Title

An Investigation into Household and Occupational Pesticide Exposures with Genetic Variants as Risk Factors for Parkinson's Disease

Author

Narayan, Shilpa

Publication Date

2015

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA

Los Angeles

An Investigation into Household and Occupational Pesticide Exposures with Genetic Variants as Risk Factors for Parkinson's Disease

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Epidemiology

by

Shilpa Narayan

2015

ABSTRACT OF THE DISSERTATION

An Investigation into Household and Occupational Pesticide Exposures with Genetic Variants as Risk Factors for Parkinson's Disease

by

Shilpa Narayan
Doctor of Philosophy in Epidemiology
University of California, Los Angeles, 2015
Professor Beate R. Ritz, Chair

Epidemiologic studies suggest that pesticides are risk factors for Parkinson's Disease (PD) but predominantly assessed pesticide exposure as a broad category and often did not examine exposure modifying factors such as personal protective equipment (PPE) use and variants in genes that encode proteins involved in pesticide metabolism and distribution in the body. Only a handful have investigated household pesticide exposures alone. This dissertation research examined the relation between PD and exposures to pesticides from multiple exposure sources, including household use, occupational use, and ambient pesticide exposures from drift in addition to genetic variation in *PON1* and *ABCB1*.

We recruited 360 incident PD cases and 827 population controls between 2001 through 2011 from central California, collected data on demographics, covariates, residential and occupational address history, and exposures to chemicals by telephone, and participants provided either whole blood or saliva samples.

Frequent use of any household pesticide increased PD risk by 47%; frequent use of products containing organophosphorus pesticides (OPs) increased risk more strongly by 71%, and frequent organothiophosphate use almost doubled the risk of PD. The largest odds ratios were estimated for frequent OP users who were carriers of the 192QQ paraoxonase genetic variant related to slower detoxification of OPs.

Ever occupational pesticide use for > 10 years doubled the risk of PD compared to never occupational pesticide users. PD risk was also increased with occupational use of fungicides, herbicides, insecticides, carbamates, OPs, and organochlorine (OC) pesticides. Surprisingly, we found higher risks among those who reported using PPE, possibly because these workers felt compelled to use PPE when handling toxic pesticides.

We replicated a prior finding of *ABCB1* polymorphisms at both rs1045642 and rs2032582 modifying PD risk from occupational OC exposures. We also newly found that PD risk from occupational OP exposures is modified by these polymorphisms. However, we did not detect multiplicative interactions.

Our results suggest that exposures to OPs, carbamates, and OCs increase PD risk, and the risk from commonly used pesticides, specifically OCs and OPs, may be increased more strongly in individuals who are genetically susceptible to neurotoxic effects of pesticides.

This dissertation of Shilpa Narayan is approved.

Leeka Kheifets

Jeff Bronstein

Haroutune Armenian

Beate R. Ritz, Committee Chair

University of California, Los Angeles

2015

Table of Contents

LIST OF TABLES AND FIGURES

ACKNOWLEDGMENTS

Chapter 3 was originally published in the International Journal of Epidemiology by Oxford University Press as: Narayan S, Liew Z, Paul K, Lee PC, Sinsheimer JS, Bronstein JM, Ritz B. Household organophosphorus pesticide use and Parkinson's disease. Int J Epidemiol. 2013 Oct;42(5):1476-85.

This dissertation research was supported in part by fellowships from the UCLA FSPH Department of Epidemiology and UCLA Graduate Division.

I gratefully thank Dr. Beate Ritz, the chair of my dissertation committee, for her advice and support throughout my doctoral studies. I have been very fortunate to work with her and learn through her guidance. Thanks to Dr. Leeka Kheifets, Dr. Haroutune Armenian, and Dr. Jeff Bronstein for serving on my dissertation committee and providing helpful suggestions and feedback.

I would like to thank all of the participants in the PEG and CGEP studies, our study movement disorder neurologists, Dr. Myles Cockburn at the University of Southern California who was instrumental in generating our GIS pesticide exposure estimates, and our field staff who recruited participants and conducted interviews for our study. I also thank the Checkoway, Farin and Zabetian laboratories at the University of Washington and the Elbaz laboratory and associates at IntegraGen in France for genotyping our samples. I would also like to acknowledge Dr. Janet S. Sinsheimer, Dr. Pei-Chen Lee, Dr. Zeyan Liew, Kimberly Paul, and Dr. Shannon L. Rhodes for advice and contributions to my projects.

I am grateful to my parents, Badari and Poornima, brother Nikhil, and many friends and family members who have been sources of support. My deepest gratitude goes to my husband Carl for

his encouragement and support throughout the process of preparing and defending my dissertation.

VITA

2005	B.S., Biology, Biophysics Concentration Haverford College Haverford, Pennsylvania
2005-2006	Research Specialist Physiology Department University of Pennsylvania School of Medicine Philadelphia, Pennsylvania
2006-2007	Autism Behavior Analyst California Psychcare Inc. Van Nuys, California
2008	Intern Los Angeles County Department of Public Health Surveillance, Epidemiology, and Evaluation Unit Los Angeles, California
2008	Intern Los Angeles County Department of Public Health Office of Women's Health El Monte, California
2009	M.P.H., Epidemiology School of Public Health University of California, Los Angeles Los Angeles, California
2009-2011	Graduate Student Researcher School of Public Health, Department of Epidemiology University of California, Los Angeles Los Angeles, California
2013	Special Reader Epidemiology 100, Principles of Epidemiology University of California, Los Angeles Los Angeles, California

PUBLICATIONS AND PRESENTATIONS

Narayan S, Liew Z, Paul K, Lee PC, Sinsheimer JS, Bronstein JM, Ritz B. Household organophosphorus pesticide use and Parkinson's disease. Int J Epidemiol. 2013 Oct;42(5):1476-85.

Gatto NM, Deapen D, Stoyanoff S, Pinder R, Narayan S, Bordelon Y, Ritz B. Lifetime exposure to estrogens and Parkinson's disease in California teachers. Parkinsonism Relat Disord. 2014 Nov;20(11):1149-56.

"Household organophosphorus pesticide use and Parkinson's disease." Shilpa Narayan, Zeyan Liew, Kimberly Paul, Pei-Chen Lee, Janet S. Sinsheimer, Jeff M. Bronstein, Beate Ritz. Poster presentation at the joint meeting of the International Society of Environmental Epidemiology, the International Society of Exposure Science, and the International Society of Indoor Air Quality and Climate in Basel, Switzerland. August 19-23, 2013.

1 Background and Introduction

Parkinson's disease (PD) is a chronic and progressive movement disorder. Estimated incidence rates vary, but one systematic review approximated the incidence rate to be 16-19 per 100,000 person-years (1). PD prevalence is approximately 1%, though estimates of PD prevalence vary between studies and depending on age group (2, 3). Though PD is a rare disease, the annual economic burden of PD in the US has been estimated to be 23 billion dollars (4). Given the negative health and economic impacts, research into the cause of PD is necessary to identify worthwhile public health interventions that would alleviate these burdens.

1.1 Parkinson's Disease risk and protective factors

Parkinson's Disease has unknown etiology, though some risk and protective factors have been identified. Increasing age is the most evident risk factor for the disease. Males are also more frequently affected than females and have 1.5 times the risk of PD (5). An unexplained yet consistent inverse relation has been found between smoking and PD (6-8), with ever smokers estimated to have half the risk of PD compared to never smokers. Another potential protective factor is coffee drinking, with a meta-analysis estimating a 30% reduction in risk for coffee drinkers compared with non-coffee drinkers (6). A meta-analysis of familial aggregation studies comparing the proportion of affected first degree relatives between cases and controls found that having a first-degree relative with PD almost triples the risk of disease (RR= 2.9 , 95% CI: 2.2–3.8) (9). Common genetic polymorphisms have been found to influence susceptibility for sporadic PD (2), and it is suspected that PD etiology is multi-factorial, involving both environmental and genetic risk factors (10, 11). Other occupational and environmental exposures have also been suggested as risk factors for PD, including pesticides and solvents (12).

1.2 PD Clinical Features and Neuropathology

Diagnosis of idiopathic PD is based on clinical assessment, and definitive diagnosis can only be obtained postmortem through histopathological studies. The four cardinal features of PD are rest tremor, cogwheel rigidity, bradykinesia, and loss of postural reflexes/postural instability (13). Other non-motor symptoms of the disease may include cognitive impairment, depression, and autonomic dysfunction.

Pathological features of PD include substantial death of the dopamine-producing nerve cells in the substantia nigra, with the loss of these cells leading to a dopamine deficit. Lewy bodies, which are inclusion bodies containing alpha-synuclein, ubiquitin, and other proteins are another pathological feature of the disease.

1.3 Previous findings on PD and Pesticides

Pesticide exposures have been of interest to PD researchers as a potential risk factor since the early 1980s when heroin addicts using a synthetic heroin laced with 1-Methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) developed parkinsonism (14). N-Methyl-4-phenylpyridine (MPP+) is a toxic metabolite of MPTP that is transported by the dopamine transporter (DAT) into dopaminergic neurons and is similar in structure to the herbicide paraquat (15).

Possible mechanisms involved in PD pathogenesis include mitochondrial dysfunction, proteasome inhibition, oxidative stress, aggregation of alpha-synuclein, and problems with dopamine homeostasis and Ca^{2+} homeostasis (16). Experimental evidence from animal, cell, and other lab based studies demonstrate that other pesticides besides paraquat are neurotoxic. Rotenone, a botanical pesticide that is a mitochondrial complex I inhibitor, damages dopaminergic neurons(17) and inhibits proteasome activity (18). The dithiocarbamate pesticide maneb has been linked to selective dopaminergic neuron degeneration (19). The organochlorine

pesticide dieldrin induces oxidative stress and depolarization of mitochondrial membrane potential leading to death of neuronal cells (20) and another organochlorine (OC), heptachlor has negative effects on the striatal dopaminergic system in mice (21). Organophosphorus (OP) pesticides are another class that have been implicated in PD. The OP pesticide chlorpyrifos increased dopamine turnover in murine striatal synaptosomes and decreased mitochondrial function (22). Some studies have examined more environmentally relevant doses of pesticide exposure (23-25). The benzimidazole/carbamate fungicide, benomyl, was selectively toxic to dopaminergic neurons and it was determined that benomyl's neurotoxic actions most likely occurred through inhibition of aldehyde dehydrogenase (ALDH) (23). The dopamine metabolite 3,4-dihydroxyphenylacetaldehyde (DOPAL) is toxic and is metabolized by ALDH. Thus ALDH inhibition is a potential mechanism that may result in selective vulnerability of dopaminergic neurons to pesticides. Despite experimental evidence on mechanisms of pesticide neurotoxicity from toxicologic studies, there is uncertainty regarding the relevance of these studies to Parkinson's Disease in humans. Routes of pesticide administration in animal studies, such as intraperitoneal injection and direct injection into the brain, are not directly comparable to the routes of human exposure by ingestion, inhalation, or dermal contact, and the dose of pesticides administered usually is not comparable to human exposure levels (16).

Over 40 epidemiologic studies have examined associations between pesticide exposures and PD. Most studies of pesticide exposure and PD have been on occupational pesticide exposures or occupational and non-occupational exposures combined. A recent meta-analysis summarized studies in this field of research through November 2010 (26). While many of these have suggested positive associations of PD with exposure to pesticides, herbicides, insecticides, and fungicides, several confidence intervals are very wide due to small sample sizes, and there

are some inconsistent results. Some studies found a negative association (27, 28) and several did not find evidence of an association (27, 29-34). The effect estimates for pesticides overall varied in magnitude from RR values of 0.75 to 7.00. The authors of the meta-analysis found a high degree of heterogeneity between studies, with large calculated I^2 values, indicating that the variations in the findings of the studies are not due to chance alone (35). Despite the substantial heterogeneity, the authors used random effects meta-analysis to pool results of the studies and obtained a summary risk ratio of 1.52 (95% CI: 1.23, 1.89) for the association between occupational pesticide exposure and PD. They explored the potential reasons for heterogeneity and found that differences in exposure assessment contributed to heterogeneity (26). Additional characteristics which might have contributed to heterogeneity of study results, but which the meta-analysis authors were not able to investigate include differences in PD diagnostic criteria, varying participation rates, differences in pesticide-PD associations between males and females, differences in the type of agriculture in the different study regions, and differences in the time-periods of the studies.

Compared to those studies that examined any occupational pesticide use, fewer studies have looked at specific pesticides alone and questions remain on whether and which specific pesticides may be responsible for the increased risk of PD seen in many studies. An occupational cohort study of workers at a paper mill who were exposed to the fungicide diphenyl found an RR of 5.6 (95 % CI: 1.8-13) for the relation between diphenyl and PD, though this study included only 5 exposed PD cases (36). In another study of professional pesticide use among agricultural workers in France, use of organochlorine insecticides and dithiocarbamate fungicides more than doubled PD risk in men (OR=2.4, 95% CI: 1.2-5.0 & OR=2.1, 95%CI: 1.0-4.3, respectively) (37). One case-control study nested within the Finnish Mobile Clinic Health Examination Survey

cohort with 101 incident PD cases, assessed serum biomarkers for exposure to the organochlorine pesticide dieldrin at baseline, and reported that increasing serum concentrations of dieldrin increase PD risk (38). However, biomarkers are feasible only for the study of persistent organochlorine pesticides. Another family based case control study of pesticide use at work or at home found organochlorines and organophosphorus pesticides to be associated with PD (39). In the Agricultural Health Study (AHS), a cohort of licensed pesticide applicators and their spouses from Iowa and North Carolina, multiple pesticides were assessed for an association with PD. Increased risk of PD was associated with self-reported uses of the pesticides dicamba, trifluralin, 2, 4, 5-T, butylate, chlorothalonil, benomyl, methyl bromide, the organochlorine pesticide lindane, and the organophosphorus pesticide phorate (40). A subsequent case control study nested within the AHS, found associations between rotenone (OR = 2.5, 95% CI: 1.3–4.7) and paraquat (OR = 2.5; 95% CI, 1.4–4.7) exposures and PD (41). Rotenone use was found to increase PD risk in a Texas case control study (OR= 10.0, 95% CI: 2.9-34.3), but no association was seen for many other pesticides including DDT, lindane, dieldrin, paraquat, the dithiocarbamates maneb/mancozeb, diazinon, or malathion. Prior investigations in Central California on well water, ambient residential, and ambient workplace pesticide exposures from a geographic information system (GIS) provide evidence suggesting that exposures to specific pesticides (paraquat, maneb, ziram, chlorpyrifos, diazinon, parathion, N-methyl carbamates, benomyl, cyanazine, dieldrin, endosulfan, metam, propargite, triflumizole, captan, mancozeb, and zineb) either alone and/or in combination with other pesticides, head trauma, and genetic susceptibility (23-25, 42-49) increase PD risk.

While some studies found positive associations between specific pesticides and PD, others did not (34, 50-52). A Canadian case-control study of self-reported occupational pesticide

exposures, found that pesticide use was associated with prevalent PD, but that exposures to specific pesticides including organochlorines, organophosphates, and carbamates were not (52). A United Kingdom based occupational cohort study of paraquat production workers did not find an association between paraquat and PD mortality, but only had 1 exposed case (51). In a Finnish population based case-control study, use of the organochlorine pesticide DDT was not found to be associated with prevalent PD (50). Finally, in a study of a primarily urban area of Washington state, investigators did not find associations between PD and specific pesticides, including the OPs parathion, diazinon, and malathion, the herbicide paraquat, DDT, and 2-4D (34, 53). Results from epidemiologic studies on PD and exposures to specific pesticides are inconsistent. Additional epidemiologic research on specific pesticide exposures should lead towards an understanding of the heterogeneity of prior findings.

1.4 PD and Susceptibility genotypes

In addition to pesticide exposures, genetic factors have been linked to PD. Monogenic PD has been linked to mutations in *SNCA*, *Parkin, PINK1, DJ-1*, and *LRRK2* (2, 54, 55). However, highly penetrant genes are estimated to only explain a minority of PD cases (55). The disease is likely multifactorial for the majority of cases (54, 56). The study of gene-environment interactions may offer insight into the varying associations found for specific pesticides and PD. Genetic polymorphisms that impact physiologic processes of pesticide metabolism and distribution are of particular interest.

Paraoxonase (PON1) is an arylesterase that contributes to the detoxification of several OP chemicals (57). Putative functional single nucleotide polymorphisms (SNPs) have been identified in the *PON1* gene and two exonic SNPs have been studied extensively: L55M (rs854560) and Q192R (rs662). The *PON1* 192 variant has been shown to affect the catalytic

efficiency of hydrolysis of toxic OP pesticide metabolites paraoxon and chlorpyrifos oxon, with the R allele having higher activity than the Q allele (58, 59). The SNP at position 55 is thought to be related to serum concentrations of PON1, with higher concentrations of PON1 seen in carriers of the L allele compared with the M allele (60, 61). The 192Q and 55M alleles were found to influence PON1 serum diazoxonase activity (62) in human serum. These *PON1* gene polymorphisms have been studied in relation to PD risk (63-69), but a recent meta-analysis concluded that there is no association (70) as expected, if *PON1* variation influences PD risk only via OP exposure such that subjects exposed to OPs who have less detoxification capabilities due to gene status would be more affected by these neurotoxins (57).

ABCB1, which encodes P-glycoprotein (P-gp), is another gene that may be relevant to PD etiology in individuals exposed to pesticides. P-gp is categorized as an ATP-binding cassette (ABC) transporter, which includes a large family of proteins involved in membrane transport. Transport substrates of P-gp include lipophilic compounds, organic cations, and organic bases (71), and P-gp acts as an efflux transporter, pumping many of its substrates out of cells (72). Multiple organochlorine and organophosphorus pesticides have also been found to be P-gp substrates, and common functional polymorphisms in *ABCB1* have been connected with PD in epidemiologic studies of individuals exposed to pesticides(73-75). Common *ABCB1* polymorphisms include rs1045642 and rs2032582, which have been linked to changes in P-gp expression and function through laboratory-based studies (76-78). P-gp is located in numerous regions of the body including the blood-brain barrier and in enterocytes in the gastrointestinal tract (72). P-glycoprotein might be involved in susceptibility to PD through interactions with pesticides which might be substrates of P-gp transport or inhibitors of P-gp function. Reduced expression and transport activity of P-gp due to functional polymorphisms in *ABCB1* could allow

increased absorption of xenobiotic neurotoxins into the bloodstream through the gastrointestinal tract and increased accumulation of these neurotoxins in the brain.

1.5 Study Objectives

Many case-control studies and cohort studies as well as basic science studies have now suggested a role for pesticides as a risk factor for Parkinson's Disease, but these studies predominantly assessed pesticide exposure as a broad category and often did not examine exposure modifying factors such as use of personal protective equipment, specific job tasks, and variants in genes that encode proteins involved in pesticide metabolism and distribution in the body. Previous epidemiologic studies examined occupational exposures to pesticides either alone or together with non-occupational exposures, and only a handful have explored household pesticide exposures (37, 53, 79) alone.

Further investigation into specific pesticides and interactions of pesticide exposures with common genetic variants should contribute to clarifying the nature of the suggested relationship between pesticides and PD risk. Thus, the objectives of this dissertation include examinations of PD associations with (1) household pesticide exposures, specifically household organophosphorus (OP) exposures, and modification of associations by genetic variants in the gene *PON1*, encoding the xenobiotic metabolizing enzyme paraoxonase, (2) occupational exposure to specific types of pesticides, and (3) the interaction of organochlorine (OC) and OP pesticide exposures with genetic variants in the *ABCB1* gene, which encodes the P-glycoprotein transporter in the blood-brain barrier that controls entry of xenobiotics into the brain. Study subjects came from the Parkinson Environment Gene (PEG) Study in Central California.

2 Parkinson Environment Gene (PEG) Study

2.1 Study Subjects

The Parkinson Environment Gene (PEG) study is a population-based case-control study of Parkinson's disease, with subjects from Kern, Fresno, and Tulare counties in central California. This region is primarily rural with a large amount of agricultural activity.

Cases diagnosed with Parkinson's disease within the three years prior to contact with us were recruited from 2001 through 2007. Cases were eligible to take part in the study if they lived in CA for at least 5 years prior to diagnosis and were residents of Fresno, Kern, or Tulare Counties, were not in the last stages of a terminal illness, had their diagnosis confirmed as clinically probable or possible PD by a UCLA movement disorder specialist, and were willing to participate. Cases were recruited in collaboration with 90% of practicing neurologists in the region, Kaiser Permanente, Kern and Visalia Medical Centers, the Veteran's Administration, Parkinson's Disease support groups, local newspapers, and radio stations that broadcast public service announcements. Case diagnoses of clinically probable or possible PD were confirmed using the following criteria: (I) presence of at least two of the following signs: bradykinesia, cogwheel rigidity resting tremor, at least one of which must have been resting tremor or bradykinesia; (II) no suggestion of a cause for another parkinsonian syndrome, such as trauma, brain tumor, infection, cerebrovascular disease, or other known neurological disease or treatment in the past with dopamine-blocking or dopamine-depleting agents; (III) No atypical features such as prominent oculomotor palsy, cerebellar signs, vocal cord paresis, severe orthostatic hypotension, pyramidal signs, amyotrophy, or limb apraxia; (IV) asymmetric onset; (V) if treatment with levodopa had been initiated, symptomatic improvement after treatment. Probable cases met criteria one through four plus/minus criterion five. Possible cases had at least one sign from criterion one and fulfilled criteria two and three (80). These cases were followed for a five

year period to better understand the progression of motor and non-motor manifestations of PD, and during that follow-up process, additional cases were identified as not having idiopathic PD (81).

Population controls must have been at least 35 years of age, did not have PD, lived in CA for at least five years prior to screening, and must have resided primarily in Fresno, Kern, or Tulare counties. Controls ages 65 or older were sampled randomly from Medicare enrollees in the three counties and from randomly selected housing units identified from tax assessor records of residential parcels during the first year of the study. After the instatement of HIPAA, all remaining controls were recruited from randomly selected parcels from tax assessor records, and two sampling strategies were used to enroll controls. First, letters were mailed to randomly selected residential units and controls were enrolled through mail and phone. In the second strategy, controls were recruited from randomly selected clusters of neighboring households during home visits by trained field staff who determined eligibility and enrolled controls at the door step. Only one person per household was allowed to enroll as a control in our study (42, 82).

2.2 Data Collection

Trained interviewers collected data by telephone. We obtained information on demographics, family history of PD, smoking, residential and occupational address history, exposures to specific chemicals (pesticides, metals, chemical solvents, etc.), a medical history, and data on other lifestyle factors such as physical activity, alcohol consumption, coffee consumption, etc. Subjects also completed the Mini Mental State Examination (MMSE) either in person or by telephone. UCLA movement disorder specialists examined cases, administering the motor exam portion of the Unified Parkinson's Disease Rating Scale (UPDRS) and assigning a

score on the Modified Hoehn and Yahr Scale. Subjects provided either whole blood or saliva samples. The UCLA Institutional Review Board approved the study, and all participants provided written informed consent.

