quality of life and for predicting mortality (Forsaa et al. 2010), identifying modifiable predictors for rate or severity of symptoms during disease course is important for both patients and for developing preventive measures.

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