3 Household Organophosphorus Pesticide Use and Parkinson's Disease

3.1 INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disease characterized clinically by both motor and non-motor symptoms and pathologically by loss of dopaminergic neurons in the midbrain and presence of Lewy bodies. Pesticides in general have been associated with an increased risk for developing Parkinson's disease (26) but most human studies focused on occupational exposures (83, 84). Household pesticide use in the USA continues to be very common, with use prevalence as high as 80-90% of households (85-87). This is of concern since persistence of pesticides inside homes can lead to prolonged exposures of household members (88-90). Until recently, many pesticide products permitted for household use contained organophosphorus (OP) chemicals; e.g. the OP insecticides chlorpyrifos and diazinon were widely used in household applications prior to the United States Environmental Protection Agency phase out from products permitted for household use in 2001 and 2004, respectively (91, 92). Organophosphorus pesticides as a class and individual OPs such as chlorpyrifos and parathion have been associated with PD in a handful of studies (39, 46, 53, 93, 94).

Here we explore whether exposures to pesticides from household use, especially those containing OPs, impact the odds of developing PD. In addition, we also assessed whether our results are consistent with genetic susceptibility expected among carriers of the 192QQ and 55MM variants in the gene encoding for the xenobiotic enzyme paraoxonase (PON1) known to detoxify several common OPs (95).

3.2 METHODS

The UCLA Institutional Review Board approved the study, and all participants provided written informed consent.

Subject Recruitment and Enrolment

This case-control study enrolled incident idiopathic PD patients from 2001 to 2007 and population-based controls between 2001 and 2011 from three mostly rural agricultural counties (Kern, Tulare, and Fresno) in central California. Subject recruitment (42, 43) and case criteria (80, 96) have been described elsewhere.

We identified 1167 PD patients through local neurologists, medical groups, and public service announcements; 397 had received a PD diagnosis >3 years prior to recruitment, 134 lived outside the area, 51 did not have a PD diagnosis, and 22 were too ill to participate. Of all eligible cases (N=563), 90 could not be examined i.e. declined, moved, became too ill or died before we examined them. Our movement disorder neurologists examined 473 eligible patients and excluded 107, because they did not meet required criteria for idiopathic PD (97). Six subjects withdrew prior to interview.

Initially, we recruited controls from the population using Medicare lists (in 2001) but, after the instatement of the Health Insurance Portability and Accountability Act (HIPAA), we solely used residential tax assessor records from the tri-county area. Two sampling strategies were implemented to maximize control enrolment success: first, we randomly selected residential parcels and enrolled via mail and phone, and second, we randomly selected clusters of neighboring households and enrolled participants during in-person visits at their door-step. Control sampling strategies have been described in detail elsewhere (42, 82).

Of 1212 potential controls contacted through the first recruitment strategy, 457 were ineligible (409 were < 35 years of age, 44 were too ill to participate, and 4 did not reside in

target counties). Furthermore, 409 eligible controls declined, became too ill or moved after screening and prior to interview leaving 346 controls recruited via phone and mail. In addition, an early mailing, for which the number of eligible subjects who declined remains unknown, produced 62 controls with home pesticide use information from interviews. We screened 4756 individuals for eligibility at their door step, finding 3515 to be ineligible (88% due to age criteria) and leaving 1241 eligible controls, of whom 634 declined participation, while 607 controls enrolled. However, 183 subjects agreed to an abbreviated questionnaire without household pesticide information and were excluded.

Of all cases and controls enrolled, in total 357 cases and 807 controls provided information necessary for analyses of household pesticide use. For 278 cases and 397 controls of Caucasian race we have both *PON1* genotype and household pesticide use information to assess modifications of OP pesticide effects on PD due to differences in OP metabolism from known functional variants.

Exposure Assessment

Trained staff collected information on demographic characteristics, smoking history, and lifetime household pesticide use. Participants self-reported personal use of pesticide products during four age periods: young adult (16-<25 years), adult (25-<45 years), middle-age (45-<65 years), and senior (≥65 years) in three micro-environments, i.e. inside the home, or outdoors on lawns and in yards, or during gardening activities. Subjects were asked to recall names of products and the pesticide targets (e.g. cockroaches, spiders, ants, termites, bees/hornets/wasps, flies, weed control, plant disease); some recalled specific chemicals (e.g. malathion, diazinon). We also elicited information about formulation of products (e.g. liquid, granules, bait, powder) and frequency of use i.e. none or rare (once a year or less), occasional (2-

11 times a year), or regular use (once a month or more; note: nobody reported more than once a week average use). We prompted interviewees who recalled a portion of the product name, with similarly sounding products with the same target and formulation. All interviews for cases and controls enrolled through our first sampling strategy were conducted from 2001 through 2007 and from 2009 through 2011 for controls enrolled through our second strategy. Throughout, we employed the same primary interviewers and supervisors.

We supplemented our interview data with information about ingredients of reported home and garden use pesticide products from the California Department of Pesticide Regulation (CDPR) product label database (98). Over 70% of products in this database have registration dates from the year 1970 and later. We compared dates of active registration listed in the CDPR database to dates of reported pesticide use to identify products for sale in California in those years. We also cross-referenced targets (e.g. ants, weeds) and formulation (e.g. liquid, granules) reported with targets, types (e.g. herbicide, insecticide, fungicide), and formulations listed in the CDPR database to identify products possibly used if product names were recalled incompletely. The active ingredient contributing the largest percentage to a product's composition was identified as the main ingredient. For some pesticides used before 1970, information on product composition was not available through CDPR; instead we identified the most likely main active ingredient with the same brand name (e.g. Black Flag) and target (e.g. ants). For some products, chemical composition varied over time, thus we considered the subject exposed to all possible main active ingredients. In addition, we also assigned chemical classes for each main active ingredient using the Pesticide Action Network (PAN) pesticide database (99).

The organophosphorus pesticides we identified in reported products included glyphosate, chlorpyrifos, bensulide, dichlorvos, diazinon, malathion, tetrachlorvinphos, oxydemeton-methyl, parathion, demeton, glufosinate-ammonium, disulfoton, and methidathion.

Genotyping Methods

Using whole blood or saliva samples from participants, genotyping for *PON1* L55M (rs854560) was conducted at the UCLA Genotyping and Sequencing Core Facility via pyrosequencing (46), and for Q192R (rs662) with the Fluidigm BioMark HD system (Fluidigm Corporation, South San Francisco, CA) at the University of Washington. Genotyping call rates for *PON1* L55M and *PON1* Q192R were 100% and 93%, respectively, and we did not detect departure from Hardy-Weinberg equilibrium in controls. We considered *PON1* 55MM and *PON1* 192QQ as 'risk' genotypes, because results for human serum analyses of PON1 diazoxonase activity suggested median metabolic activity in carriers of these homozygous variants is lowest (62).

Statistical Methods

We included only household pesticide products that subjects reported having personally used in their home, in yards and on lawns, or for gardening. We present results for progressively more specific pesticide usage beginning with (i) any use of household pesticides, then for types/classes of main active ingredients including (ii) any organophosphorus pesticide, (iii) subclasses of organophosphate (e.g. dichlorvos, tetrachlorvinphos) and organothiophosphates (e.g. chlorpyrifos, diazinon, malathion, oxydemeton-methyl, parathion, demeton, disulfoton, methidathion), and finally, (iv) the most commonly used insecticides, diazinon and chlorpyrifos.

We also excluded exposures reported for the last 10 years prior to the index age to account for the extended pre-clinical state of PD (100). We calculated a weighted average

frequency of pesticide use, first multiplying the midpoint of the reported pesticide use frequency category (i.e. for rare use: 0.5 times/year, occasional use: 6.5 times/year, regular use: 32 times/year) by years in each age period except for 10 years prior to index date, and summing across the periods before dividing by the total number of years between age 16 and the index age minus 10. We also calculated weighted averages without lagging, and using the same method, calculated weighted averages for exposures at younger ages only (16-<45 years). Similarly, we calculated weighted averages for each of the four age periods of exposure. We dichotomized household pesticide use into "frequent use" as an average frequency at or above the median of the exposure distribution in exposed controls and "never use/rare use" for an average frequency below the median. We also examine indoor and outdoor (i.e. yards, lawns, gardening) use separately. Subjects who reported use but did not specify a product name were excluded in analyses of organophosphorus use, but included as 'exposed' for any type of household pesticide use.

We calculated odds ratios (ORs) and 95% confidence intervals (CIs) in unconditional logistic regression analysis adjusting for age (continuous) at index date (i.e. year of diagnosis for cases and year of interview for controls), sex, race (white/non-white), smoking (ever/never), education (<12 years, 12 years, and >12 years), and family history of PD in first degree relatives (yes/no). We assessed effects for any pesticide use as well as for each organophosphorus pesticide group. As reference group for all comparisons we used "never use/rare use" of any household pesticides, thereby excluding those who used other types/classes of pesticides from the comparison when considering specific sub-categories of pesticides.

In sensitivity analyses, we excluded 62 controls from an unknown base of eligible subjects and stratified by gender. Additionally, we adjusted for ambient pesticide exposures at

residences and workplaces based on a Geographic Information System (GIS) model we developed using the California Pesticide Use Reporting system during 1974-1999 (42, 43), by weighting annual pounds of pesticide applied by proportion of acreage treated within a 500 meter buffer around addresses and summing exposures over the 26 year period. We created indicator variables, one for residential and one for workplace exposures, for ever having greater than median exposure (in exposed controls) for four types of pesticides (organochlorines (OC), organophosphorus, dithiocarbamates (DTC) and paraquat (PQ)). We also adjusted for a Job Exposure Matrix (JEM) derived life-time cumulative occupational pesticide measure (none, low, medium, high) based on work history and detailed job tasks information (82). Finally, we assessed modification of the effect estimate for PD from home pesticide use by *PON1* 192QQ genotype, and also the combined *PON1* diplotype (55MM, 192QQ), to identify low metabolizers (62). We used SAS Version 9.2 to conduct all analyses.

3.3 RESULTS

Our study participants were mostly older than 60 years of age, cases were more likely male, less educated than controls, and more often never smokers (Table 3-1).

Frequent household pesticide use, increased the odds of developing Parkinson's disease by 47% (95% CI: 1.13, 1.92). However, for organophosphorus and organothiophosphate classes of chemicals associations were larger (70-100% increase), and both common active ingredients chlorpyrifos and diazinon contributed to the increase (Table 3-2). Point estimates for unlagged exposures were slightly attenuated (Appendix Table 7-1) and for OP exposure at younger ages (16-<45) slightly increased. Susceptibility window analyses in the four age periods yielded smaller estimates in the older ages (Appendix Table 7-2).

Adjustment for ambient pesticide exposures at residences or work places attenuated estimates for household OP pesticide use minimally [OR=1.59 (95% CI: 1.12, 2.25)]; similarly

adjustment for life-time occupational pesticide exposures using our JEM estimates made no difference [OR=1.69 (95% CI: 1.19, 2.40)] (Table 3-3).

Separating indoor and outdoor household pesticide use resulted in similar size 50-70% increases in the odds ratio, but more participants reported use of organophosphorus pesticides outdoors on lawns, yards, or in gardens (25.9% of cases and 17.4% of controls outdoors vs. 3.0% and 1.8% indoors). Odds ratio estimates for use of any household pesticide were not different for men and women.

The influence of *PON1* 192QQ genotype (Table 3-4) was assessed in Caucasians only. As expected, we observed no increase in the OR with any *PON1* genotype in never/rare users of household pesticides, and a small 41% increase for subjects reporting frequent use of any pesticide who carried 192RR and QR genotypes; but we observed much larger ORs (2.62-3.71) in frequent users of OPs who carried the 192QQ genotype compared with never/rare users who were carriers of 192RR and QR genotypes. Carriers of the *PON1* diplotype, 55MM-192QQ, had almost 6-fold increase in the odds of PD, though this estimate was based on small numbers [OR=5.75 (95% CI: 1.41, 23.40)].

Our estimates for household pesticide use and joint analyses of household pesticide use and *PON1* 192 genotype were similar after excluding the 62 controls recruited in early mailings.

3.4 DISCUSSION

Our population-based case-control study of PD conducted in California's Central Valley suggests that household pesticide use increases the odds of developing PD especially for products that contain OPs as active ingredients independent of occupational and ambient exposures. Moreover, our results are corroborated by our finding that carriers of the *PON1* 192QQ variant or the 55MM-192QQ diplotype using household pesticides are at higher risk than non-carriers who are rarely or un-exposed.

Few previous studies have analyzed personal household pesticide use in relation to PD risk. In contrast to our results, a case-control study in Washington State did not find an association of PD with personal use of any household pesticide product (including those containing OPs) (53). A French case-control study found a 40% increase in the OR for PD for gardening related pesticide exposures but 95% CIs included the null (95% CI: 0.90, 2.30) (37). A recent PD meta-analysis reported a summary risk ratio of 1.18 (95% CI: 0.86, 1.63) for household pesticide use relying on three studies –including the two we referenced above (26). Our study is unique, since we used information on main active ingredients from CDPR to augment detailed questionnaire data. CDPR registers all pesticide products, including those meant for household use, before they can be sold in California.

Organophosphorus pesticides are still used in large amounts agriculturally (101, 102). Chlorpyrifos is permitted for use in ant and roach bait in homes (103), and other organophosphorus pesticides with similar mechanisms of toxicity, such as bensulide, are also still permitted as ingredients in household pesticide products (104). Thus, it is important to consider contributions of household organophosphorus pesticide use in PD studies since decades of past use exposed a large proportion of the US population. While in general OP elimination from the body is fast, for more lipophilic agents such as chlorpyrifos and diazinon some proportion stored in body fat may be more gradually released into circulation and eliminated (105, 106). Pesticides may also persist for longer periods in carpet dust (88). A recent study in the Salinas Valley of California suggests that household pesticide use may contribute a considerable proportion to pesticide exposures from indoor dust even in agricultural areas, with the finding that concentrations of chlorpyrifos and diazinon in household dust samples were 40-80% lower in 2006 than in 2000-2002 when both pesticides were ingredients of household

pesticide products (107). We recently reported that behaviors such as ventilation and cleaning of pesticide treated areas that would minimize pesticide exposures after in-home treatment and use of personal protective equipment during applications are uncommon (85). Animal studies indicated that OPs, such as chlorpyrifos, may affect dopaminergic neurotransmission (22, 108), and chronic low exposure to some OPs may result in mitochondrial dysfunction and apoptosis of neurons (109). Moreover, it has been suggested that neurotoxicity from OPs such as diazinon and chlorpyrifos may occur at levels lower than those eliciting acute toxicity (110).

Our analyses of organophosphorus pesticide use only account for main active ingredients and no other active or inert pesticide ingredients in products. Indeed, other active ingredients tend to change more frequently over time making it more difficult to identify them accurately, and inert ingredients are not required to be reported. To limit exposure misclassification for OP pesticide usage, we excluded participants with frequent use who could not recall a specific product name or enough information to identify a product and active ingredient.

A particular strength of our study is the disease characterization largely limiting misclassification error since cases were diagnosed by UCLA movement disorder specialists, and a majority of cases were re-evaluated over time. However, as with all other case control studies we assessed exposures only retrospectively, possibly resulting in differential recall bias if cases ruminate about causes for disease and over-report or more accurately recall and report past household pesticide use than controls. Our exposure assessment for OP pesticides depended only partially on recall and in large part on information on active ingredients retrieved from the CDPR. We relied on this database to identify products and periods when they contained OP pesticides as main ingredients, information participants would be unlikely to recall or differentially recall. In addition, no study participant would have been able to report use

consistent with *PON1* genotype carrier status which was unknown to them. Similar to our recent finding for ambient organophosphorus pesticide exposures (47), we estimated the highest risk of PD in carriers of the 55MM-192QQ diplotype who were frequent users of household OPs. Given extensive evidence that the *PON1* Q192R single nucleotide polymorphism is functional (58, 59, 111-113) and experimental data from human serum analyses that showed the Q allele influences PON1 serum diazoxonase activity under close to physiological conditions (62), finding the expected influence of slow organophosphate metabolizer status on PD provides support that the associations are not solely attributable to recall bias. While a smaller proportion of eligible controls compared to cases participate in our study, this would only result in selection bias if household pesticide use was related to participation. However, it is less likely that selection bias would affect our results from joint analyses of home pesticide use and genotype, since subjects would not have been able to select themselves into our study based on *PON1* genotype and household pesticide use.

Although many epidemiologic studies have assessed associations between pesticides and Parkinson Disease, few have focused on household pesticide use or organophosphorus pesticides. Household pesticide use is highly prevalent in the US, and organophosphorus pesticides are still used in household pesticide products. We enhanced our exposure assessment and limited recall bias by using the CDPR product label database to identify major active ingredients in products. Our findings for household pesticide use and PD were strongest in carriers of genetic variants associated with slow metabolism for OPs. This study contributes important evidence for an association between PD and household pesticide use, specifically OP pesticide use.

Table 3-1. Characteristics of Study Population.

Characteristic	Cases (N=357) n (%)	Controls (N=807) n (%)
Sex (male)	205 (57.4)	371 (46.0)
Age*		
mean ± SD	68.3 ± 10.2	66.2 ± 11.6
range	34-88	35-99
≤60 years	75 (21.0)	254 (31.5)
>60 years	282 (79.0)	553 (68.5)
Cigarette smoking		
Never	187 (52.4)	389 (48.2)
Former	150 (42.0)	328 (40.6)
Current	20 (5.6)	90 (11.2)
Race		
White	287 (80.4)	564 (69.9)
Non-White	70 (19.6)	242 (30.0)
Unspecified		1 (0.1)
Education		
0-<12 years	66 (18.5)	116 (14.4)
12 years	96 (26.9)	166 (20.6)
>12 years	195 (54.6)	525 (65.0)
First-degree relative with PD†		
No	305 (85.4)	742 (91.9)
Yes	52 (14.6)	65 (8.1)

*This is the age at diagnosis for cases and age at interview for controls.

† We assumed that 26 controls who did not report family history of PD did not have first-degree relatives with PD.

Table 3-2. Parkinson's Disease Association with Average Household Pesticide Use Frequency from age 16 until 10 years prior to Index Age in the Central Valley of California.

	Cases n (%)	Controls n (%)	Crude OR	Adjusted OR* (95% CI)
Any Household Pesticide Usage				
Never Use/Rare Use	196 (54.9)	504 (62.5)	1.00	1.00
Frequent Use†	161 (45.1)	303 (37.5)	1.37	1.47 (1.13, 1.92)
Any Organophosphorus (OP) pesticide use‡				
Never Use/Rare Use	196 (70.3)	504 (80.6)	1.00	1.00
Frequent Use†	83 (29.7)	121 (19.4)	1.76	1.71 (1.21, 2.41)
Chemical classes within OP pesticides				
Organophosphate				
Never Use/Rare Use	196 (75.1)	504 (84.1)	1.00	1.00
Frequent Use†	65 (24.9)	95 (15.9)	1.76	1.72 (1.18, 2.51)
Organothiophosphate				
Never Use/Rare Use	196 (85.2)	504 (92.3)	1.00	1.00
Frequent Use†	34 (14.8)	42 (7.7)	2.08	1.95 (1.17, 3.23)
Individual Organothiophosphate pesticides				
chlorpyrifos				
Never Use/Rare Use	196 (95.6)	504 (98.2)	1.00	1.00
Frequent Use†	9 (4.4)	9 (1.8)	2.57	2.73 (1.03, 7.24)
diazinon				
Never Use/Rare Use	196 (90.3)	504 (94.0)	1.00	1.00
Frequent Use†	21 (9.7)	32 (6.0)	1.69	1.58 (0.87, 2.88)

*Adjusted for age (continuous), sex, smoking, race, PD family history, and education.

†Subjects with an average frequency of use per year during ages 16-<10 years prior to index age that was at or above the median in exposed controls were assigned to the "Frequent Use" category. For all comparisons, those in the "Never Use/Rare 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.

‡Subjects may be counted in multiple sub-categories of organophosphorus pesticide usage.

Table 3-3. Parkinson's Disease Associations with Average Household Pesticide Use Frequency from age 16 until 10 years prior to Index Age; Additional Adjustment for Other Sources of Pesticide Exposure.

	Cases n (%)	Controls n (%)	Crude OR	ORIGINAL MODEL Adjusted OR*,† (95% CI)	MODEL 1 Adjusted OR*,‡ (95% CI)	MODEL 2 Adjusted OR*,§ (95% CI)
Any Household Pesticide Usage						
Never Use/Rare Use	196 (54.9)	504 (62.5)	1.00	1.00	1.00	1.00
Frequent Use‖	161 (45.1)	303 (37.5)	1.37	1.47 (1.13, 1.92)	1.41 (1.08, 1.84)	1.45 (1.11, 1.90)
Any Organophosphorus (OP) pesticide use¶						
Never Use/Rare Use	196 (70.3)	504 (80.6)	1.00	1.00	1.00	1.00
Frequent Use‖	83 (29.7)	121 (19.4)	1.76	1.71 (1.21, 2.41)	1.59 (1.12, 2.25)	1.69 (1.19, 2.40)
Chemical classes within OP pesticides **Organophosphate**						
Never Use/Rare Use	196 (75.1)	504 (84.1)	1.00	1.00	1.00	1.00
Frequent Use‖	65 (24.9)	95 (15.9)	1.76	1.72 (1.18, 2.51)	1.57 (1.07, 2.30)	1.70 (1.16, 2.50)
Organothiophosphate						
Never Use/Rare Use	196 (85.2)	504 (92.3)	1.00	1.00	1.00	1.00
Frequent Use‖	34 (14.8)	42 (7.7)	2.08	1.95 (1.17, 3.23)	1.95 (1.17, 3.25)	2.00 (1.18, 3.39)
Individual Organothiophosphate pesticides **chlorpyrifos**						
Never Use/Rare Use	196 (95.6)	504 (98.2)	1.00	1.00	1.00	1.00
Frequent Use‖	9 (4.4)	9 (1.8)	2.57	2.73 (1.03, 7.24)	2.55 (0.96, 6.75)	2.81 (1.02, 7.71)
diazinon						
Never Use/Rare Use	196 (90.3)	504 (94.0)	1.00	1.00	1.00	1.00
Frequent Use‖	21 (9.7)	32 (6.0)	1.69	1.58 (0.87, 2.88)	1.61 (0.88, 2.95)	1.58 (0.86, 2.90)

*All models are adjusted for age (continuous), sex, smoking, race, education, and PD Family History. Additional adjustments for other pesticide exposures are listed below.

†ORIGINAL MODEL: Unadjusted for other pesticide exposures.

‡MODEL 1: Additionally adjusted for ambient residential and ambient workplace exposures to pesticides (organophosphorus, organochlorine, dithiocarbamates, and/or paraquat) from nearby agricultural applications.

§MODEL 2: Additionally adjusted for a job exposure matrix (JEM) derived exposure to any pesticide.

‖ Subjects with an average frequency of use per year during ages 16-<10 years prior to index age that was at or above the median in exposed controls were assigned to the "Frequent Use" category. For all comparisons, those in the "Never Use/Rare 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.

¶Subjects may be counted in multiple sub-categories of organophosphorus pesticide usage.

Table 3-4. Combined Effects of *PON1* Q192R and Household Pesticide Usage from age 16 until 10 years prior to Index Age in Association With Parkinson's Disease, Caucasians Only.

	Never Use/Rare Use				Frequent Use*			
	Case n	Control n	Crude OR	Adjusted OR† (95% CI)	Case n	Control n	Crude OR	Adjusted OR† (95% CI)
Any Household Pesticide Use								
PON1 Q192R								
RR+RQ	74	133	1.00	1.00	62	84	1.33	1.41 (0.90, 2.21)
QQ	75	120	1.12	1.09 (0.72, 1.65)	67	60	2.01	1.96 (1.23, 3.11)
OR for interaction							*1.35*	*1.27 (0.67, 2.42)*
Any Organophosphorus (OP) pesticide use‡								
PON1 Q192R								
RR+RQ	74	133	1.00	1.00	28	48	1.05	1.03 (0.58, 1.82)
QQ	75	120	1.12	1.09 (0.72, 1.65)	37	24	2.77	2.62 (1.42, 4.83)
OR for interaction							*2.35*	*2.34 (1.02, 5.35)*
Chemical classes within OP pesticides								
Organophosphate Use								
PON1 Q192R								
RR+RQ	74	133	1.00	1.00	24	36	1.20	1.26 (0.68, 2.33)
QQ	75	120	1.12	1.09 (0.72, 1.66)	28	19	2.65	2.51 (1.28, 4.94)
OR for interaction							*1.97*	*1.82 (0.74, 4.51)*
Organothiophosphate Use								
PON1 Q192R								
RR+RQ	74	133	1.00	1.00	11	20	0.99	0.93 (0.41, 2.10)
QQ	75	120	1.12	1.09 (0.72, 1.66)	16	7	4.11	3.71 (1.42, 9.68)
OR for interaction							*3.70*	*3.67 (1.05, 12.78)*

* Subjects with an average frequency of use per year during ages 16-<10 years prior to index age that was at or above the median in exposed controls were assigned to the "Frequent Use" category. For all comparisons, those in the "Never Use/Rare 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.

†Adjusted for age (continuous), sex, smoking, and education. We did not adjust for family history of PD to avoid the issue of over-adjustment due to possible correlations of family history with *PON1* genotype.

‡Subjects may be counted in multiple sub-categories of organophosphorus pesticide usage.

4 Occupational Pesticide Use and Parkinson's Disease

4.1 INTRODUCTION

Parkinson's disease (PD) is a chronic and progressive movement disorder. Many previous epidemiologic investigations identified occupational pesticide exposures as risk factors for PD. Studies reporting associations of PD with occupational exposures to pesticides, herbicides, insecticides, and fungicides, however, are of varying quality, size, and consistency in terms of agents examined. Also, some studies assessed exposures rather crudely (ever/never occupational exposure), or employed self-reports only (16), with little more than a handful of studies creating job exposure matrixes (JEMs) based on various types of information and levels of detail (37, 82, 114-117), and the Agricultural Health Study (AHS) being the only cohort of licensed pesticide applicators and spouses with a prospective design and detailed assessment of pesticide use (40).

In our large California based case control study of PD, we conducted a detailed historical assessment of active use of occupational pesticides and personal protective equipment (PPE). In addition, we also have available extensive information on additional sources of pesticide exposure in this population, specifically ambient pesticide exposures from agricultural applications at work places and residences and household pesticide use. Here, we report for the first time on PD risk from primarily farming-related occupational pesticide use based on self-reports complemented by information on chemicals from the California pesticide registration system. Different from most previous studies, we are able to adjust for other pesticide exposure (gardening and household use and ambient bystander exposures) common in agricultural environments and examine whether personal protective equipment (PPE) use modifies risk.

4.2 METHODS

Study Subjects

The Parkinson Environment Gene (PEG) study is a population-based case-control study of Parkinson's disease, with participants recruited from the mostly rural California counties Kern, Fresno, and Tulare. Cases were enrolled within three years of PD diagnosis, from 2001 through 2007, and population controls were enrolled between 2001 and 2011. Descriptions of PD case diagnostic criteria (80, 96) and subject recruitment (42, 43) may be found in our prior publications.

Briefly, through local neurologists, medical groups, and public service announcements, we identified 1167 PD patients. We excluded 397 diagnosed >3 years before contact, 134 not living in the target counties, 51 without a PD diagnosis, and 22 who were too ill to participate. Of 563 remaining eligible cases, 90 declined, moved, became too ill or died before we could examine them. We further excluded 107 who did not meet criteria for idiopathic PD at exam (118-120), and six withdrew prior to interview leaving us with 360 patients.

Controls 65 years or older were initially from Medicare enrollee lists for all three counties but after the Health Insurance Portability and Accountability Act (HIPAA) was instated, controls were randomly selected from residential parcel listings on tax assessor records. We used two strategies to enroll controls. First, we mailed letters to selected residential units and enrolled through mail and phone only. Using a second strategy, we recruited controls from randomly selected clusters of five neighboring households from parcel listings, and trained field staff conducted home visits to determine eligibility and enrolled controls at the door step. Only one eligible person per household was allowed to enroll as a control in our study (42, 82).

Using the first sampling strategy, we contacted 1,212 potential controls of whom 457 were ineligible (409 were < 35 years of age, 44 too ill to participate, and 4 lived outside target

counties). We recruited 346 controls via phone and mail, since an additional 409 eligible controls declined, became too ill, or moved after screening and prior to interview. Through an early mailing, for which the number of eligible subjects who declined remains unknown, we recruited and interviewed 62 controls. We screened 4,753 individuals for eligibility at their door step and found 3,512 to be ineligible (88% due to age criteria), leaving 1,241 eligible controls, of whom 634 declined participation and 607 enrolled. Of the 607 controls enrolled through the second sampling strategy, 183 subjects agreed to participate in an abbreviated interview only and did not provide occupational information. Altogether, we have 827 controls available.

This study was approved by the UCLA Institutional Review Board, and we obtained written informed consent from all participants.

Data Collection

Trained interviewers collected information by telephone on demographic characteristics, smoking, household pesticide use, lifetime residential addresses, lifetime occupations and addresses, and screened for jobs with exposures of interest, i.e. fertilizers, pesticides, metals, wood, paint strippers, and solvents. PD cases (228 out of 360) and controls (457 out of 827), who screened positive i.e. reported (1) ever having worked with any one of the agents of interest or who reported having ever (2) lived on a farm, or (3) worked on a farm, were additionally interviewed to collect more details on specific occupational exposures. We did not interview 269 participants who screened negative, and 9 who did not respond to our screening questions. Of those who screened positive for fertilizers or pesticides, or ever worked or lived on a farm (N=754), 21.3% (52/244) cases and 20% (102/510) controls refused to participate in the detailed occupational interview. Based on respondent data, it is unlikely that those who screened positive for other chemicals only (paint strippers, etc.), and refused to participate in occupational

interviews (10 cases and 60 controls) would have used pesticides occupationally. Of those who did not screen positive, 7 subjects were nevertheless interviewed and, as expected, none of them reported pesticide exposures.

All of our patients were seen at least once – many multiple times over a period of 10 years – by our UCLA movement specialists to confirm idiopathic PD according to UK Brain Bank, CAPIT Rating Scale, and Gelb criteria (118-120). We also conducted a Mini-Mental State Examination over the phone or in person, with phone scores converted into predicted in-person scores as recommended (121).

Occupational Pesticide Exposures

Here, we newly utilize extensive information from the additional interview in which participants self-reported occupational pesticide use of fungicides, herbicides, insecticides, and other pesticides (rodenticides, defoliants) including the name of pesticide products used, purpose or site of usage (e.g. crop, plant, animal, insect), duration (years) of use, location of use (Fresno, Kern, or Tulare counties; California; United States or abroad), whether subjects mixed or loaded pesticides, application methods (tractor with/out an enclosed cab, hand sprayer, backpack or aerial application, etc.), and personal protective equipment (PPE) use (gloves, mask, coveralls, boots, goggles, respirator, etc.). In order to reduce subject burden and recall issues, we limited collection of all data to pesticide group (fungicides/herbicides/insecticides/other pesticides).

We identified the main active ingredient of each self-reported pesticide product, relying on the California Department of Pesticide Regulation (CDPR) product label database (98), which lists the active ingredients of all pesticide products sold on the California market. We obtained the main active ingredient (in terms of product weight), by comparing the reported pesticide

product name and purpose of use with CDPR database names, purposes (e.g. crop, plant, animal, insect), use types (e.g. fungicides, herbicides, insecticides), and product registration dates during the years of reported use.

When information on product composition was not available through CDPR (i.e. use prior to 1970), the most probable main active ingredient was identified based on products with the same brand names (e.g. Lannate) and purposes/sites of usage (e.g. cotton, alfalfa). If the chemical composition of a product varied over time, we considered the user as exposed to all main active ingredients the product contained in the period of its use. To identify the chemical classes of the main active ingredients (e.g. dicarboximide, inorganic, amide, etc.), we used the Pesticide Action Network (PAN) pesticide database (99) and the Compendium of Pesticide Common Names (122). When the reported information was inadequate to identify chemical class we still were able to identify pesticide use type (fungicide/insecticide/herbicide/other pesticides).

From the self-reported occupational pesticide use information we derived ever/never use for each main active ingredient. We additionally summarized over the categories of all pesticides, pesticide use types (fungicides, insecticides, herbicides, and other pesticides), and chemical classes (carbamates, organochlorines, organophosphorus). We considered 'ever users' those who used any ingredient within the category.

We considered subjects who screened negative, or refused to participate in the occupational interview, or who participated but did not provide responses to questions about pesticide use as unexposed if during screening they reported no regular work (i.e. once a week or more) with fertilizers or pesticides. For those without a screening question value, or responding yes to our screening question but refusing the occupational interview, or missing responses to

questions about pesticide use (7 cases and 13 controls from all three groups combined), we considered the occupational pesticide use data as missing.

Ambient Pesticide Exposures

A geographic information system (GIS) was used to obtain estimates of ambient workplace and ambient residential pesticide exposures. The lifetime occupational and residential addresses for the period 1974-1999 were geocoded and combined with data on pesticide use records from CDPR and land use maps from the California Department of Water Resources (123, 124). We estimated the pounds per acre per year of pesticides applied within a 500 meter radius surrounding each address. We then summed the exposures over the 26-year period and calculated 26-year average exposures. Those subjects with exposure at workplace or residential addresses greater than or equal to the median 26-year average exposure in exposed controls for four types of pesticides (organochlorines (OC), organophosphorus, dithiocarbamates (DTC) and paraquat (PQ)) were assigned a value of 1 for workplace and residential exposure, respectively. Those with exposures at workplace or residential addresses below the median 26-year average exposure in exposed controls for all four types of pesticides were considered unexposed and assigned a 0 for workplace and residential exposure, respectively.

Household Pesticide Exposures

We previously created a measure of household pesticide use frequency (125), identifying main active ingredients of reported home and garden use pesticide products from the CDPR product label database in the manner described for occupational products. We calculated the lifetime average frequency of any household pesticide use (personal application indoors or outdoors in yards, on lawns, or in gardens), considering use at or above the median value in exposed controls ‘frequent use’ and use below the median ‘never/rare use’.

Statistical analyses

We calculated odds ratios and 95% confidence intervals using unconditional logistic regression for ever use of any occupational pesticide, pesticide use types (i.e. fungicides/insecticides/herbicides/ other pesticides), and exposure to specific chemicals and classes as reported. To allow for comparison to prior studies on occupational pesticides, we used a reference group of never occupational pesticide users throughout, which included participants with other types of pesticide exposures (i.e. frequent household pesticide use and/or ambient pesticide exposures). We report on chemicals and chemical classes with at least 5 exposed cases and 5 exposed controls for analyses and examined specifically carbamates, OPs, and OCs. We conducted analyses of self-reported duration of work with pesticides in years, examining those with 1) >0 and ≤ 10 years and 2) >10 years of work with pesticides, and calculating a p-trend based on the median of each category. We also analyzed PPE use (yes/no, type of PPE used, frequency of PPE use) and job tasks of mixing or loading pesticides or applying pesticides at work.

Analyses were adjusted for sex, smoking (ever/never), age at index date (continuous), education (<12 years, 12 years, and >12 years), and race (white/non-white). For sensitivity analyses, we adjusted for other sources of pesticide exposure (frequent household use, ambient workplace, ambient residential) or for occupational use (yes/no) of other types of pesticides (i.e. OPs, OCs, DTCs, paraquat, rotenone, carbamates, triflumizole, captan, and propargite, pesticides for which we have previously seen associations of ambient exposures with PD (24, 25, 42, 43, 48)). We additionally adjusted for PD family history (yes/no), MMSE scores, other farming related exposures (includes regular, i.e. once a week or more, work with metals, wood, chemical solvents, or paint strippers), estimated associations for males only, excluded controls who were

interviewed later than cases (i.e. between 2009-2011), excluded the 62 controls from an unknown base population, and excluded participants with low MMSE scores (less than 27). We additionally analyzed occupational pesticide use with a 'low exposure' reference group. Participants in this reference category 1) did not use pesticides occupationally, 2) were unexposed to ambient residential or workplace OP, OC, DTC pesticides and paraquat (i.e. exposed below the median of exposed controls), and 3) were never/rare users of household pesticides. They may have had lower ambient exposures to pesticides at workplaces or residences or lower household pesticide exposures from infrequent use. All analyses were conducted in SAS version 9.3.

4.3 RESULTS

The majority of our participants were older than 60 years of age and of European ancestry. Cases were more often male, less educated than controls, and more likely to be never smokers than controls (Table 4-1). Men (28%) reported occupational pesticide use more frequently than women (4.2%).

Those ever using any occupational pesticides, fungicides, insecticides, and herbicides had 29 to 89% increased risk for PD (Table 4-2). On average, cases used pesticides longer than controls and most effect estimates were much larger for those having used pesticides for more than 10 years. Adjusting for other sources of pesticide exposure (i.e. frequent household use, ambient residential and ambient workplace) attenuated our estimates. Concerning pesticide groups, we estimated the strongest association for use of carbamates (OR=3.45, 95% CI: 1.19, 10.02). When we conducted analyses with the 'low exposure' reference group, we found our ORs to be elevated for all categories of occupational pesticide use (Table 4-4). Results did not change in other sensitivity analyses. Active occupational users who also reported using PPE were

at increased risk, while our data suggested a smaller risk increase for ever pesticide users without PPE, and the highest OR for those always using PPE (Table 4-3). We also saw a positive association for the job task of mixing/loading pesticides (OR=1.61, 95% CI: 1.00, 2.59).

4.4 DISCUSSION

Our findings for occupational pesticide use are in agreement with earlier studies showing an increase in PD risk. Our results are also consistent with expectations in terms of duration of exposure such that longer years of use were associated with higher risk, and the highest risks were estimated for job activities (mixing/loading) known to result in particularly high exposures (126). Interestingly, those who reported PPE use, especially always use of PPE, were at highest risk of PD, possibly because these farm workers felt compelled to use PPE when handling toxic pesticides; however, the types of PPEs they used failed to protect them adequately. Different from previous studies, our estimates are adjusted for all other sources of pesticide exposure in addition to all major confounders.

Toxicologic studies in animals, cells, and *in vitro* experiments with pesticides provided evidence of neurotoxicity in support of the hypothesis that pesticides are involved in PD pathogenesis. Mechanisms by which pesticides may be related to PD pathogenesis include oxidative stress and inhibition of mitochondrial complex I (127).

Paraquat neurotoxicity causes dopaminergic cell death in the substantia nigra pars compacta (SNpc) of mice (128, 129). Rotenone inhibits mitochondrial complex I in rats (130). Additionally, pesticides were found to accelerate the formation of α-synuclein fibrils *in vitro*, including rotenone, DDT, 2,4-dichlorophenoxyacetic acid (2,4-D), dieldrin, diethyldithiocarbamate, paraquat, maneb, trifluralin, parathion, and imidazoldinethione (131), and mice exposed to paraquat had increases in brain levels of α-synuclein and α-synuclein

containing aggregates in the SNpc (132). Lab and epidemiologic studies from our group show that benomyl inhibits aldehyde dehydrogenase, which detoxifies the dopamine metabolite DOPAL, in mesencephalic rat neurons and inhibits the ubiquitin-proteasome system in SK-N-MC neuroblastoma cells (23-25, 133).

Previously, ten cohort studies – six occupational - examined associations between PD and occupational pesticide exposures or work in occupations involving pesticide exposures (30, 36, 40, 51, 115, 116, 134-137), and reported relative risk estimates ranging from 0.66 to 5.6. However, since PD is a rare event in all but very large cohorts, these studies relied on as few as 1 and a maximum of 134 exposed incident PD cases. Exposure assessment in these studies was based on self-report, broad occupational categories listed in national databases, and few used employee records (36, 51) or job-exposure matrices (115, 116). The Agricultural Health Study (40) and a French (PAQUID) study (116) performed the most detailed exposure assessments, but still only had 68 and 8 exposed PD cases available for analysis, respectively. Some studies collected exposure information only once at baseline, possibly ignoring long periods of exposure during follow-up and prior to diagnosis that might be relevant (30, 115, 134, 137, 138). Case control studies enrolling larger numbers of PD cases might have higher diagnostic accuracy if patients are examined by experts, but many were small (<200 cases) and included prevalent cases with long (>5 years) or unspecified disease duration (28, 32, 33, 37, 50, 52, 114, 139-144). Few included incident cases (34, 37), raising concerns about survivor bias, differential recall due to cognitive impairment in prevalent cases, and temporal ambiguity.

Strengths of our California case control study are that it is to date the largest in terms of the number of PD cases with a high exposure prevalence (21%) and that we enrolled incident PD cases diagnosed by UCLA movement disorder specialists and re-evaluated most patients at

multiple follow-up occasions, limiting misclassification of disease status. Our study is one of few that evaluated risk of PD from exposure to specific pesticides (34, 36-38, 40, 50-52, 93) and also duration and intensity/type of exposure, only the second occupational study which assessed use of personal protective equipment, and the first that controlled for other sources of pesticide exposures in residents of largely agricultural counties in which few can be considered completely unexposed. Since participants often do not know or remember what active ingredients the product they used contained, we compared reported pesticide brand names, purposes, and dates of use with information in the CDPR database to identify the main active pesticide ingredients in reported pesticide products. Restricting analyses to subjects with high MMSE score indicated that our results were not affected by impaired cognition. Furthermore, we collected detailed information about PPE use during occupational work with pesticides. Findings from the Agricultural Health Study suggested that PPE use in pesticide applicators may reduce PD risk (40), while a family-based case-control study found PPE use to not alter associations between pesticide use at home and work and PD (39). In contrast, our findings suggest that PPE use did not protect against risk but rather may even be a surrogate marker for the use of more toxic pesticides or, alternatively, the PPEs they used did not protect from exposure to the agents handled. Indeed, most of our study participants did not report using highly protective PPE (e.g. respirators, chemically resistant rubber gloves). Additional research targeting PPEs when assessing health risks from chronic pesticide exposures is needed.

Our subjects reported occupational use of 148 different pesticides, with 42% and 37.8% of exposed cases and controls, respectively, reporting use of more than one pesticide up to a maximum of 29 different pesticides, limiting our ability to estimate effects for single pesticide exposures of interest for PD based on animal, cell, or previous human data. Of cases and controls

who reported occupational pesticide use, 35.1% of cases and 25.4% of controls did not recall the specific products used. Chemicals our participants commonly used include DDT, 2,4-D, malathion, and glyphosate, but these have not been previously linked to PD. Our finding for carbamates is consistent with our prior finding of an increased PD risk in consumers of well-water possibly contaminated with N-methyl carbamates (48). Our difficulty in interpreting results as pesticide specific is due to co-exposure to multiple pesticides applied simultaneously or sequentially by study participants. When we mutually adjusted for occupational use of other pesticides we previously identified as relevant for PD, estimates for occupational carbamate use remained elevated, but confidence intervals widened (OR=4.46, 95% CI: 0.66, 30.25). Importantly, in our reference group of non-occupationally exposed subjects a majority were exposed to other sources of pesticides including household and gardening pesticides or ambient exposures at residences or workplaces from agricultural applications in these counties. Of note, in sensitivity analyses we created an alternate reference group of 'low exposure', excluding subjects with other sources of pesticide exposure, and found even more strongly increased risks with occupational use of carbamates, organochlorines, and organophosphorus pesticides (Table 4-4).

In this population based study of incident PD, we found evidence of increased risk with occupational pesticide use, increasing years of pesticide use, and job tasks resulting in the highest exposures to pesticides such as mixing and loading pesticides. We also found some evidence for specific pesticides including carbamates, OPs, and OCs. Finally, personal protective equipment use may not result in a reduced risk from pesticide exposures at the workplace.

Table 4-1. Characteristics of subjects.

Variable	Cases (360) mean(sd) or n(%)	Controls (827) mean(sd) or n(%)
Age		
mean (sd)	68.3 (10.2)	66 (11.7)
<=60 years	76 (21.1)	264 (31.9)
>60 years	284 (78.9)	563 (68.1)
range	34-88	35-99
Sex		
Male	206 (57.2)	382 (46.2)
Female	154 (42.8)	445 (53.8)
Race		
missing	0	2 (0.2)
White	290 (80.6)	569 (68.8)
Black	3 (0.83)	28 (3.4)
Latino	47 (13.1)	160 (19.4)
Asian	4 (1.1)	25 (3)
Native American	16 (4.4)	43 (5.2)
Education		
<12 years	67 (18.6)	123 (14.9)
12 years	96 (26.7)	172 (20.8)
>12 years	197 (54.7)	532 (64.3)
Family History of PD		
positive	53 (14.7)	65 (7.9)
negative	307 (85.3)	762 (92.1)
Smoking Status		
Never	188 (52.2)	400 (48.4)
Former	152 (42.2)	333 (40.3)
Current	20 (5.6)	94 (11.4)
Years of Occupational Pesticide use		
mean (sd)	18.2 ± 15.4	13.0 ± 13.2

Table 4-2. OR (95% CI) for self-reported occupational pesticide use, years of use, and PD risk.

Variable*: n(%) or mean ± SD	Cases (360)	Controls (827)	Unadjusted OR	Adjusted[a] OR (95%CI)	Adjusted[b] OR (95%CI)
Any Occupational Pesticides					
Missing	7 (1.9)	13 (1.6)			
No Occupational Pesticide Use[c]	279 (77.5)	700 (84.6)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Ever Occupational Pesticide Use	74 (20.6)	114 (13.8)	1.63	1.50 (1.05, 2.14)	1.36 (0.95, 1.95)
Duration of use in years					
Zero years[c]	279 (77.5)	700 (84.6)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
>0 and ≤ 10 years	29 (8.1)	55 (6.7)	1.32	1.27 (0.77, 2.09)	1.22 (0.74, 2.02)
> 10 years	35 (9.7)	40 (4.8)	2.20	1.98 (1.20, 3.28)	1.69 (1.01, 2.83)
p-trend[d]			*0.0009*	*0.0073*	*0.0426*
Pesticide Product Type					
Fungicides					
No Occupational Pesticide Use[c]	279 (77.5)	700 (84.6)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Ever Fungicide Use	31 (8.6)	39 (4.7)	2.00	1.89 (1.12, 3.19)	1.62 (0.95, 2.76)
Duration of use in years					
Zero years[c]	279 (77.5)	700 (84.6)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
>0 and ≤ 10 years	14 (3.9)	18 (2.2)	1.95	1.97 (0.93, 4.17)	1.86 (0.87, 3.95)
> 10 years	13 (3.6)	16 (1.9)	2.04	1.82 (0.83, 3.97)	1.46 (0.66, 3.23)
p-trend[d]			*0.0363*	*0.0969*	*0.2776*
Insecticides					
No Occupational Pesticide Use[c]	279 (77.5)	700 (84.6)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Ever Insecticide Use	51 (14.2)	87 (10.5)	1.47	1.29 (0.87, 1.94)	1.15 (0.76, 1.74)
Duration of use in years					
Zero years[c]	279 (77.5)	700 (84.6)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
>0 and ≤ 10 years	20 (5.6)	41 (5.0)	1.22	1.12 (0.62, 1.99)	1.05 (0.58, 1.90)
> 10 years	23 (6.4)	29 (3.5)	1.99	1.71 (0.94, 3.10)	1.45 (0.79, 2.65)
p-trend[d]			*0.0146*	*0.0771*	*0.2315*
Herbicides					
No Occupational Pesticide Use[c]	279 (77.5)	700 (84.6)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Ever Herbicide Use	41 (11.4)	60 (7.3)	1.72	1.51 (0.96, 2.36)	1.34 (0.84, 2.12)
Duration of use in years					
Zero years[c]	279 (77.5)	700 (84.6)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
>0 and ≤ 10 years	8 (2.2)	31 (3.8)	0.65	0.65 (0.29, 1. 46)	0.59 (0.26, 1.35)
> 10 years	26 (7.2)	22 (2.7)	2.97	2.41 (1.31, 4.44)	2.07 (1.12, 3.85)
p-trend[d]			*0.0005*	*0.0070*	*0.0290*
Other Pesticides (rodenticides,defoliants,etc)					
No Occupational Pesticide Use[c]	279 (77.5)	700 (84.6)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Ever Use of Other Pesticides	20 (5.6)	39 (4.7)	1.29	1.27 (0.71, 2.29)	1.19 (0.66, 2.17)
Duration of use in years					
Zero years[c]	279 (77.5)	700 (84.6)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
>0 and ≤ 10 years	6 (1.7)	18 (2.2)	0.84	0.98 (0.37, 2.59)	1.01 (0.38, 2.69)
> 10 years	9 (2.5)	8 (1.0)	2.82	2.60 (0.95, 7.12)	2.05 (0.74, 5.69)
p-trend[d]			*0.0536*	*0.0764*	*0.1829*

**Note that subjects may be counted in multiple sub-categories of pesticide usage.*

Table 4-2. OR (95% CI) for self-reported occupational pesticide use, years of use, and PD risk.

[a]Adjusted for sex, smoking(ever/never), age(continuous), education(<12 years, 12 years, and >12 years), race(white/non-white).
[b]Adjusted for sex, smoking(ever/never), age(continuous), education(<12 years, 12 years, and >12 years), race(white/non-white), household pesticide use frequency (frequent vs never/rare), and ambient residential and work address pesticide exposures.
[c]Reference group composed of never users of occupational pesticides by self-report. These subjects may have other pesticide exposures (based on frequent household pesticide use, ambient residential, and/or ambient workplace pesticide exposures).
[d]Based on median of each category.

Table 4-3. OR (95% CI) for occupational pesticide use with/out Personal protective equipment (PPE) and PD risk.

	Cases (360)		Controls (827)			
	n	%	n	%	Unadjusted OR	Adjusted[a] OR (95%CI)
PPE[b]						
No Occupational Pesticide Use[c]	279	(77.5)	700	(84.6)	1.00 (Ref)	1.00 (Ref)
Occ pesticide use without PPE	28	(7.8)	49	(5.9)	1.43	1.33 (0.80, 2.20)
Occ pesticide use with PPE use	46	(12.8)	65	(7.9)	1.78	1.64 (1.06, 2.53)
Types of PPE						
gloves						
No Occupational Pesticide Use[c]	279	(77.5)	700	(84.6)	1.00 (Ref)	1.00 (Ref)
Occ pesticide use without gloves	34	(9.4)	61	(7.4)	1.40	1.25 (0.78, 1.99)
Occ pesticide use with gloves	40	(11.1)	53	(6.4)	1.89	1.82 (1.14, 2.90)
mask						
No Occupational Pesticide Use[c]	279	(77.5)	700	(84.6)	1.00 (Ref)	1.00 (Ref)
Occ pesticide use without mask	43	(11.9)	68	(8.2)	1.59	1.54 (1.00, 2.37)
Occ pesticide use with mask	31	(8.6)	46	(5.6)	1.69	1.45 (0.87, 2.41)
coveralls						
No Occupational Pesticide Use[c]	279	(77.5)	700	(84.6)	1.00 (Ref)	1.00 (Ref)
Occ pesticide use without coveralls	50	(13.9)	77	(9.3)	1.63	1.51 (1.01, 2.27)
Occ pesticide use with coveralls	24	(6.7)	37	(4.5)	1.63	1.48 (0.84, 2.62)
tractor with enclosed cab						
No Occupational Pesticide Use[c]	279	(77.5)	700	(84.6)	1.00 (Ref)	1.00 (Ref)
Occ pesticide use without enclosed cab	66	(18.3)	104	(12.6)	1.59	1.51 (1.05, 2.18)
Occ pesticide use with enclosed cab	8	(2.2)	10	(1.2)	2.01	1.42 (0.53, 3.77)
PPE use frequency[d]						
No Occupational Pesticide Use[c]	279	(77.5)	700	(84.6)	1.00 (Ref)	1.00 (Ref)
No PPE and Occ pesticide use	28	(7.8)	49	(5.9)	1.43	1.33 (0.80, 2.21)
Sometimes PPE use when Occ pesticide use	23	(6.4)	39	(4.7)	1.48	1.40 (0.79, 2.45)
Always PPE use when Occ pesticide use	20	(5.6)	21	(2.5)	2.39	2.21 (1.14, 4.30)

[a]Adjusted for sex, smoking(ever/never), age(continuous), education(<12 years, 12 years, and >12 years), race(white/non-white).

[b]PPE includes gloves, masks, coveralls, applying pesticides in an enclosed cab, and other sorts of protection, such as boots, goggles, etc. For 14 subjects who used pesticides, information on PPE use was not available, and we assigned them to no PPE.

[c] Reference group composed of never users of occupational pesticides by self-report. These subjects may have other pesticide exposures (based on frequent household pesticide use, ambient residential, and/or ambient workplace pesticide exposures).

[d]The PPE use frequency does not incorporate information on use of a tractor with an enclosed cab. Note that 3 cases and 5 controls reported using PPE, but did not provide a frequency of PPE use.

Table 4-4. OR (95% CI) for self-reported occupational pesticide use and PD risk. Reference group has low exposure to pesticides.

	Cases (360)		Controls (827)			
Variable*	**n**	**%**	**n**	**%**	**Unadjusted OR**	**Adjusted[a] OR (95%CI)**
Any Occupational Pesticides						
Missing	2	(0.6)	16	(1.9)		
low exposure[b]	34	(9.4)	134	(16.2)	1.00 (Ref)	1.00 (Ref)
Exposure to other sources of pesticides[c]	250	(69.4)	563	(68.1)	1.75	1.89 (1.25, 2.87)
Ever Occupational Pesticide Use	74	(20.6)	114	(13.8)	2.56	2.50 (1.50, 4.15)
Pesticide Product Type						
Fungicides						
low exposure[b]	34	(9.4)	134	(16.2)	1.00 (Ref)	1.00 (Ref)
Exposure to other sources of pesticides[c]	293	(81.4)	638	(77.2)	1.81	1.92 (1.27, 2.91)
Ever Fungicide Use	31	(8.6)	39	(4.7)	3.13	3.11 (1.65, 5.88)
Insecticides						
low exposure[b]	34	(9.4)	134	(16.2)	1.00 (Ref)	1.00 (Ref)
Exposure to other sources of pesticides[c]	273	(75.8)	590	(71.3)	1.82	1.97 (1.30, 2.98)
Ever Insecticide Users	51	(14.2)	87	(10.5)	2.31	2.10 (1.22, 3.60)
Herbicides						
low exposure[b]	34	(9.4)	134	(16.2)	1.00 (Ref)	1.00 (Ref)
Exposure to other sources of pesticides[c]	283	(78.6)	617	(74.6)	1.81	1.94 (1.28, 2.94)
Ever Herbicide Use	41	(11.4)	60	(7.3)	2.69	2.45 (1.37, 4.36)
Other Pesticides (rodenticides,defoliants,etc)						
low exposure[b]	34	(9.4)	134	(16.2)	1.00 (Ref)	1.00 (Ref)
Exposure to other sources of pesticides[c]	304	(84.4)	638	(77.2)	1.88	1.98 (1.31, 2.99)
Ever Use of Other Pesticides	20	(5.6)	39	(4.7)	2.02	2.06 (1.03, 4.09)
Chemical Class of Main Active Ingredients						
Carbamates						
low exposure[b]	34	(9.4)	134	(16.2)	1.00 (Ref)	1.00 (Ref)
Exposure to other sources and/or other occupational pesticides[c]	314	(87.2)	671	(81.1)	1.84	1.95 (1.29, 2.95)
Ever Carbamate Use	10	(2.8)	6	(0.7)	6.57	5.55 (1.81, 17.04)
Organochlorines						
low exposure[b]	34	(9.4)	134	(16.2)	1.00 (Ref)	1.00 (Ref)
Exposure to other sources and/or other occupational pesticides[c]	314	(87.2)	660	(79.8)	1.88	1.98 (1.31, 2.99)
Ever Organochlorine Use	10	(2.8)	17	(2.1)	2.32	1.97 (0.81, 4.82)
Organophosphorus Pesticides						
low exposure[b]	34	(9.4)	134	(16.2)	1.00 (Ref)	1.00 (Ref)
Exposure to other sources and/or other occupational pesticides[c]	308	(85.6)	647	(78.2)	1.88	1.98 (1.31, 2.99)
Ever Organophosphorus Use	16	(4.4)	30	(3.6)	2.10	2.01 (0.95, 4.23)

**Note that subjects may be counted in multiple sub-categories of pesticide usage.*

[a]Adjusted for sex, smoking(ever/never), age(continuous), education(<12 years, 12 years, and >12 years), race(white/non-white).

[b] Reference category subjects are unexposed to ambient residential or workplace pesticides (OPs, OCs, DTCs, & paraquat), are never/rare users of household pesticides, and did not use pesticides occupationally.

[c]These subjects did not self-report occupational pesticide use for the specific category of pesticides but were exposed to pesticides. They reported using other pesticides occupationally and/or were exposed to pesticides based on other measures of pesticide exposure (frequent household pesticide use, ambient residential, and/or ambient workplace pesticide exposures).

5 Genetic Variability in *ABCB1*, Occupational Pesticide Exposure, and Parkinson's Disease

5.1 INTRODUCTION

Parkinson's disease (PD), the second most common neurodegenerative disease, is considered to have a multifactorial etiology. There is substantial evidence suggesting that pesticide exposures increase risk of PD (26), and it has recently been suggested that the risk attributable to these exposures may be modified by regulators of xenobiotic uptake and their distribution throughout the body, such as P-glycoprotein (P-gp), encoded by the *ABCB1* gene (73-75), since P-gp is involved in efflux of xenobiotics across the blood brain barrier (BBB) (71).

Certain lipophilic pesticides, such as the organophosphorus pesticide (OP) chlorpyrifos, organochlorines (OCs), and rotenone, easily cross the blood brain barrier (145-147). Animal and cell studies suggest that pesticides are removed from BBB endothelial cells by P-glycoprotein; e.g., mice deficient in P-gp had higher brain concentrations of a lipophilic pesticide (148, 149). Many lipophilic and amphipathic xenobiotic compounds, including several OC and OP pesticides, are not only P-gp substrates, but also dose dependently either stimulate or inhibit transport activity or modulate P-gp expression (150-155).

The *ABCB1* gene is highly polymorphic with thousands of putative single nucleotide polymorphisms (156); the two most studied are a synonymous mutation in rs1045642 (c.3435C/T) in exon 26 and a missense mutation in rs2032582 (c.2677G/T/A) in exon 21 (157). These polymorphisms have been shown to affect P-gp function in a substrate dependent fashion (76). The mutation at rs1045642 possibly alters substrate specificity by changing the timing of co-translational folding (77); additionally, the homozygous TT genotype has been associated with

lower P-gp expression levels (78, 158), possibly through a reduction of mRNA stability (159). P-gp expression has also been lower for carriers of the TT genotype at rs2032582 (158).

A French study (75), reported that exposed carriers of the TT genotype for rs1045642 exhibited the highest risk of PD when exposed to organochlorine pesticides compared to unexposed C allele carriers (OR=7.2, 95% CI: 2.1, 24.8). Results for rs2032582 were similar, with exposed men carrying the T-allele exhibiting 7.9 times the risk of developing PD compared to unexposed G-allele carriers (95% CI: 2.2, 28.9), and multiplicative interaction terms were statistically significant. Additionally, a multiplicative interaction was also observed in case-only analyses for rs2032582 and cumulative lifetime hours of organochlorine exposure.

We have previously found that exposures ambiently at residences and workplaces to OCs dieldrin and endosulfan (24, 25) and to OPs (46, 47, 49) as well as consumption of well water possibly contaminated with OPs(48) and frequent household use of OPs (125) increase PD risk. Several other epidemiologic (37-39, 53, 93, 94), as well as toxicologic (20-22, 108-110), studies have implicated OCs and OPs in PD, and pesticides in these chemical classes have been found to impact P-gp function. Here, we re-examine the influence of the two *ABCB1* polymorphisms and occupational OC and OP pesticide exposures on PD risk in an attempt to replicate and expand on prior epidemiologic findings reported by Dutheil et al. (2010).

5.2 METHODS

All research procedures for this study were approved by the University of California, Los Angeles (UCLA) Institutional Review Board, with written informed consent provided by all participants.

Study Subject Recruitment

We conducted a population-based case-control study of Parkinson's disease, with participants recruited from Kern, Fresno, and Tulare counties in Central California. From 2001 through 2007, we enrolled cases within three years of PD diagnosis, and from 2001 to 2011 we enrolled population controls. Our prior publications describe PD case diagnostic criteria (80, 96) and subject recruitment (42, 43).

Through local neurologists, medical groups, and public service announcements, we identified 1167 PD patients, of whom 604 were ineligible (397 were diagnosed >3 years before contact, 134 did not live in target counties, 51 did not have PD, and 22 were too ill). Among eligible cases (n=563), 90 declined, moved, became too ill or died and therefore could not be examined. We excluded 107 patients not meeting idiopathic PD criteria at exam (118-120), and six cases withdrew before being interviewed.

Prior to the instatement of the Health Insurance Portability and Accountability Act (HIPAA), we enrolled controls 65 years or older from Medicare enrollee lists, but afterwards we selected all controls randomly from residential tax assessor records. We enrolled controls using two approaches. We first mailed letters to selected residential units and enrolled controls by mail and phone. With a second expanded approach, we recruited controls randomly selected and living in clusters of five neighboring households in the three counties at the door step during home visits. We permitted enrolment of one eligible person per household as a control (42, 82).

We contacted 1,212 potential controls with our first approach; 457 were ineligible (409 were too young, 44 too ill to participate, and 4 lived outside the counties). Additionally, 409 eligible controls declined, became too ill, or moved after screening and prior to interview; thus, we recruited 346 remaining eligible controls via phone and mail. Through an early mailing, we

recruited and interviewed another 62 randomly selected controls for whom the proportion of eligible subjects declining participation remains unknown. Using our second recruitment approach, we screened 4,753 individuals for eligibility and found 3,512 ineligible (88% due to age criteria). Of the remaining 1,241 eligible controls, 634 declined participation, and 607 enrolled at the door step, but a subset (N=183) agreed only to an abridged interview and did not provide information needed to determine occupational pesticide exposures.

For 351 cases and 725 controls of all races, and 282 cases and 514 controls of European ancestry, we have both *ABCB1* genotype and pesticide exposure information to assess modifications of occupational OC and OP pesticide associations with PD by the two functional variants of interest.

Data Collection

Our trained staff conducted telephone interviews to collect data on demographic characteristics, smoking, lifetime occupations and addresses, household pesticide use, lifetime residential addresses, and screened for jobs with exposures of interest, i.e. fertilizers or pesticide exposures, metals, wood, paint strippers, and solvents. PD cases (290 out of 360) and controls (619 out of 827) screened positive in that they reported (1) work with any one of the exposures of interest, (2) ever having lived or (3) having worked on a farm. While most of these subjects, 228 cases (78.6%) and 457 controls (73.8%), agreed to interviews about specific occupational exposures, 62 cases and 162 controls refused this additional interview. Among those screening positive for regular work (i.e. once a week or more) with fertilizers or pesticides, having worked on a farm, or lived on a farm (N=754), 21.3% (52/244) of cases and 20% (102/510) of controls refused the detailed occupational interview.

Our UCLA movement disorder specialists confirmed idiopathic PD in all cases based on UK Brain Bank, CAPIT Rating Scale, and Gelb criteria (118-120), and a majority were seen multiple times over a 10 year period. Our interviewers conducted the Mini-Mental State Examination in person or over the phone; we converted phone scores to predicted in-person scores as recommended (121).

Occupational Pesticide Exposures

Using occupational histories and self-reported job tasks (pesticide mixing and application, planting and ploughing, field and non-fieldwork, and work with farming supplies, etc.) for each farming related job held for 6 months or longer, we previously created a job exposure matrix (JEM) measure of lifetime cumulative workplace pesticide exposure (82). We assigned weights representing the intensity of probable pesticide exposure, multiplied by the years in the job, and calculated lifetime cumulative exposure by summing over all jobs. We consider those with a lifetime cumulative exposure above the 75th percentile of exposed controls occupationally exposed to pesticides.

Using our detailed occupational interview, we collected occupational pesticide use information for fungicides, herbicides, insecticides, and other pesticides, eliciting the name of each pesticide product whenever possible, purpose or site of usage (e.g. crop, plant, animal, insect), and years of use.

We used the California Department of Pesticide Regulation (CDPR) product label database (98) to identify the main active ingredients (based on product weight) of self-reported pesticide products, comparing reported pesticide product names and purposes of use with CDPR database information (names, purposes, use types (e.g. fungicides, herbicides, insecticides), and

product registration dates) on products sold on the California market in the reported years of use. Products with the same brand names (e.g. Lannate) and purposes/sites of usage (e.g. cotton, alfalfa) were used to identify the most likely main active ingredient for products used prior to 1970 for which product details were unavailable through CDPR. Participants were considered exposed to all main active ingredients in the product throughout the reported time span of use if pesticide products changed chemical composition over time. We used the Pesticide Action Network (PAN) pesticide database (99) and the Compendium of Pesticide Common Names (122) to determine chemical classes (e.g. organophosphorus, organochlorine) of main active ingredients.

We distinguished ever/never use of each main active ingredient in self-reported products and ever/never use of any occupational pesticide or of any main active ingredient in a chemical class (organochlorines include DDT, toxaphene, aldrin, dieldrin,chlordane, lindane, methoxychlor, chlorothalonil, dicofol; organophosphorus chemicals include malathion, methyl parathion, parathion, diazinon, demeton, phosmet, TEPP, mevinphos, phorate, chlorpyrifos, dimethoate, acephate, disulfoton, naled, methamidophos, ethion, bensulide).

We considered participants who refused the extended occupational interview, screened negative, or those who participated without providing occupational pesticide use information 'never users' of occupational pesticides if they had reported 'no use' for our screening question about ever regular work with fertilizers or pesticides. A minority of subjects (7 cases and 13 controls) did not provide the information necessary to identify their occupational pesticide use status and were considered to have missing occupational pesticide use values.

Ambient Pesticide Exposures

Using a geographic information system (GIS), we combined CDPR pesticide use reports, California Department of Water Resources land use maps, and geocoded lifetime occupational and residential addresses from 1974 through 1999 to obtain estimates of pounds per acre per year of pesticides applied within 500 meters around each address (123, 124). We computed 26-year average exposures to individual pesticides within the OC, OP, and dithiocarbamate (DTC) classes, as well as for paraquat (PQ). Participants with exposure levels at or above the median value of the 26-year average in exposed controls at workplaces or residences for each pesticide group (OCs, OPs, DTCs, and PQ) were considered "exposed" ambiently at workplaces or residences, respectively.

Our measure for ambient workplace OC pesticide exposure included exposure to chlorothalonil, camphechlor, toxaphene, dienochlor, methoxychlor, lindane, dicofol, dieldrin, endosulfan, and chlordane. Our workplace GIS-based measure for OP pesticide exposure included exposure to 36 OP pesticides (49).

Household Pesticide Exposures

We previously used the CDPR product label database to identify main active ingredients in household pesticide products (personal application indoors or outdoors in yards, on lawns, or in gardens) (125). We computed an average frequency of any household pesticide use over the lifetime and defined 'frequent users' as those with lifetime average frequencies greater than or equal to the median for exposed controls. Those using them less frequently and never users were included in a 'never/rare users' group.

Genotyping methods

Whole blood or saliva samples from participants were genotyped at rs1045642 (C3435T) and rs2032582 (G2677(A,T)) at IntegraGen in France by allele-specific PCR (AS-PCR) using the Fluidigm BioMark system (Fluidigm Corporation, South San Francisco, CA). Genotyping call rates for rs1045642 and rs2032582 in those of European ancestry were 98.8% and 98.0%, respectively. Therefore, 10 and 17 subjects who failed genotyping for rs1045642 and rs2032582, respectively, are not included in tests for marginal associations of *ABCB1* genotypes with PD (14 and 35 subjects from all race/ethnicities, respectively, failed genotyping). We tested for and did not detect departures from Hardy-Weinberg equilibrium in controls.

Statistical analyses

We separately examined professional exposure to organochlorine (OC) and organophosphorus (OP) pesticides according to occupational self-report and ambient GIS-derived workplace exposure. Subjects in the reference category (i.e. 'low exposure') include those unexposed to 1) pesticides occupationally according to self-report; 2) ambiently to workplace pesticides (OPs, OCs, DTCs, and paraquat; i.e. exposed below the median of exposed controls); 3) our JEM score (i.e. exposed at or below the 75th percentile); and 4) never/rare users of household pesticides. Thus, these reference group subjects may be exposed to lower exposures due to active occupational use, or from ambient exposures to pesticides at workplace or residential addresses, or infrequently using pesticides at home. Similar to Dutheil et al. (2010), who created a separate category for those with pesticide exposures from gardening/home use only, we also grouped into a separate category subjects who reported frequent household pesticide use but were low or un-exposed to all other occupational sources of pesticides as listed above. Unlike the French study, our 'occupationally OC exposed' and 'occupationally OP exposed' groups included those exposed to OCs or OPs 1) ambiently (at or above the median of

exposed controls) at workplace addresses and/or 2) to self-reported occupational pesticides. We also created a separate category for subjects with workplace exposure to other pesticides (based on JEM, ambient workplace exposures, and self-reported active use as defined above). All exposures had to have occurred prior to the index time.

Using unconditional logistic regression to calculate odds ratios (ORs) and 95% confidence intervals (CIs), we estimated marginal associations for occupational OC and OP pesticides (exposed ambiently at workplaces and/or due to self-reported active use) separately and used a recessive genetic model to assess associations for each SNP. For rs1045642, the reference group includes any C allele carriers, for rs2032582 any G allele carriers. We considered the 'TT' genotype the risk genotype for both SNPs as done by Dutheil et al. (2010) in interaction analyses. We also created an *ABCB1* risk score in which participants were assigned a score of 1 for each homozygous variant genotype at each SNP and a score of 0 otherwise (1=homozygous variant genotype at one of the *ABCB1* SNPs, 2=homozygous variant genotype at both SNPs). We also estimated marginal associations of each *ABCB1* polymorphism with PD, using dummy variables to compare heterozygous and homozygous variant genotypes to homozygous wild-types.

We then examined each *ABCB1* polymorphism and occupational pesticide exposures together, using subjects without the risk genotype and with 'low exposure' as reference groups. We conducted gene-environment interaction analyses separately for rs1045642, rs2032582, and a 'double recessive' genetic model based on our risk score comparing those with a score of 2 to those with scores of 0 or 1, using product terms to assess interactions on a multiplicative scale. We confirmed with logistic regression that pesticide exposures were independent of susceptibility genotypes in controls and conducted case-only analyses, which provide increased

statistical power to detect departure from multiplicativity compared to case-control analyses (160).

To address concerns about population stratification, we conducted analyses restricted to European ancestry participants only; in sensitivity analyses we included all subjects and adjusted for race (white/nonwhite). We adjusted in all analyses for age at index date (continuous and using age at diagnosis for cases and at interview for controls), sex, county (Fresno/Kern/Tulare), smoking (never/former/current), and total in-person MMSE score (MMSE score ≤ 25/ MMSE score >25). We mutually adjusted combined analyses of each *ABCB1* polymorphism and occupational pesticide exposures for the homozygous variant genotype at the other SNP. Multicollinearity is unlikely to cause a problem, because the LD among the SNPs is not strong ($r^2 = 0.53$). We previously found that *PON1* 55MM (at rs854560) variant genotype modifies PD risk from ambient residential and workplace OP pesticide exposures (47). Therefore, we adjusted all analyses of OP pesticides for *PON1* slow metabolizer genotype status. In sensitivity analyses, we examined associations adjusted for ambient residential exposures (to OPs, OCs, DTCs, and paraquat), excluded controls recruited from an unknown base of eligible subjects, or excluded controls obtained with the second sampling approach. All statistical analyses were conducted using SAS 9.3. We conducted power analyses using Quanto Version 1.2.4 (161, 162) with two-sided tests and an alpha level of 0.05.

5.3 RESULTS

Most of our study participants were older than 60 years of age. We found more males and more never smokers among cases than controls (Table 5-1). *ABCB1* SNP genotype frequencies were similar in those of European ancestry and all races together (Appendix Table 7-4).

We observed 47-54% increases in PD risk for rs2032582 and rs1045642 using recessive genetic models for participants of European ancestry (Table 5-2), and similar results for all subjects combined (Appendix Table 7-5). Occupational pesticide exposures to OCs and OPs increased PD risk by 72-88% (Table 5-3), and nearly doubled PD risk in all races (Appendix Table 7-6). A larger percentage of subjects were exposed to OP pesticides, though approximately 64% of occupationally exposed subjects were exposed to both types of pesticides (104 cases and 146 controls).

For occupational pesticide exposure and *ABCB1* SNP rs1045642, we estimated the largest ORs of 2.09-3.84 for OP and/or OC exposed carriers of the homozygous variant TT genotype and other pesticides compared with 'low exposure' non-variant genotype carriers. Adjusting for the variant TT genotype at rs2032582 did not appreciably alter results (Table 5-4). We observed similar increases in risk for carriers of the TT genotype for *ABCB1* SNP rs2032582 (ORs: 2.44-3.36) with occupational OC and/or OP exposures, though results were attenuated following adjustment for carrying the homozygous variant genotype at rs1045642. Among those occupationally exposed to OC and/or OP pesticides, participants with the highest risk had homozygous variant genotypes for both rs1045642 and rs2032582 [OR= 3.71, 95% CI: (1.96, 7.02)] (Appendix Table 7-3). Results were similar when adjusting for ambient residential pesticide exposures and for all races combined (Appendix Table 7-7). We determined that we had at least 80% power to detect interaction ORs of 4.1-5.4 or greater as reported in Dutheil et al. (2010). However, we did not detect interactions on the multiplicative scale using case-control and case-only analyses.

Marginal and joint associations for occupational pesticide exposure and *ABCB1* genotypes were similar after we excluded controls recruited early from an unknown base of eligible subjects and controls recruited with the second approach.

5.4 DISCUSSION

Our population based case-control study corroborated a previous report that two common genetic variants in the *ABCB1* gene, which codes for P-glycoprotein, act together with pesticide exposures to increase PD risk. Specifically, PD risk from occupational exposures to organochlorine pesticides is being modified by variant alleles of the *ABCB1* gene at both rs1045642 and rs2032582. We newly report that associations between occupational organophosphorus pesticide exposures and PD are also modified by polymorphisms in both SNPs. Our findings also suggest that the highest PD risk occurs in carriers of the TT genotype at both *ABCB*1 polymorphic sites when occupationally exposed to pesticides, either from ambient exposures at the workplace and/or through active work with these chemicals. Our study had sufficient power to detect interaction ORs of the magnitude reported in Dutheil et al. (2010), but we did not replicate these findings of large interactions on a multiplicative scale.

Human P-glycoprotein, encoded by the *ABCB1* gene, is the most studied of the ATP-binding cassette (ABC) transporters, and is involved in efflux of a large variety of substrates from cells (163). In human brain tissue samples, P-glycoprotein has been found to be expressed by endothelial cells in central nervous system capillaries (164), primarily on the luminal but also on the basal side of BBB endothelial cells facing the brain interstitial fluid and on intracellular organelle membranes (165). The transporter is also expressed in other areas of the body important for xenobiotic uptake and distribution including the apical surface of enterocytes, the

nose-brain barrier, proximal tubular kidney cells, and the biliary canalicular membrane of hepatocytes (72, 166, 167).

Several studies examined associations between PD and polymorphisms in the *ABCB1* gene in European and Asian populations, without accounting for environmental exposures (see Appendix Tables 7-8 and 7-9). The results of these studies have been inconsistent. An Italian hospital based case control study, found no association between PD and rs1045642 or rs2032582, but reported an association between the TT genotype at rs1045642 and the T allele at rs2032582 and early onset PD (168). A Polish population based case control study found suggestive evidence of a protective association between the 2677G-3435C haplotype (G allele at rs2032582 and C allele at rs1045642) and PD (169). A German clinic based case control study that only included PD cases with evidence of increased iron content in the substantia nigra, found no association between 10 SNPs in *ABCB1* and PD (170). A Swedish hospital based case control study did not find evidence of an association of either rs1045642 or rs2032582 with PD (171). The recent meta-analysis of PD genome-wide association studies in individuals of European ancestry identified associations in the discovery phase between PD and two *ABCB1* SNPs, rs28746490 and rs2235043, though these did not reach genome-wide significance, with meta p-values between $1x10^{-4}$ and 0.05 (172). Our genetic marginal effect estimates (Table 5-2) suggest an increased risk of PD for homozygous variant carriers of either SNP, but due to small numbers of subjects completely unexposed according to all of our pesticide measures, we cannot estimate genetic effects in a truly pesticide unexposed population.

Three prior studies in European populations have provided evidence for a gene-environment interaction between pesticide exposure and polymorphisms in *ABCB1* (73-75). Two studies (Drozdzik et al. 2003; Zschiedrich et al. 2009) conducted case-only analyses but did not

examine specific pesticides, nor did they present information on whether pesticide exposure and the rs1045642 polymorphism were independent in controls. Assumptions of independence along with disease rarity are both required in order to estimate departures from multiplicative interaction using a case-only analysis (173).

A Japanese hospital based case control study did not find evidence of interaction (174), and unlike the French case control study (Dutheil et al. 2010) which used a recessive genetic model, they examined interactions for rs1045642 using a dominant genetic model. Genotype frequencies were different in these two populations (CC, CT, and TT genotypes 37.4%, 45.1%, and 17.4%, in Japanese controls and 24%, 50%, and 26% in French controls).

When using genetic markers in association studies, there is the possibility that the marker studied is associated with a causal variant and is not itself the causal variant due to linkage disequilibrium. We also can only speculate that our results might be due to dysfunctional P-gp activity or reduced P-gp expression. Our subjects are exposed to a large variety of environmental toxins that may upregulate or downregulate P-gp expression and activity, and we most likely did not account for all possible modulators in our analyses. However, we have employed some of the most comprehensive pesticide exposure assessment. We adjusted our analyses for smoking; the polycyclic aromatic hydrocarbon benzo[a]pyrene, a component of cigarette smoke, has been shown to modulate P-gp expression in cell studies (175), and smoking is inversely associated with PD.

In this study, we relied partially on self-reported information to construct our exposure measures, though we used the CDPR database to identify whether our subjects worked with OC or OP pesticides instead of asking our subjects to recall exposures to specific chemicals. In Dutheil et al. (2010), enrolled participants worked in agriculture or related occupations, and all

subjects considered exposed to OC pesticides had worked with these pesticides. In our study, approximately 30% of subjects worked in jobs related to farming, fishing, or forestry (82), but fewer reported working directly with pesticides. Therefore, the majority of subjects we considered occupationally exposed to OC or OP pesticides were exposed ambiently at the workplace. It is important to note that our participants who had ambient workplace pesticide exposures did not necessarily work in occupations related to agriculture or pesticide application. In both studies, participants actively used or were exposed to a large variety of pesticides. While the French study had slightly more subjects who worked with OC pesticides compared to OPs, in our California population a larger percentage had been occupationally exposed to OP pesticides and a majority to both.

Our GIS based measures of specific pesticide exposures do not depend on recall, a unique strength of our study. There is the possibility of some nondifferential exposure misclassification due to variations in wind patterns and tracking of pesticide residues into workplaces as well as geocoding problems due to incomplete addresses; this however, affected exposure assessment for similar proportions of cases and controls (49). In an effort to reduce exposure misclassification, we increased the specificity of our GIS based exposure assignments, considering subjects exposed to OC or OP pesticides only when their exposure levels were at or above the median level in exposed controls. Another key strength of our study is that UCLA movement disorder specialists diagnosed PD and even repeated evaluations for most of our cases over time.

Multiple investigations highlighted the role of P-gp for the xenobiotic efflux function of the blood-brain barrier and the biological interaction of pesticides with P-gp as transport substrates and inhibitors. Using GIS based exposure assessment of ambient pesticide exposures from drift in addition to self-reported occupational pesticide use, our results support prior

findings that genetic variants at rs1045642 and rs2032582 in *ABCB1* modify PD risk from occupational exposures to organochlorines. Additionally, we found evidence suggesting that variants at these loci also modify risk from organophosphorus (OP) pesticide exposures, and together, homozygous variant genotypes at both positions appear to confer the greatest PD risk in those with both OC and OP exposures.

Table 5-1. Characteristics of Study Population, participants with European ancestry (n=866).

	Cases (N=286) No. (%)	Controls (N=580) No. (%)
Sex (male)	161 (56.3)	290 (50)
Age[a]		
mean +/- SD	69.1 +/- 10.4	67.5 +/- 11.6
range	34-88	35-99
≤60 years	58 (20.3)	151 (26.0)
>60 years	228 (79.7)	429 (74)
Cigarette smoking		
Never	157 (54.9)	260 (44.8)
Former	117 (40.9)	254 (43.8)
Current	12 (4.2)	66 (11.4)
County		
Fresno	134 (46.9)	246 (42.4)
Kern	100 (35)	236 (40.7)
Tulare	52 (18.2)	98 (16.9)
Education		
0-<12 years	32 (11.2)	40 (6.9)
12 years	84 (29.4)	119 (20.5)
>12 years	170 (59.4)	421 (72.6)
First-degree relative with PD		
No	245 (85.7)	528 (91.0)
Yes	41 (14.3)	52 (9.0)
***ABCB1* rs2032582 genotype**[b]		
GG	95 (33.2)	186 (32.1)
GT	118 (41.3)	278 (47.9)
TT	68 (23.8)	104 (17.9)
***ABCB1* rs1045642 genotype**[b]		
CC	62 (21.7)	122 (21.0)
CT	128 (44.8)	308 (53.1)
TT	95 (33.2)	141 (24.3)

[a] This is the age at diagnosis for cases and age at interview for controls.

[b]Genotyping failed for 17 and 10 subjects, respectively, for rs2032582 and rs1045642.

Table 5-2. Parkinson Disease associations with *ABCB1* rs2032582 (n=849), *ABCB1* rs1045642 (n=856), and *ABCB1* risk score (n=846), participants with European ancestry.

	Cases No. (%)	Controls No. (%)	Unadjusted OR	Adjusted[a] OR (95% CI)	*Dutheil et al. 2010* *OR[b] (95% CI)*
ABCB1-rs2032582					
GA	0	0	*NC*	*NC*	*0.6 (0.2- 2.3)*
GG	95 (33.8)	186 (32.7)	1.00	1.00	*1.00*
GT	118 (42)	278 (48.9)	0.83	0.82 (0.59, 1.15)	*1.0 (0.7-1.4)*
TA	0	0	*NC*	*NC*	*NC*
TT	68 (24.2)	104 (18.3)	1.28	1.31 (0.88, 1.96)	*1.0 (0.6-1.6)*
ABCB1-rs2032582					
GG+GT	213 (75.8)	464 (81.7)	1.00	1.00	*1.00*
TT	68 (24.2)	104 (18.3)	1.42	1.47 (1.04, 2.10)	*NC*
ABCB1- rs1045642					
CC	62 (21.8)	122 (21.4)	1.00	1.00	*1.00*
CT	128 (44.9)	308 (53.9)	0.82	0.82 (0.56, 1.19)	*1.2 (0.8-1.9)*
TT	95 (33.3)	141 (24.7)	1.33	1.34 (0.89, 2.02)	*1.0 (0.6-1.6)*
ABCB1- rs1045642					
CC+CT	190 (66.7)	430 (75.3)	1.00	1.00	*1.00*
TT	95 (33.3)	141 (24.7)	1.53	1.54 (1.12, 2.12)	*NC*
ABCB1 risk score[c]					
0	182 (64.8)	415 (73.5)	1.00	1.00	*1.00*
1	36 (12.8)	58 (10.3)	1.42	1.33 (0.84, 2.11)	*NC*
2	63 (22.4)	92 (16.3)	1.56	1.63 (1.12, 2.37)	*NC*
p-Value for trend				*0.0076*	

NC: not calculated

[a]Adjusted for age(continuous),sex, county, smoking (never/former/current).

[b]OR from conditional logistic regression on matched sets (matched on age (±2 years), sex, and region of residency). Adjusted for pack-years (never smoker/ever smoker <=17 pack years (their median)/ever smoker > 17 pack-years) and MMSE (total in-person MMSE score <=26/MMSE score 27-28/MMSE score >=29). One case and one control had the TA genotype for rs2032582.

[c]We assigned a score of 1 for each homozygous variant genotype at each SNP and a score of 0 otherwise. We then summed scores for the two SNPs to obtain the final *ABCB1* risk score (1=homozygous variant genotype at one of the ABCB1 SNPs, 2=homozygous variant genotype at both SNPs).

Table 5-3. PD associations with workplace exposure to Organochlorine and Organophosphorus (n=804) pesticides, participants with European ancestry.

Exposure	Cases No. (%)	Controls No. (%)	Unadjusted OR	Adjusted[a] OR (95% CI)
Workplace Organochlorine Exposure				
low exposure[b]	56 (19.8)	149 (28.6)	1.00	1.00
frequent household pesticide use without workplace pesticide exposure	28 (9.9)	78 (15.0)	0.96	1.00 (0.58, 1.72)
workplace exposure to other pesticides	76 (26.9)	111 (21.3)	1.82	1.84 (1.19, 2.84)
ambient workplace OC exposure and/or self reported OC use[c]	123 (43.5)	183 (35.1)	1.79	1.72 (1.15, 2.58)
Workplace Organophosphorus Exposure				
low exposure[b]	56 (19.8)	149 (28.6)	1.00	1.00
frequent household pesticide use without workplace pesticide exposure	28 (9.9)	78 (15)	0.96	1.01 (0.58, 1.73)
workplace exposure to other pesticides	38 (13.4)	67 (12.9)	1.51	1.42 (0.85, 2.38)
ambient workplace OP exposure and/or self reported OP use[c]	161 (56.9)	227 (43.6)	1.89	1.88 (1.28, 2.76)

[a]Adjusted for age(continuous), sex, county, smoking (never/former/current), and total in-person MMSE score (MMSE score ≤ 25/ MMSE score >25).

[b] Reference category subjects are unexposed to any ambient workplace pesticides (OPs, OCs, DTCs, & paraquat), did not use pesticides occupationally, are unexposed according to JEM score,and are never/rare users of household pesticides. They may have ambient residential pesticide exposures.

[c]Many cases (n=104) and controls (n=146) are occupationally exposed to both OC and OP pesticides.

Table 5-4. *ABCB1* SNP rs1045642 and Exposure to Workplace Organochlorine & Organophosphorus Pesticides in Association With Parkinson Disease, participants with European ancestry.

	ABCB1-rs1045642						
	CC+CT			TT			
	N case/control	**Crude OR (95% CI)**	**Adjusted[a] OR (95% CI)**	**N case/control**	**Crude OR (95% CI)**	**Adjusted[a] OR (95% CI)**	***Product p-value***
Workplace Organochlorine Exposure							
low exposure[b]	38/119	1.00	1.00	18/30	1.88	1.78 (0.80, 3.98)	-
frequent household pesticide use without workplace pesticide exposure	21/55	1.20	1.30 (0.69, 2.46)	7/22	1.00	0.95 (0.34, 2.67)	*0.16*
workplace exposure to other pesticides	46/82	1.76	1.84 (1.08, 3.14)	30/26	3.61	3.63 (1.73, 7.62)	*0.84*
ambient workplace OC exposure and/or self reported OC use	84/131	2.01	1.94 (1.20, 3.13)	38/49	2.43	2.33 (1.19, 4.55)	*0.38*
Workplace Organophosphorus Exposure							
low exposure[b]	38/119	1.00	1.00	18/30	1.88	1.97 (0.86, 4.51)	-
frequent household pesticide use without workplace pesticide exposure	21/55	1.20	1.60 (0.81, 3.13)	7/22	1.00	0.93 (0.33, 2.67)	*0.06*
workplace exposure to other pesticides	28/53	1.65	1.81 (0.96, 3.42)	10/13	2.41	2.07 (0.73, 5.84)	*0.39*
ambient workplace OP exposure and/or self reported OP use	102/160	2.00	2.08 (1.29, 3.37)	58/62	2.93	3.00 (1.58, 5.72)	*0.49*

[a]Adjusted for age(continuous), sex, county, smoking (never/former/current), and total in-person MMSE score (MMSE score ≤ 25/ MMSE score >25), and the variant TT genotype at rs2032582. Results for OP pesticides are additionally adjusted for *PON1* 55MM (at rs854560) variant genotype.

[b]Reference category subjects are unexposed to any ambient workplace pesticides (OPs, OCs, DTCs, & paraquat), did not use pesticides occupationally, are unexposed according to JEM score, and are never/rare users of household pesticides. They may have ambient residential pesticide exposures.

6 Conclusion and Public Health Implications

This dissertation examined the relation between Parkinson's disease and exposures to pesticides from multiple exposure sources, including household use, occupational use, and ambient pesticide exposures from drift in addition to genetic variation in *PON1* and *ABCB1* that may render individuals to be more susceptible to neurotoxic effects of pesticides.

We found evidence that household pesticide use, particularly of OP pesticides increases risk of PD. These findings are in accord with other prior findings for OP pesticides. In a clinic-based case control study in Texas, use of chlorpyrifos products was associated with a doubling in PD risk (OR = 2.0; 95% CI = 1.02-3.8) (93). A family-based case control study found ever use of organophosphorus pesticides increased PD risk by 89% (OR=1.89; 95% CI=1.11-3.25) (39), and an older German case control study found ever use of alkylated phosphates associated with an 80% increase in PD risk (OR=1.8; 95% CI=0.9-3.3) (94). We are the first to report these associations after adjusting for ambient pesticide exposures in agricultural areas with high levels of commercial pesticide applications. Our results are further supported by our finding that carriers of the *PON1* variants for slow metabolism of OPs are at much higher risk when using household OP pesticides. In addition, we found the same gene-environment interaction for household OP pesticide use that we saw for ambient exposures to agricultural OP pesticides in earlier PEG studies (46, 47), providing more support that PON1 slow metabolizer genotype status and OP pesticide exposures interact to increase PD risk.

Our findings for occupational pesticide use corroborate findings of increased risk of PD with use (26), and confirm prior findings for a link between increased risk of PD and exposure to

specific pesticides, such as carbamates, organochlorines, and organophosphorus pesticides. Our results may be due to differential recall bias. We also saw an increased risk of PD with use of personal protective equipment, which, though unexpected, may reflect a situation in which those with exposure to more toxic pesticides wore PPE. Understanding how occupational tasks and behaviors affect health is important to protect the health of agricultural workers. Further studies on use of personal protective equipment may have ramifications for agricultural worker protection standards.

As we would expect based on a majority of US households reporting use and storage of household pesticides (85-87, 176), a larger percentage of PEG study participants were frequent household pesticide users (45.1% of cases and 37.5% of controls) compared to occupational pesticide users (20.6% of cases and 13.8% of controls). We estimated similar sized odds ratios for both types of pesticide exposures, for which there are many possible explanations. For example, if we were better at assessing household pesticide use than occupational pesticide use, the misclassification of our occupational pesticide use variable could result in reduced OR estimates. When we improved upon our occupational pesticide exposure assessment using details on duration, those with longer durations of occupational pesticide use had larger risk of PD. This population has many different types of pesticide exposures, and few study participants are truly unexposed to pesticides. Thus, the reference groups of "never/rare household pesticide use" in the household pesticide analyses and "no occupational pesticide use" in the occupational pesticide use analyses include participants with other pesticide exposures, which could lead to similar estimates. In support of this explanation, we observed elevated OR estimates for ever occupational pesticide users when we excluded frequent household pesticide users and those

with ambient pesticide exposures at residences and workplaces from our reference group (Table 4-4).

We reported a replication of a prior finding (75) of *ABCB1* polymorphisms modifying PD risk from occupational organochlorine pesticide exposures. We also found that PD risk from occupational organophosphorus pesticide exposures is modified by *ABCB1* polymorphisms. These results provide further evidence that pesticides which are P-gp substrates or inhibitors may greatly increase PD risk. Our study suggests that *ABCB1* polymorphisms are possibly relevant in PD etiology for those who are ambiently exposed to pesticides at their workplaces in addition to those who actively use pesticides.

Though the etiology of PD is unknown, we can imagine possible causal mechanisms for PD. We can use the sufficient-component cause model (177) to represent possible sets of sufficient causes (see Figure 6-1). The moment that all of the components of a sufficient cause are present will be the beginning of PD onset for an individual. Increasing age is an undisputed risk factor for PD, and we add age in all sufficient causes for the disease. In addition, being male could be a component in some causal constellations. Genetic factors, such as autosomal dominant or recessive mutations could result in monogenic PD (2), represented by the causal pie in Figure 6-1a, or two single nucleotide polymorphisms together may result in PD in males, which could be represented by the pie in Figure 6-1b. Some PD cases may occur without the involvement of genetic risk factors, requiring the presence of environmental/exogenous risk factors (Figures 6-1c and 6-1d). Finally, other sufficient causes for PD may require both genetic and environmental risk factors to be present (Figures 6-1e and 6-1f).

Less than 5% of PD is currently explained by genes (55, 178), and we did not look at the public health role of genetic risk variants in our research. Instead, we examined genetic variants to identify possible mechanisms for the involvement of pesticides as PD risk factors. Gaining a better understanding of the mechanisms and pathways involved in PD etiology will aid the development of therapeutics and neuroprotective agents.

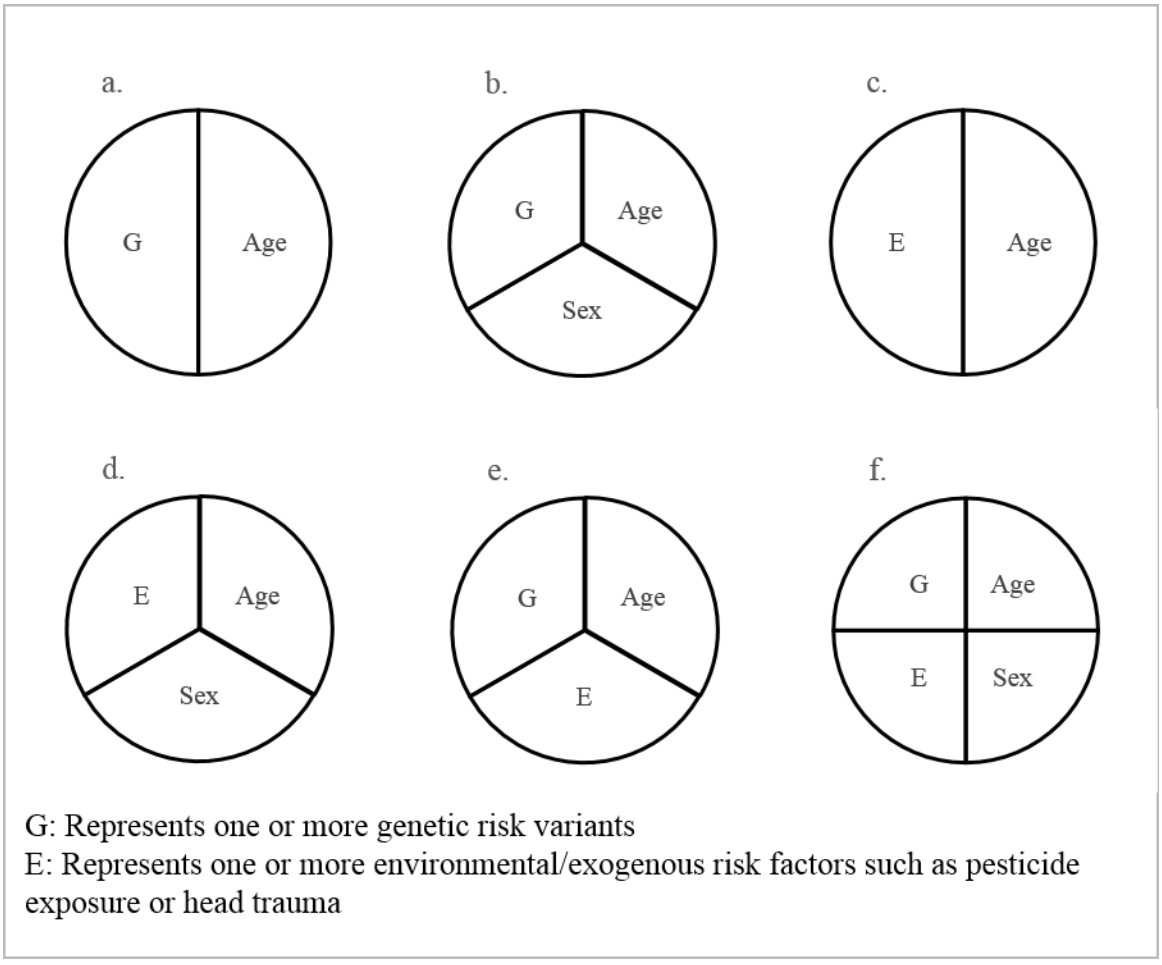

Figure 6-1: A representation of some possible sets of sufficient causes for Parkinson's

Studies in animals and cells have suggested that pesticides could be involved in PD etiology. What is now needed to guide epidemiologic studies of specific pesticides are more laboratory studies to identify mechanisms of neurotoxicity for dosages and modes of pesticide exposure that reflect the human experience. In addition, examining possible mechanisms of pesticide neurotoxicity in laboratory models more relevant to human physiology, such as human induced pluripotent stem cells, would be informative for epidemiologic investigations. Finally, better pesticide exposure assessment that accounts for multiple sources of pesticide exposure, such as dietary sources, household and occupational exposures, ambient exposures, and bystander exposures from job tasks that don't directly involve mixing, loading or applying pesticides, such as farm fieldwork, planting, or ploughing, is necessary for better understanding of possible risks from pesticides.

With projected worldwide increases in the population over the age of 65 and higher PD incidence rates in the elderly, the negative health and economic burdens of PD are bound to increase. One study projected that the number of PD cases over the age of 50 in the world's most populous countries is expected to double by 2030 to approximately 9 million cases (179). It is imperative that epidemiologic research efforts are directed towards understanding the etiology of this debilitating disease now in order to identify those at risk and prevent future PD cases.

Given the large number of epidemiologic studies that have suggested pesticides as a risk factor for PD and the widespread use of pesticides in agriculture and in households, further research into the role of pesticide exposures is necessary. If specific offending chemicals are identified through research, efforts can be made to implement legislation that bans the production, sale, and use of the particular pesticide.

7 Appendix

Table 7-1. Parkinson's Disease Association with Average Household Pesticide Use Frequency from Age 16 Years to Index Age in the Central Valley of California, ALL RACES.

		Cases n (%)	Controls n (%)	Crude OR	Adjusted OR* (95% CI)
Any Household Pesticide Usage					
	Never Use/Rare Use	206 (57.7)	498 (61.7)	1.00	1.00
	Frequent Use†	151 (42.3)	309 (38.3)	1.18	1.31 (1.00, 1.71)
Any Organophosphorus (OP) pesticide use‡					
	Never Use/Rare Use	206 (71.8)	498 (80.2)	1.00	1.00
	Frequent Use†	81 (28.2)	123 (19.8)	1.59	1.61 (1.14, 2.28)
Chemical classes within OP pesticides					
Organophosphate					
	Never Use/Rare Use	206 (77.2)	498 (83.7)	1.00	1.00
	Frequent Use†	61 (22.8)	97 (16.3)	1.52	1.55 (1.06, 2.28)
Organothiophosphate					
	Never Use/Rare Use	206 (86.2)	498 (92.2)	1.00	1.00
	Frequent Use†	33 (13.8)	42 (7.8)	1.90	1.89 (1.14, 3.14)
Individual Organothiophosphate pesticides					
chlorpyrifos					
	Never Use/Rare Use	206 (95.8)	498 (97.8)	1.00	1.00
	Frequent Use†	9 (4.2)	11 (2.2)	1.98	2.07 (0.82, 5.23)
diazinon					
	Never Use/Rare Use	206 (91.6)	498 (93.6)	1.00	1.00
	Frequent Use†	19 (8.4)	34 (6.4)	1.35	1.37 (0.75, 2.52)

*Adjusted for age (continuous), sex, smoking, race, PD family history and education.

† Subjects with an average frequency of use per year during ages 16-<index age that was at or above the 50th percentile in exposed controls were assigned to the "Frequent Use" category. Those in the "Never Use/Rare Use" category had an average frequency of use per year during ages 16-<index age that was below the 50th percentile for ANY PESTICIDE.

‡Subjects may be counted in multiple sub-categories of organophosphorus pesticide usage.

Table 7-2. Parkinson's Disease Association with Average Household Pesticide Use Frequency in Each of Four Age Periods in the Central Valley of California, ALL RACES.

	Cases n (%)	Controls n (%)	Crude OR	Adjusted OR* (95% CI)
YOUNG ADULT (16-<25 YEARS OF AGE) ; 357 case, 807 controls				
Any Household Pesticide Usage				
Never Use/Rare Use	281 (78.7)	656 (81.3)	1.00	1.00
Frequent Use†	76 (21.3)	151 (18.7)	1.18	1.39 (1.00, 1.94)
Any Organophosphorus (OP) pesticide use				
Never Use/Rare Use	281 (94)	656 (95.9)	1.00	1.00
Frequent Use†	18 (6.0)	28 (4.1)	1.50	1.54 (0.81, 2.92)
ADULT (25-<45 YEARS OF AGE); 357 case, 807 controls				
Any Household Pesticide Usage				
Never Use/Rare Use	201 (56.3)	501 (62.1)	1.00	1.00
Frequent Use†	156 (43.7)	306 (37.9)	1.27	1.40 (1.07, 1.82)
Any Organophosphorus (OP) pesticide use				
Never Use/Rare Use	201 (72.6)	501 (82.3)	1.00	1.00
Frequent Use†	76 (27.4)	108 (17.7)	1.75	1.77 (1.23, 2.55)
MIDDLE AGE (45-<65 YEARS OF AGE); 348 case, 767 controls				
Any Household Pesticide Usage				
Never Use/Rare Use	199 (57.2)	502 (65.5)	1.00	1.00
Frequent Use†	149 (42.8)	265 (34.6)	1.42	1.32 (1.01, 1.74)
Any Organophosphorus (OP) pesticide use				
Never Use/Rare Use	199 (72.1)	502 (81.4)	1.00	1.00
Frequent Use†	77 (27.9)	115 (18.6)	1.69	1.38 (0.96, 1.97)
SENIOR (≥ 65 YEARS OF AGE-<index age); 246 case, 457 controls				
Any Household Pesticide Usage				
Never Use/Rare Use	167 (67.9)	319 (69.8)	1.00	1.00
Frequent Use†	79 (32.1)	138 (30.2)	1.09	1.11 (0.77, 1.58)
Any Organophosphorus (OP) pesticide use				
Never Use/Rare Use	167 (81.5)	319 (83.5)	1.00	1.00
Frequent Use†	38 (18.5)	63 (16.5)	1.15	1.08 (0.66, 1.74)

*Adjusted for age (continuous), sex, smoking, race, PD family history and education.

†Subjects with a frequency of use per year during the age period that was at or above the 50th percentile in exposed controls were assigned to the "Frequent Use" category. Those in the "Never Use/Rare Use" category had an average frequency of use per year during the same age period that was below the 50th percentile for ANY PESTICIDE.

NOTE: *9 cases and 40 controls were younger than 45 years at index age; 111 cases and 350 controls were younger than 65 years at index age.*

Table 7-3. *ABCB1* SNP rs2032582 and *ABCB1* risk score Exposure to Workplace Organochlorine & Organophosphorus Pesticides in Association With Parkinson Disease, participants with European ancestry.

	ABCB1-rs2032582						
	GG+GT			TT			
	N case/ control	**Crude OR (95% CI)**	**Adjusted[a] OR (95% CI)**	**N case/ control**	**Crude OR (95% CI)**	**Adjusted[a] OR (95% CI)**	***Product p-value***
Workplace Organochlorine Exposure							
low exposure[b]	41/124	1.00	1.00	14/24	1.76	1.33 (0.56, 3.16)	-
frequent household pesticide use without workplace pesticide exposure	22/60	1.11	1.21 (0.65, 2.25)	6/18	1.01	0.76 (0.26, 2.24)	*0.26*
workplace exposure to other pesticides	54/89	1.84	1.82 (1.10, 3.01)	21/17	3.74	3.07 (1.32, 7.12)	*0.66*
ambient workplace OC exposure and/or self reported OC use	94/147	1.93	1.83 (1.16, 2.90)	26/32	2.46	1.85 (0.88, 3.91)	*0.57*
Workplace Organophosphorus Exposure							
low exposure[b]	41/124	1.00	1.00	14/24	1.76	1.51 (0.62, 3.68)	-
frequent household pesticide use without workplace pesticide exposure	22/60	1.11	1.45 (0.75, 2.77)	6/18	1.01	0.76 (0.25, 2.31)	*0.13*
workplace exposure to other pesticides	30/55	1.65	1.73 (0.94, 3.19)	8/9	2.69	1.60 (0.52, 4.88)	*0.48*
ambient workplace OP exposure and/or self reported OP use	118/181	1.97	1.96 (1.24, 3.10)	39/40	2.95	2.62 (1.26, 5.42)	*0.80*
	ABCB1 risk score[c]						
	≤1			2			
	N case/ control	**Crude OR (95% CI)**	**Adjusted[a] OR (95% CI)**	**N case/ control**	**Crude OR (95% CI)**	**Adjusted[a] OR (95% CI)**	***Product p-value***
Workplace Organochlorine Exposure							
low exposure[b]	41/126	1.00	1.00	14/22	1.96	1.99 (0.92, 4.28)	-
frequent household pesticide use without workplace pesticide exposure	24/59	1.25	1.34 (0.73, 2.46)	4/18	0.68	0.69 (0.22, 2.21)	*0.06*
workplace exposure to other pesticides	56/90	1.91	1.91 (1.16, 3.15)	19/15	3.89	4.29 (1.95, 9.45)	*0.83*
ambient workplace OC exposure and/or self reported OC use	95/151	1.93	1.85 (1.18, 2.92)	25/27	2.85	2.87 (1.47, 5.61)	*0.62*
Workplace Organophosphorus Exposure							
low exposure[b]	41/126	1.00	1.00	14/22	1.96	2.25 (1.01, 5.02)	-
frequent household pesticide use without workplace pesticide exposure	24/59	1.25	1.62 (0.86, 3.07)	4/18	0.68	0.68 (0.21, 2.20)	*0.02*
workplace exposure to other pesticides	30/57	1.62	1.69 (0.92, 3.10)	8/7	3.51	2.62 (0.87, 7.86)	*0.60*
ambient workplace OP exposure and/or self reported OP use	121/184	2.02	2.05 (1.30, 3.22)	36/35	3.16	3.71 (1.96, 7.02)	*0.66*

Table 7-3. *ABCB1* SNP rs2032582 and *ABCB1* risk score Exposure to Workplace Organochlorine & Organophosphorus Pesticides in Association With Parkinson Disease, participants with European ancestry.

[a]Adjusted for age(continuous), sex, county, smoking (never/former/current), and total in-person MMSE score (MMSE score ≤ 25/ MMSE score >25). Results for OP pesticides are additionally adjusted for *PON1* 55MM (at rs854560) variant genotypes. Analyses for rs2032582 are additionally adjusted for the variant TT genotype at rs1045642.

[b]Reference category subjects are unexposed to any ambient workplace pesticides (OPs, OCs, DTCs, & paraquat), did not use pesticides occupationally, are unexposed according to JEM score,and are never/rare users of household pesticides. They may have ambient residential pesticide exposures.

[c]We assigned a score of 1 for each homozygous variant genotype at each SNP and a score of 0 otherwise. We then summed scores for the two SNPs to obtain the final ABCB1 risk score (1=homozygous variant genotype at one of the ABCB1 SNPs, 2=homozygous variant genotype at both SNPs).

Table 7-4. Characteristics of Study Population, all races (n=1212).

	Cases (N=356) No.(%)	Controls (N=856) No.(%)
Sex (male)	205 (57.6)	411 (48.0)
Age[a]		
mean +/- SD	68.3 +/- 10.2	66.2 +/- 11.6
range	34-88	35-99
≤60 years	74 (20.8)	264 (30.8)
>60 years	282 (79.2)	592 (69.2)
Cigarette smoking		
Never	186 (52.2)	404 (47.2)
Former	151 (42.4)	348 (40.7)
Current	19 (5.3)	104 (12.1)
Race		
White	286 (80.3)	580 (67.8)
Black	3 (0.8)	26 (3.0)
Latino	47 (13.2)	181 (21.1)
Asian	4 (1.1)	24 (2.8)
Native American	16 (4.5)	43 (5.0)
Unspecified	0	2 (0.2)
County		
Fresno	162 (45.5)	350 (40.9)
Kern	126 (35.4)	349 (40.8)
Tulare	68 (19.1)	157 (18.3)
Education		
0-<12 years	66 (18.5)	146 (17.1)
12 years	95 (26.7)	180 (21.0)
>12 years	195 (54.8)	530 (61.9)
First-degree relative with PD		
No	303 (85.1)	791 (92.4)
Yes	53 (14.9)	65 (7.6)
***ABCB1* rs2032582 genotype**		
GG	117 (32.9)	292 (34.1)
GT	157 (44.1)	386 (45.1)
TT	76 (21.3)	149 (17.4)
***ABCB1* rs1045642 genotype**		
CC	82 (23.0)	217 (25.4)
CT	162 (45.5)	438 (51.2)
TT	111 (31.2)	188 (22.0)

[a] This is the age at diagnosis for cases and age at interview for controls.

[b]Genotyping failed for 14 and 35 subjects, respectively, for rs1045642 and rs2032582.

Table 7-5. Parkinson Disease associations with *ABCB1* rs2032582 (n=1177), *ABCB1* rs1045642 (n=1198), and ABCB1 risk score (n=1173), all races included.

	Cases No. (%)	Controls No. (%)	Unadjusted OR	Adjusted[a] OR (95% CI)
ABCB1-rs2032582				
GG	117 (33.4)	292 (35.3)	1.00	1.00
GT	157 (44.9)	386 (46.7)	1.02	0.96 (0.71, 1.29)
TT	76 (21.7)	149 (18.0)	1.27	1.34 (0.92, 1.93)
ABCB1-rs2032582				
GG+GT	274 (78.3)	678 (82)	1.00	1.00
TT	76 (21.7)	149 (18.0)	1.26	1.37 (0.99, 1.90)
ABCB1- rs1045642				
CC	82 (23.1)	217 (25.7)	1.00	1.00
CT	162 (45.6)	438 (52)	0.98	0.85 (0.61, 1.18)
TT	111 (31.3)	188 (22.3)	1.56	1.42 (0.98, 2.05)
ABCB1- rs1045642				
CC+CT	244 (68.7)	655 (77.7)	1.00	1.00
TT	111 (31.3)	188 (22.3)	1.59	1.59 (1.18, 2.12)
ABCB1 risk score[b]				
0	235 (67.1)	617 (75.0)	1.00	1.00
1	44 (12.6)	79 (9.6)	1.46	1.41 (0.93, 2.14)
2	71 (20.3)	127 (15.4)	1.47	1.56 (1.10, 2.21)
p-Value for trend				*0.0062*

[a]Adjusted for age(continuous),sex, county, smoking (never/former/current), total in-person MMSE score (MMSE score ≤ 25/ MMSE score >25), race (white/non-white).

[b]We assigned a score of 1 for each homozygous variant genotype at each SNP and a score of 0 otherwise. We then summed scores for the two SNPs to obtain the final ABCB1 risk score (1=homozygous variant genotype at one of the ABCB1 SNPs, 2=homozygous variant genotype at both SNPs).

Table 7-6. PD associations with workplace exposure to Organochlorine and Organophosphorus pesticides (n=1088), all races.

Exposure	Cases No. (%)	Controls No. (%)	Unadjusted OR	Adjusted OR[a] (95% CI)
Workplace Organochlorine Exposure				
low exposure[b]	61 (17.3)	198 (26.9)	1.00	1.00
frequent household pesticide use without workplace pesticide exposure	34 (9.7)	104 (14.1)	1.06	1.13 (0.69, 1.84)
workplace exposure to other pesticides	94 (26.7)	168 (22.8)	1.82	1.85 (1.24, 2.75)
ambient workplace OC exposure and/or self reported OC use[c]	163 (46.3)	266 (36.1)	1.99	1.93 (1.34, 2.78)
Workplace Organophosphorus Exposure				
low exposure[b]	61 (17.3)	198 (26.9)	1.00	1.00
frequent household pesticide use without workplace pesticide exposure	34 (9.7)	104 (14.1)	1.06	1.13 (0.69, 1.85)
workplace exposure to other pesticides	48 (13.6)	91 (12.4)	1.71	1.57 (0.98, 2.51)
ambient workplace OP exposure and/or self reported OP use[c]	209 (59.4)	343 (46.6)	1.98	2.00 (1.41, 2.85)

[a]Adjusted for age(continuous), sex, county, smoking (never/former/current), total in-person MMSE score (MMSE score ≤ 25/ MMSE score >25), and race (white/non-white).

[b] Reference category subjects are unexposed to any ambient workplace pesticides (OPs, OCs, DTCs, & paraquat), did not use pesticides occupationally, are unexposed according to JEM score,and are never/rare users of household pesticides.

[c]Several cases (n=142) and controls (n=222) are occupationally exposed to both OC and OP pesticides.

Table 7-7. ABCB1 polymorphisms and Exposure to Workplace Organochlorine & Organophosphorus Pesticides in Association With Parkinson Disease, all races.

	ABCB1-rs2032582						
	GG+GT			TT			
	N case/ control	**Crude OR (95% CI)**	**Adjusted[a] OR (95% CI)**	**N case/ control**	**Crude OR (95% CI)**	**Adjusted[a] OR (95% CI)**	***Product p-value***
Workplace Organochlorine Exposure							
low exposure[b]	46/161	1.00	1.00	14/29	1.69	1.12 (0.50, 2.51)	-
frequent household pesticide use without workplace pesticide exposure	26/81	1.12	1.23 (0.70, 2.17)	8/23	1.22	0.86 (0.33, 2.22)	*0.43*
workplace exposure to other pesticides	69/131	1.84	1.83 (1.16, 2.89)	23/26	3.10	2.32 (1.09, 4.93)	*0.80*
ambient workplace OC exposure and/or self reported OC use	131/216	2.12	2.05 (1.36, 3.10)	29/44	2.31	1.53 (0.77, 3.02)	*0.37*
Workplace Organophosphorus Exposure							
low exposure[b]	46/161	1.00	1.00	14/29	1.69	1.10 (0.47, 2.58)	-
frequent household pesticide use without workplace pesticide exposure	26/81	1.12	1.28 (0.70, 2.31)	8/23	1.22	0.85 (0.31, 2.32)	*0.42*
workplace exposure to other pesticides	38/72	1.85	1.84 (1.04, 3.26)	9/15	2.10	1.49 (0.52, 4.22)	*0.63*
ambient workplace OP exposure and/or self reported OP use	162/275	2.06	2.14 (1.40, 3.26)	43/55	2.74	2.06 (1.04, 4.06)	*0.77*
	ABCB1-rs1045642						
	CC+CT			TT			
	N case/ control	**Crude OR (95% CI)**	**Adjusted[a] OR (95% CI)**	**N case/ control**	**Crude OR (95% CI)**	**Adjusted[a] OR (95% CI)**	***Product p-value***
Workplace Organochlorine Exposure							
low exposure[b]	42/162	1.00	1.00	19/34	2.16	2.25 (1.06, 4.76)	-
frequent household pesticide use without workplace pesticide exposure	25/75	1.29	1.38 (0.77, 2.46)	9/28	1.24	1.42 (0.58, 3.50)	*0.17*
workplace exposure to other pesticides	61/130	1.81	1.88 (1.17, 3.04)	33/34	3.74	4.11 (2.07, 8.15)	*0.95*
ambient workplace OC exposure and/or self reported OC use	115/198	2.24	2.18 (1.42. 3.35)	47/64	2.83	2.93 (1.62, 5.31)	*0.21*
Workplace Organophosphorus Exposure							
low exposure[b]	42/162	1.00	1.00	19/34	2.16	2.18 (1.00, 4.78)	-
frequent household pesticide use without workplace pesticide exposure	25/75	1.29	1.45 (0.78, 2.67)	9/28	1.24	1.34 (0.51, 3.48)	*0.15*
workplace exposure to other pesticides	37/72	1.98	1.93 (1.07, 3.47)	11/17	2.50	2.78 (1.02, 7.55)	*0.49*
ambient workplace OP exposure and/or self reported OP use	139/256	2.09	2.23 (1.43, 3.49)	69/81	3.29	3.73 (2.04, 6.81)	*0.52*

Table 7-7. ABCB1 polymorphisms and Exposure to Workplace Organochlorine & Organophosphorus Pesticides in Association With Parkinson Disease, all races.

	ABCB1 risk score[c]						
	≤1			2			
	N case/ control	**Crude OR (95% CI)**	**Adjusted[a] OR (95% CI)**	**N case/ control**	**Crude OR (95% CI)**	**Adjusted[a] OR (95% CI)**	***Product p-value***
Workplace Organochlorine Exposure							
low exposure[b]	46/165	1.00	1.00	14/25	2.01	1.92 (0.91, 4.03)	-
frequent household pesticide use without workplace pesticide exposure	28/82	1.22	1.34 (0.77, 2.32)	6/21	1.02	1.06 (0.40, 2.84)	*0.17*
workplace exposure to other pesticides	71/134	1.90	1.90 (1.21, 2.99)	21/22	3.42	3.85 (1.89, 7.84)	*0.92*
ambient workplace OC exposure and/or self reported OC use	132/221	2.14	2.11 (1.40, 3.16)	28/37	2.71	2.64 (1.44, 4.85)	*0.36*
Workplace Organophosphorus Exposure							
low exposure[b]	46/165	1.00	1.00	14/25	2.01	1.98 (0.91, 4.32)	-
frequent household pesticide use without workplace pesticide exposure	28/82	1.22	1.42 (0.79, 2.55)	6/21	1.02	0.98 (0.35, 2.71)	*0.12*
workplace exposure to other pesticides	38/76	1.79	1.78 (1.01, 3.12)	9/11	2.93	3.09 (1.07, 8.95)	*0.85*
ambient workplace OP exposure and/or self reported OP use	165/279	2.12	2.24 (1.47, 3.41)	40/48	2.99	3.43 (1.89, 6.24)	*0.59*

[a]Adjusted for age(continuous), sex, county, smoking (never/former/current), total in-person MMSE score (MMSE score ≤ 25/ MMSE score >25), and race (white/non-white). Results for OP pesticides are additionally adjusted for *PON1* 55MM (at rs854560) variant genotypes. Analyses for rs2032582 are additionally adjusted for variant TT genotype at rs1045642, and analyses for rs1045642 are additionally adjusted for variant TT genotype at rs2032582.

[b]Reference category subjects are unexposed to any ambient workplace pesticides (OPs, OCs, DTCs, & paraquat), did not use pesticides occupationally, are unexposed according to JEM score,and are never/rare users of household pesticides. They may have ambient residential pesticide exposures.

[c]We assigned a score of 1 for each homozygous variant genotype at each SNP and a score of 0 otherwise. We then summed scores for the two SNPs to obtain the final ABCB1 risk score (1=homozygous variant genotype at one of the ABCB1 SNPs, 2=homozygous variant genotype at both SNPs).

Table 7-8. Review of studies examining the association between PD, *ABCB1* polymorphisms, & pesticides.

Study	Ancestry	*ABCB1* polymorphisms examined	Cases	Controls	Findings
Kiyohara et al. 2013	Asian (Japanese)	**rs1045642(c.3435C/T)**	**238**	**368**	Results suggested a slightly elevated risk of PD among those subjects with the CT or TT genotypes compared to those with the CC genotype (OR=1.33, 95% CI=0.93-1.90). Authors did not detect an association with occupational pesticide use and PD, home pesticide use and PD, and did not detect an interaction between occupational pesticide use or home pesticide use and the polymorphism. Never smokers with a CT or TT genotype had 4.01 times the risk of PD compared to smokers with the CC genotype (95% CI=2.05-7.83). Ever drinkers with at least one T allele had 1.83 times the risk of PD compared to never drinkers with the CC genotype (95% CI 1.07-3.15).
Dutheil et al. 2010	European (French)	**rs2032582(c.2677G/T/A) & rs1045642(c.3435C/T)**	**101**	**234**	The two *ABCB1* polymorphisms were not found to be associated with PD. Those men occupationally exposed to organochlorine pesticides who were homozygous variant carriers for either SNP were at the highest risk for PD. Case only analyses identified associations between rs2032582 and organochlorine exposure (OR=5.4, 95% CI=1.1-27.5) as well as cumulative hours of pesticide exposure. Case only analyses identified associations between rs1045642 and organochlorine exposure (OR=4.1, 95% CI= 1.0-17.0).
Zschiedrich et al. 2009	European (German and Serbian)	**rs2032582 (c.2677G/T/A) , rs1045642 (c.3435C/T),** & rs55852620 (c.3320A/C)	**86**	**307**	No association found between PD and any of the three *ABCB1* polymorphisms. Found an association between pesticide exposure and the polymorphism at rs1045642 in German cases.
Droździk et al. 2003	European (Polish)	**rs1045642 (c.3435C/T)**	**107**	**103**	No association found between the polymorphism and PD. Found more 3435CC homozygotes among cases not exposed to pesticides. Found more heterozygotes among cases exposed to pesticides.

Table 7-9. Review of studies examining the association between PD and *ABCB1* polymorphisms.

Study	Ancestry	*ABCB1* polymorphisms examined	Cases	Controls	Findings
Westerlund et al. 2009	European (Sweden)	**rs2032582 (c.2677G/T/A) , rs1045642 (c.3435C/T)**, rs1128503 (c.1236T/C)	**288**	**313**	No association of rs2032582 or rs1045642 with PD found. rs1128503 genotype was associated with PD. 1236C–2677G haplotype associated with PD, & evidence suggesting the 1236C–2677G–3435C haplotype associated with PD.
Funke et al. 2009	European (German)	**rs2032582 (c.2677G/T/A) , rs1045642 (c.3435C/T)**, rs28401798 (c.3151C/G), rs28381804 (c.49T/C), rs1202183 (c.131T/C), rs9282565 (c.239C/A), rs2229109 (c.1199G/A), rs1128503 (c.1236T/C), rs28381902 (c.1696G/A), & rs28381914 (c.1777C/T)	**300**	**302**	Did not find an association between PD and any of the ten listed *ABCB1* polymorphisms.
Mizuta et al. 2008 *	Asian (Japanase)	rs2235035	**858**	**917**	Analyses suggest association of rs2235035 T allele with PD OR=1.15, 95%CI: 1.00,1.32;
Mizuta et al. 2006*	Asian (Japanase)	rs2235048,rs1202169 (-41A/G)	**190**	**190**	Analyses suggest an association of rs1202169 G allele with PD (OR=1.28, 95%CI:0.96,1.72).
Tan et al. 2005	Asian (Chinese)	**rs2032582 (c.2677G/T/A) , rs1045642 (c.3435C/T),** rs1202169 (-41A/G), rs34976462 (c.-145C/G), rs3213619 (c.-129T/C), rs1128503 (c.1236T/C), and rs3842 (4036A/G).	**185**	**206**	No evidence of an association between any of the SNPs and PD. Found evidence of a protective association of 2677G-3435C haplotype and 2677T-3435T haplotype with PD. The two haplotypes 2677T-3435C and 2677G-3435T were found to increase PD risk.
Tan et al. 2004	European (Polish)	**rs2032582 (c.2677G/T/A) , rs1045642 (c.3435C/T)**, rs3213619 (c.-129T/C), rs1202169 (-41A/G), rs34976462 (c.-145C/G), rs1128503 (c.1236T/C), rs3842 (4036A/G)	**158**	**139**	No finding between individual SNPs and PD. Association between 2677G-3435C haplotype and not having PD (OR=0.25, 95%CI: 0.06-1.08).

Table 7-9. Review of studies examining the association between PD and *ABCB1* polymorphisms.

Lee et al. 2004	Asian (Chinese)	**rs2032582 (c.2677G/T/A) , rs1045642 (c.3435C/T)**, rs1202169 (-41A/G), rs1128503 (c.1236T/C), rs3842 (4036A/G), rs34976462 (c.-145C/G), rs3213619 (c.-129T/C)	**206**	**224**	SNPs at rs2032582, rs1045642, & rs1128503 were found to be associated with PD. Evidence suggesting an association of several haplotypes with PD including protective association of the 3435T-2677T haplotype with PD. Associations of PD with rs2032582, rs1045642, and several haplotypes were found in men & in those at or over the age of 60 at index date.
Momose et al. 2002 &Toda et al. 2003*	Asian (Japanese)	**rs1045642 (c.3435C/T)**	**232**	**249**	Authors did not find evidence of an association of the SNP with PD.
Furuno et al. 2002	European (Italian)	**rs2032582 (c.2677G/T/A) , rs1045642 (c.3435C/T)**, & rs3213619 (c.-129T/C)	**95**	**106**	No finding for PD. 3435TT genotype and 2677T allele were associated with early onset PD (≤ 45 years of age at onset). Confirmed rs2032582 and rs1045642 in LD.

***Studies Overlap & data and results for tested associations obtained from PDGene database (180).**

REFERENCES

1. Twelves D, Perkins KS, Counsell C. Systematic review of incidence studies of Parkinson's disease. Mov Disord. 2003 Jan;18(1):19-31.

2. Wirdefeldt K, Adami HO, Cole P, Trichopoulos D, Mandel J. Epidemiology and etiology of Parkinson's disease: a review of the evidence. Eur J Epidemiol. 2011 Jun;26 Suppl 1:S1-58.

3. de Lau LM, Breteler MM. Epidemiology of Parkinson's disease. Lancet Neurol. 2006 Jun;5(6):525-35.

4. Huse DM, Schulman K, Orsini L, Castelli-Haley J, Kennedy S, Lenhart G. Burden of illness in Parkinson's disease. Mov Disord. 2005 Nov;20(11):1449-54.

5. Wooten GF, Currie LJ, Bovbjerg VE, Lee JK, Patrie J. Are men at greater risk for Parkinson's disease than women? J Neurol Neurosurg Psychiatry. 2004 Apr;75(4):637-9.

6. Hernan MA, Takkouche B, Caamano-Isorna F, Gestal-Otero JJ. A meta-analysis of coffee drinking, cigarette smoking, and the risk of Parkinson's disease. Ann Neurol. 2002 Sep;52(3):276-84.

7. Ritz B, Ascherio A, Checkoway H, Marder KS, Nelson LM, Rocca WA, et al. Pooled analysis of tobacco use and risk of Parkinson disease. Arch Neurol. 2007 Jul;64(7):990-7.

8. Ritz B, Rhodes SL. After half a century of research on smoking and PD, where do we go now? Neurology. 2010 Mar 16;74(11):870-1.

9. Thacker EL, Ascherio A. Familial aggregation of Parkinson's disease: a meta-analysis. Mov Disord. 2008 Jun 15;23(8):1174-83.

10. Ross CA, Smith WW. Gene-environment interactions in Parkinson's disease. Parkinsonism Relat Disord. 2007;13 Suppl 3:S309-15.

11. Di Monte DA, Lavasani M, Manning-Bog AB. Environmental Factors in Parkinson’s Disease. Neurotoxicology. 2002;23(4–5):487-502.

12. Pezzoli G, Cereda E. Exposure to pesticides or solvents and risk of Parkinson disease. Neurology. 2013 May 28;80(22):2035-41.

13. Jankovic J. Parkinson's disease: clinical features and diagnosis. J Neurol Neurosurg Psychiatry. 2008 Apr;79(4):368-76.

14. Langston JW, Ballard P, Tetrud JW, Irwin I. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. Science. 1983 Feb 25;219(4587):979-80.

15. Richardson JR, Quan Y, Sherer TB, Greenamyre JT, Miller GW. Paraquat neurotoxicity is distinct from that of MPTP and rotenone. Toxicol Sci. 2005 Nov;88(1):193-201.

16. Brown TP, Rumsby PC, Capleton AC, Rushton L, Levy LS. Pesticides and Parkinson's disease--is there a link? Environ Health Perspect. 2006 Feb;114(2):156-64.

17. Testa CM, Sherer TB, Greenamyre JT. Rotenone induces oxidative stress and dopaminergic neuron damage in organotypic substantia nigra cultures. Brain Res Mol Brain Res. 2005 Mar 24;134(1):109-18.

18. Chou AP, Li S, Fitzmaurice AG, Bronstein JM. Mechanisms of rotenone-induced proteasome inhibition. Neurotoxicology. 2010 Aug;31(4):367-72.

19. Zhang J, Fitsanakis VA, Gu G, Jing D, Ao M, Amarnath V, et al. Manganese ethylene-bis-dithiocarbamate and selective dopaminergic neurodegeneration in rat: a link through mitochondrial dysfunction. J Neurochem. 2003 Jan;84(2):336-46.

20. Kitazawa M, Anantharam V, Kanthasamy AG. Dieldrin-induced oxidative stress and neurochemical changes contribute to apoptopic cell death in dopaminergic cells. Free Radic Biol Med. 2001 Dec 1;31(11):1473-85.

21. Kirby ML, Barlow RL, Bloomquist JR. Neurotoxicity of the organochlorine insecticide heptachlor to murine striatal dopaminergic pathways. Toxicol Sci. 2001 May;61(1):100-6.

22. Karen DJ, Li W, Harp PR, Gillette JS, Bloomquist JR. Striatal dopaminergic pathways as a target for the insecticides permethrin and chlorpyrifos. Neurotoxicology. 2001 Dec;22(6):811-7.

23. Fitzmaurice AG, Rhodes SL, Lulla A, Murphy NP, Lam HA, O'Donnell KC, et al. Aldehyde dehydrogenase inhibition as a pathogenic mechanism in Parkinson disease. Proc Natl Acad Sci U S A. 2013 Jan 8;110(2):636-41.

24. Fitzmaurice AG, Rhodes SL, Cockburn M, Ritz B, Bronstein JM. Aldehyde dehydrogenase variation enhances effect of pesticides associated with Parkinson disease. Neurology. 2014 Feb 4;82(5):419-26.

25. Rhodes SL, Fitzmaurice AG, Cockburn M, Bronstein JM, Sinsheimer JS, Ritz B. Pesticides that inhibit the ubiquitin-proteasome system: effect measure modification by genetic variation in SKP1 in Parkinsons disease. Environ Res. 2013 Oct;126:1-8.

26. van der Mark M, Brouwer M, Kromhout H, Nijssen P, Huss A, Vermeulen R. Is pesticide use related to Parkinson disease? Some clues to heterogeneity in study results. Environ Health Perspect. 2012 Mar;120(3):340-7.

27. Nuti A, Ceravolo R, Dell'Agnello G, Gambaccini G, Bellini G, Kiferle L, et al. Environmental factors and Parkinson's disease: a case-control study in the Tuscany region of Italy. Parkinsonism Relat Disord. 2004 Dec;10(8):481-5.

28. Chan DK, Woo J, Ho SC, Pang CP, Law LK, Ng PW, et al. Genetic and environmental risk factors for Parkinson's disease in a Chinese population. J Neurol Neurosurg Psychiatry. 1998 Nov;65(5):781-4.

29. Wong GF, Gray CS, Hassanein RS, Koller WC. Environmental risk factors in siblings with Parkinson's disease. Arch Neurol. 1991 Mar;48(3):287-9.

30. Petrovitch H, Ross GW, Abbott RD, Sanderson WT, Sharp DS, Tanner CM, et al. Plantation work and risk of Parkinson disease in a population-based longitudinal study. Arch Neurol. 2002 Nov;59(11):1787-92.

31. Werneck AL, Alvarenga H. Genetics, drugs and environmental factors in Parkinson's disease. A case-control study. Arq Neuropsiquiatr. 1999 Jun;57(2B):347-55.

32. Koller W, Vetere-Overfield B, Gray C, Alexander C, Chin T, Dolezal J, et al. Environmental risk factors in Parkinson's disease. Neurology. 1990 Aug;40(8):1218-21.

33. Petersen MS, Halling J, Bech S, Wermuth L, Weihe P, Nielsen F, et al. Impact of dietary exposure to food contaminants on the risk of Parkinson's disease. Neurotoxicology. 2008 Jul;29(4):584-90.

34. Firestone JA, Lundin JI, Powers KM, Smith-Weller T, Franklin GM, Swanson PD, et al. Occupational factors and risk of Parkinson's disease: A population-based case-control study. Am J Ind Med. 2010 Mar;53(3):217-23.

35. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ. 2003 Sep 6;327(7414):557-60.

36. Wastensson G, Hagberg S, Andersson E, Johnels B, Barregard L. Parkinson's disease in diphenyl-exposed workers--a causal association? Parkinsonism Relat Disord. 2006 Jan;12(1):29-34.

37. Elbaz A, Clavel J, Rathouz PJ, Moisan F, Galanaud JP, Delemotte B, et al. Professional exposure to pesticides and Parkinson disease. Ann Neurol. 2009 Oct;66(4):494-504.

38. Weisskopf MG, Knekt P, O'Reilly EJ, Lyytinen J, Reunanen A, Laden F, et al. Persistent organochlorine pesticides in serum and risk of Parkinson disease. Neurology. 2010 Mar 30;74(13):1055-61.

39. Hancock DB, Martin ER, Mayhew GM, Stajich JM, Jewett R, Stacy MA, et al. Pesticide exposure and risk of Parkinson's disease: a family-based case-control study. BMC Neurol. 2008;8:6.

40. Kamel F, Tanner C, Umbach D, Hoppin J, Alavanja M, Blair A, et al. Pesticide exposure and self-reported Parkinson's disease in the agricultural health study. Am J Epidemiol. 2007 Feb 15;165(4):364-74.

41. Tanner CM, Kamel F, Ross GW, Hoppin JA, Goldman SM, Korell M, et al. Rotenone, paraquat, and Parkinson's disease. Environ Health Perspect. 2011 Jun;119(6):866-72.

42. Costello S, Cockburn M, Bronstein J, Zhang X, Ritz B. Parkinson's disease and residential exposure to maneb and paraquat from agricultural applications in the central valley of California. Am J Epidemiol. 2009 Apr 15;169(8):919-26.

43. Wang A, Costello S, Cockburn M, Zhang X, Bronstein J, Ritz B. Parkinson's disease risk from ambient exposure to pesticides. Eur J Epidemiol. 2011 Jul;26(7):547-55.

44. Ritz BR, Manthripragada AD, Costello S, Lincoln SJ, Farrer MJ, Cockburn M, et al. Dopamine transporter genetic variants and pesticides in Parkinson's disease. Environ Health Perspect. 2009 Jun;117(6):964-9.

45. Lee PC, Bordelon Y, Bronstein J, Ritz B. Traumatic brain injury, paraquat exposure, and their relationship to Parkinson disease. Neurology. 2012 Nov 13;79(20):2061-6.

46. Manthripragada AD, Costello S, Cockburn MG, Bronstein JM, Ritz B. Paraoxonase 1, agricultural organophosphate exposure, and Parkinson disease. Epidemiology. 2010 Jan;21(1):87-94.

47. Lee PC, Rhodes SL, Sinsheimer JS, Bronstein J, Ritz B. Functional paraoxonase 1 variants modify the risk of Parkinson's disease due to organophosphate exposure. Environ Int. 2013 Jun;56:42-7.

48. Gatto NM, Cockburn M, Bronstein J, Manthripragada AD, Ritz B. Well-water consumption and Parkinson's disease in rural California. Environ Health Perspect. 2009 Dec;117(12):1912-8.

49. Wang A, Cockburn M, Ly TT, Bronstein JM, Ritz B. The association between ambient exposure to organophosphates and Parkinson's disease risk. Occup Environ Med. 2014 Apr;71(4):275-81.

50. Kuopio AM, Marttila RJ, Helenius H, Rinne UK. Environmental risk factors in Parkinson's disease. Mov Disord. 1999 Nov;14(6):928-39.

51. Tomenson JA, Campbell C. Mortality from Parkinson's disease and other causes among a workforce manufacturing paraquat: a retrospective cohort study. BMJ Open. 2011 Jan 1;1(2):e000283.

52. Hertzman C, Wiens M, Snow B, Kelly S, Calne D. A case-control study of Parkinson's disease in a horticultural region of British Columbia. Mov Disord. 1994 Jan;9(1):69-75.

53. Firestone JA, Smith-Weller T, Franklin G, Swanson P, Longstreth WT, Jr., Checkoway H. Pesticides and risk of Parkinson disease: a population-based case-control study. Arch Neurol. 2005 Jan;62(1):91-5.

54. Gao HM, Hong JS. Gene-environment interactions: key to unraveling the mystery of Parkinson's disease. Prog Neurobiol. 2011 Jun;94(1):1-19.

55. Klein C, Schlossmacher MG. The genetics of Parkinson disease: Implications for neurological care. Nat Clin Pract Neurol. 2006 Mar;2(3):136-46.

56. Vance JM, Ali S, Bradley WG, Singer C, Di Monte DA. Gene-environment interactions in Parkinson's disease and other forms of parkinsonism. Neurotoxicology. 2010 Sep;31(5):598-602.

57. Costa LG, Furlong CE. Paraoxonase 1: Structure, Function, and Polymorphisms. Anticholinesterase Pesticides: John Wiley & Sons, Inc.; 2011. p. 85-95.

58. Mackness B, Mackness MI, Arrol S, Turkie W, Durrington PN. Effect of the molecular polymorphisms of human paraoxonase (PON1) on the rate of hydrolysis of paraoxon. Br J Pharmacol. 1997 Sep;122(2):265-8.

59. Li WF, Costa LG, Richter RJ, Hagen T, Shih DM, Tward A, et al. Catalytic efficiency determines the in-vivo efficacy of PON1 for detoxifying organophosphorus compounds. Pharmacogenetics. 2000 Dec;10(9):767-79.

60. Garin MC, James RW, Dussoix P, Blanche H, Passa P, Froguel P, et al. Paraoxonase polymorphism Met-Leu54 is associated with modified serum concentrations of the enzyme. A possible link between the paraoxonase gene and increased risk of cardiovascular disease in diabetes. J Clin Invest. 1997 Jan 1;99(1):62-6.

61. Leviev I, James RW. Promoter polymorphisms of human paraoxonase PON1 gene and serum paraoxonase activities and concentrations. Arterioscler Thromb Vasc Biol. 2000 Feb;20(2):516-21.

62. O'Leary KA, Edwards RJ, Town MM, Boobis AR. Genetic and other sources of variation in the activity of serum paraoxonase/diazoxonase in humans: consequences for risk from exposure to diazinon. Pharmacogenet Genomics. 2005 Jan;15(1):51-60.

63. Kondo I, Yamamoto M. Genetic polymorphism of paraoxonase 1 (PON1) and susceptibility to Parkinson's disease. Brain Res. 1998 Sep 28;806(2):271-3.

64. Akhmedova S, Anisimov S, Yakimovsky A, Schwartz E. Gln --> Arg 191 polymorphism of paraoxonase and Parkinson's disease. Hum Hered. 1999 Jun;49(3):178-80.

65. Taylor MC, Le Couteur DG, Mellick GD, Board PG. Paraoxonase polymorphisms, pesticide exposure and Parkinson's disease in a Caucasian population. J Neural Transm. 2000;107(8-9):979-83.

66. Wang J, Liu Z. No association between paraoxonase 1 (PON1) gene polymorphisms and susceptibility to Parkinson's disease in a Chinese population. Mov Disord. 2000 Nov;15(6):1265-7.

67. Akhmedova SN, Yakimovsky AK, Schwartz EI. Paraoxonase 1 Met--Leu 54 polymorphism is associated with Parkinson's disease. J Neurol Sci. 2001 Mar 1;184(2):179-82.

68. Carmine A, Buervenich S, Sydow O, Anvret M, Olson L. Further evidence for an association of the paraoxonase 1 (PON1) Met-54 allele with Parkinson's disease. Mov Disord. 2002 Jul;17(4):764-6.

69. Kelada SN, Costa-Mallen P, Checkoway H, Viernes HA, Farin FM, Smith-Weller T, et al. Paraoxonase 1 promoter and coding region polymorphisms in Parkinson's disease. J Neurol Neurosurg Psychiatry. 2003 Apr;74(4):546-7.

70. Liu YL, Yang J, Zheng J, Liu DW, Liu T, Wang JM, et al. Paraoxonase 1 polymorphisms L55M and Q192R were not risk factors for Parkinson's disease: a HuGE review and meta-analysis. Gene. 2012 Jun 15;501(2):188-92.

71. Mahringer A, Ott M, Reimold I, Reichel V, Fricker G. The ABC of the blood-brain barrier - regulation of drug efflux pumps. Curr Pharm Des. 2011;17(26):2762-70.

72. Marzolini C, Paus E, Buclin T, Kim RB. Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. Clin Pharmacol Ther. 2004 Jan;75(1):13-33.

73. Drozdzik M, Bialecka M, Mysliwiec K, Honczarenko K, Stankiewicz J, Sych Z. Polymorphism in the P-glycoprotein drug transporter MDR1 gene: a possible link between environmental and genetic factors in Parkinson's disease. Pharmacogenetics. 2003 May;13(5):259-63.

74. Zschiedrich K, Konig IR, Bruggemann N, Kock N, Kasten M, Leenders KL, et al. MDR1 variants and risk of Parkinson disease. Association with pesticide exposure? J Neurol. 2009 Jan;256(1):115-20.

75. Dutheil F, Beaune P, Tzourio C, Loriot MA, Elbaz A. Interaction between ABCB1 and professional exposure to organochlorine insecticides in Parkinson disease. Arch Neurol. 2010 Jun;67(6):739-45.

76. Salama NN, Yang Z, Bui T, Ho RJ. MDR1 haplotypes significantly minimize intracellular uptake and transcellular P-gp substrate transport in recombinant LLC-PK1 cells. J Pharm Sci. 2006 Oct;95(10):2293-308.

77. Kimchi-Sarfaty C, Oh JM, Kim IW, Sauna ZE, Calcagno AM, Ambudkar SV, et al. A "silent" polymorphism in the MDR1 gene changes substrate specificity. Science. 2007 Jan 26;315(5811):525-8.

78. Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmoller J, Johne A, et al. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. Proc Natl Acad Sci U S A. 2000 Mar 28;97(7):3473-8.

79. Chaturvedi S, Ostbye T, Stoessl AJ, Merskey H, Hachinski V. Environmental exposures in elderly Canadians with Parkinson's disease. Can J Neurol Sci. 1995 Aug;22(3):232-4.

80. Kang GA, Bronstein JM, Masterman DL, Redelings M, Crum JA, Ritz B. Clinical characteristics in early Parkinson's disease in a central California population-based study. Mov Disord. 2005 Sep;20(9):1133-42.

81. Ritz B, Rhodes SL, Bordelon Y, Bronstein J. alpha-Synuclein genetic variants predict faster motor symptom progression in idiopathic Parkinson disease. PLoS One. 2012;7(5):e36199.

82. Liew Z, Wang A, Bronstein J, Ritz B. Job Exposure Matrix (JEM) derived estimates of life-time occupational pesticide exposure and the risk of Parkinson's Disease. Archives of Environmental & Occupational Health. 2013:null-null.

83. Freire C, Koifman S. Pesticide exposure and Parkinson's disease: Epidemiological evidence of association. Neurotoxicology. 2012 May 22.

84. Van Maele-Fabry G, Hoet P, Vilain F, Lison D. Occupational exposure to pesticides and Parkinson's disease: A systematic review and meta-analysis of cohort studies. Environ Int. 2012 Oct;46:30-43.

85. Armes MN, Liew Z, Wang A, Wu X, Bennett DH, Hertz-Picciotto I, et al. Residential pesticide usage in older adults residing in Central California. Int J Environ Res Public Health. 2011 Aug;8(8):3114-33.

86. Savage EP, Keefe TJ, Wheeler HW, Mounce L, Helwic L, Applehans F, et al. Household pesticide usage in the United States. Arch Environ Health. 1981 Nov-Dec;36(6):304-9.

87. Stout DM, 2nd, Bradham KD, Egeghy PP, Jones PA, Croghan CW, Ashley PA, et al. American Healthy Homes Survey: a national study of residential pesticides measured from floor wipes. Environ Sci Technol. 2009 Jun 15;43(12):4294-300.

88. Gunier RB, Ward MH, Airola M, Bell EM, Colt J, Nishioka M, et al. Determinants of agricultural pesticide concentrations in carpet dust. Environ Health Perspect. 2011 Jul;119(7):970-6.

89. Gurunathan S, Robson M, Freeman N, Buckley B, Roy A, Meyer R, et al. Accumulation of chlorpyrifos on residential surfaces and toys accessible to children. Environ Health Perspect. 1998 Jan;106(1):9-16.

90. Obendorf SK, Lemley AT, Hedge A, Kline AA, Tan K, Dokuchayeva T. Distribution of pesticide residues within homes in central New York State. Arch Environ Contam Toxicol. 2006 Jan;50(1):31-44.

91. Stone DL, Sudakin DL, Jenkins JJ. Longitudinal trends in organophosphate incidents reported to the National Pesticide Information Center, 1995-2007. Environ Health. 2009;8:18.

92. Williams MK, Rundle A, Holmes D, Reyes M, Hoepner LA, Barr DB, et al. Changes in pest infestation levels, self-reported pesticide use, and permethrin exposure during pregnancy after the 2000-2001 U.S. Environmental Protection Agency restriction of organophosphates. Environ Health Perspect. 2008 Dec;116(12):1681-8.

93. Dhillon AS, Tarbutton GL, Levin JL, Plotkin GM, Lowry LK, Nalbone JT, et al. Pesticide/environmental exposures and Parkinson's disease in East Texas. J Agromedicine. 2008;13(1):37-48.

94. Seidler A, Hellenbrand W, Robra BP, Vieregge P, Nischan P, Joerg J, et al. Possible environmental, occupational, and other etiologic factors for Parkinson's disease: A case-control study in Germany. Neurology. 1996 May;46(5):1275-84.

95. Costa LG, Furlong CE. Paraoxonase 1: Structure, Function, and Polymorphisms. In: RC STaG, editor. Anticholinesterase Pesticides: Metabolism, Neurotoxicity, and Epidemiology. Hoboken, N.J.: John Wiley & Sons, Inc.; 2010. p. 85-95.

96. Jacob EL, Gatto NM, Thompson A, Bordelon Y, Ritz B. Occurrence of depression and anxiety prior to Parkinson's disease. Parkinsonism Relat Disord. 2010 Nov;16(9):576-81.

97. Hughes AJ, Ben-Shlomo Y, Daniel SE, Lees AJ. What features improve the accuracy of clinical diagnosis in Parkinson's disease: a clinicopathologic study. 1992. Neurology. 2001 Nov;57(10 Suppl 3):S34-8.

98. California Department of Pesticide Regulation. 2010. California Department of Pesticide Regulation Product/Label Database. 2010; Available from: http://www.cdpr.ca.gov/docs/label/prodnam.htm.

99. Pesticide Action Network. Pesticide Action Network PAN Pesticide Database - Chemicals. 2000 [updated June, 2014; cited 2010, 2012]; Available from: www.pesticideinfo.org/Search_Chemicals.jsp.

100. Siderowf A, Lang AE. Premotor Parkinson's disease: concepts and definitions. Mov Disord. 2012 Apr 15;27(5):608-16.

101. Barr DB, Wong LY, Bravo R, Weerasekera G, Odetokun M, Restrepo P, et al. Urinary concentrations of dialkylphosphate metabolites of organophosphorus pesticides: National Health and Nutrition Examination Survey 1999-2004. Int J Environ Res Public Health. 2011 Aug;8(8):3063-98.

102. California Department of Pesticide Regulation. Pesticide Use Reporting - 2008 Summary Data. 2008 [cited 2012 August 1]; Available from: http://www.cdpr.ca.gov/docs/pur/pur08rep/08_pur.htm.

103. Agency USEP. Reregistration Eligibility Decision (RED) for Chlorpyrifos. EPA 738-R-01-007. In: Office of Prevention P, and Toxic Substances, editor. Washington, DC: U.S. Environmental Protection Agency; 2006b.

104. Agency. USEP. Reregistration Eligibility Decision (RED) for Bensulide. EPA 738-R-00-002. In: Office of Prevention P, and Toxic Substances, editor. Washington, DC: U.S. Environmental Protection Agency; 2006a.

105. Eaton DL, Daroff RB, Autrup H, Bridges J, Buffler P, Costa LG, et al. Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. Crit Rev Toxicol. 2008;38 Suppl 2:1-125.

106. Huen K, Bradman A, Harley K, Yousefi P, Boyd Barr D, Eskenazi B, et al. Organophosphate pesticide levels in blood and urine of women and newborns living in an agricultural community. Environ Res. 2012 Jun 8;117:8-16.

107. Quiros-Alcala L, Bradman A, Nishioka M, Harnly ME, Hubbard A, McKone TE, et al. Pesticides in house dust from urban and farmworker households in California: an observational measurement study. Environ Health. 2011;10:19.

108. Eddins D, Cerutti D, Williams P, Linney E, Levin ED. Zebrafish provide a sensitive model of persisting neurobehavioral effects of developmental chlorpyrifos exposure: Comparison with nicotine and pilocarpine effects and relationship to dopamine deficits. Neurotoxicol Teratol. 2010 Jan-Feb;32(1):99-108.

109. Kaur P, Radotra B, Minz RW, Gill KD. Impaired mitochondrial energy metabolism and neuronal apoptotic cell death after chronic dichlorvos (OP) exposure in rat brain. Neurotoxicology. 2007 Nov;28(6):1208-19.

110. Slotkin TA, Levin ED, Seidler FJ. Comparative developmental neurotoxicity of organophosphate insecticides: effects on brain development are separable from systemic toxicity. Environ Health Perspect. 2006 May;114(5):746-51.

111. Cole TB, Walter BJ, Shih DM, Tward AD, Lusis AJ, Timchalk C, et al. Toxicity of chlorpyrifos and chlorpyrifos oxon in a transgenic mouse model of the human paraoxonase (PON1) Q192R polymorphism. Pharmacogenet Genomics. 2005 Aug;15(8):589-98.

112. Jansen KL, Cole TB, Park SS, Furlong CE, Costa LG. Paraoxonase 1 (PON1) modulates the toxicity of mixed organophosphorus compounds. Toxicol Appl Pharmacol. 2009 Apr 15;236(2):142-53.

113. Richter RJ, Jarvik GP, Furlong CE. Determination of paraoxonase 1 status without the use of toxic organophosphate substrates. Circ Cardiovasc Genet. 2008 Dec;1(2):147-52.

114. Baldi I, Cantagrel A, Lebailly P, Tison F, Dubroca B, Chrysostome V, et al. Association between Parkinson's disease and exposure to pesticides in southwestern France. Neuroepidemiology. 2003 Sep-Oct;22(5):305-10.

115. Feldman AL, Johansson AL, Nise G, Gatz M, Pedersen NL, Wirdefeldt K. Occupational exposure in parkinsonian disorders: a 43-year prospective cohort study in men. Parkinsonism Relat Disord. 2011 Nov;17(9):677-82.

116. Baldi I, Lebailly P, Mohammed-Brahim B, Letenneur L, Dartigues JF, Brochard P. Neurodegenerative diseases and exposure to pesticides in the elderly. Am J Epidemiol. 2003 Mar 1;157(5):409-14.

117. Dick FD, De Palma G, Ahmadi A, Osborne A, Scott NW, Prescott GJ, et al. Gene-environment interactions in parkinsonism and Parkinson's disease: the Geoparkinson study. Occup Environ Med. 2007 Oct;64(10):673-80.

118. Gelb DJ, Oliver E, Gilman S. Diagnostic criteria for Parkinson disease. Arch Neurol. 1999 Jan;56(1):33-9.

119. Hughes AJ, Ben-Shlomo Y, Daniel SE, Lees AJ. What features improve the accuracy of clinical diagnosis in Parkinson's disease: a clinicopathologic study. Neurology. 1992 Jun;42(6):1142-6.

120. Langston JW, Widner H, Goetz CG, Brooks D, Fahn S, Freeman T, et al. Core assessment program for intracerebral transplantations (CAPIT). Mov Disord. 1992;7(1):2-13.

121. Newkirk LA, Kim JM, Thompson JM, Tinklenberg JR, Yesavage JA, Taylor JL. Validation of a 26-point telephone version of the Mini-Mental State Examination. J Geriatr Psychiatry Neurol. 2004 Jun;17(2):81-7.

122. Wood A. Compendium of Pesticide Common Names. 1995-2014 [updated 2014; cited 2012 December 12]; Available from: http://www.alanwood.net/pesticides/.

123. Goldberg DW, Wilson JP, Knoblock CA, Ritz B, Cockburn MG. An effective and efficient approach for manually improving geocoded data. Int J Health Geogr. 2008;7:60.

124. Rull RP, Ritz B. Historical pesticide exposure in California using pesticide use reports and land-use surveys: an assessment of misclassification error and bias. Environ Health Perspect. 2003 Oct;111(13):1582-9.

125. Narayan S, Liew Z, Paul K, Lee PC, Sinsheimer JS, Bronstein JM, et al. Household organophosphorus pesticide use and Parkinson's disease. Int J Epidemiol. 2013 Oct;42(5):1476-85.

126. Rutz R, Krieger RI. Exposure to pesticide mixer/loaders and applicators in California. Rev Environ Contam Toxicol. 1992;129:121-39.

127. Sherer TB, Betarbet R, Greenamyre JT. Environment, mitochondria, and Parkinson's disease. Neuroscientist. 2002 Jun;8(3):192-7.

128. Peng J, Mao XO, Stevenson FF, Hsu M, Andersen JK. The herbicide paraquat induces dopaminergic nigral apoptosis through sustained activation of the JNK pathway. J Biol Chem. 2004 Jul 30;279(31):32626-32.

129. Castello PR, Drechsel DA, Patel M. Mitochondria are a major source of paraquat-induced reactive oxygen species production in the brain. J Biol Chem. 2007 May 11;282(19):14186-93.

130. Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nat Neurosci. 2000 Dec;3(12):1301-6.

131. Uversky VN, Li J, Bower K, Fink AL. Synergistic effects of pesticides and metals on the fibrillation of alpha-synuclein: implications for Parkinson's disease. Neurotoxicology. 2002 Oct;23(4-5):527-36.

132. Manning-Bog AB, McCormack AL, Li J, Uversky VN, Fink AL, Di Monte DA. The herbicide paraquat causes up-regulation and aggregation of alpha-synuclein in mice: paraquat and alpha-synuclein. J Biol Chem. 2002 Jan 18;277(3):1641-4.

133. Wang XF, Li S, Chou AP, Bronstein JM. Inhibitory effects of pesticides on proteasome activity: implication in Parkinson's disease. Neurobiol Dis. 2006 Jul;23(1):198-205.

134. Ascherio A, Chen H, Weisskopf MG, O'Reilly E, McCullough ML, Calle EE, et al. Pesticide exposure and risk for Parkinson's disease. Ann Neurol. 2006 Aug;60(2):197-203.

135. Hofmann J, Guardado J, Keifer M, Wesseling C. Mortality among a cohort of banana plantation workers in Costa Rica. Int J Occup Environ Health. 2006 Oct-Dec;12(4):321-8.

136. Kenborg L, Lassen CF, Lander F, Olsen JH. Parkinson's disease among gardeners exposed to pesticides--a Danish cohort study. Scand J Work Environ Health. 2012 Jan;38(1):65-9.

137. Tuchsen F, Jensen AA. Agricultural work and the risk of Parkinson's disease in Denmark, 1981-1993. Scand J Work Environ Health. 2000 Aug;26(4):359-62.

138. Li X, Sundquist J, Sundquist K. Socioeconomic and occupational groups and Parkinson's disease: a nationwide study based on hospitalizations in Sweden. Int Arch Occup Environ Health. 2009 Jan;82(2):235-41.

139. Semchuk KM, Love EJ, Lee RG. Parkinson's disease and exposure to agricultural work and pesticide chemicals. Neurology. 1992 Jul;42(7):1328-35.

140. McCann SJ, LeCouteur DG, Green AC, Brayne C, Johnson AG, Chan D, et al. The epidemiology of Parkinson's disease in an Australian population. Neuroepidemiology. 1998;17(6):310-7.

141. Fall PA, Fredrikson M, Axelson O, Granerus AK. Nutritional and occupational factors influencing the risk of Parkinson's disease: a case-control study in southeastern Sweden. Mov Disord. 1999 Jan;14(1):28-37.

142. Baldereschi M, Di Carlo A, Vanni P, Ghetti A, Carbonin P, Amaducci L, et al. Lifestyle-related risk factors for Parkinson's disease: a population-based study. Acta Neurol Scand. 2003 Oct;108(4):239-44.

143. Fong CS, Wu RM, Shieh JC, Chao YT, Fu YP, Kuao CL, et al. Pesticide exposure on southwestern Taiwanese with MnSOD and NQO1 polymorphisms is associated with increased risk of Parkinson's disease. Clin Chim Acta. 2007 Mar;378(1-2):136-41.

144. Gorell JM, Johnson CC, Rybicki BA, Peterson EL, Richardson RJ. The risk of Parkinson's disease with exposure to pesticides, farming, well water, and rural living. Neurology. 1998 May;50(5):1346-50.

145. Dauer W, Przedborski S. Parkinson's disease: mechanisms and models. Neuron. 2003 Sep 11;39(6):889-909.

146. Escuder-Gilabert L, Villanueva-Camanas RM, Sagrado S, Medina-Hernandez MJ. Permeability and toxicological profile estimation of organochlorine compounds by biopartitioning micellar chromatography. Biomed Chromatogr. 2009 Apr;23(4):382-9.

147. Yang J, Aschner M. Developmental Aspects of Blood–Brain Barrier (BBB) and Rat Brain Endothelial (RBE4) Cells as in vitro Model for Studies on Chlorpyrifos Transport. Neurotoxicology. 2003;24(4–5):741-5.

148. Schinkel AH, Smit JJ, van Tellingen O, Beijnen JH, Wagenaar E, van Deemter L, et al. Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. Cell. 1994 May 20;77(4):491-502.

149. Lankas GR, Cartwright ME, Umbenhauer D. P-glycoprotein deficiency in a subpopulation of CF-1 mice enhances avermectin-induced neurotoxicity. Toxicol Appl Pharmacol. 1997 Apr;143(2):357-65.

150. Bain LJ, LeBlanc GA. Interaction of structurally diverse pesticides with the human MDR1 gene product P-glycoprotein. Toxicol Appl Pharmacol. 1996 Nov;141(1):288-98.

151. Bain LJ, McLachlan JB, LeBlanc GA. Structure-activity relationships for xenobiotic transport substrates and inhibitory ligands of P-glycoprotein. Environ Health Perspect. 1997 Aug;105(8):812-8.

152. Agarwala S, Chen W, Cook TJ. Effect of chlorpyrifos on efflux transporter gene expression and function in Caco-2 cells. Toxicol In Vitro. 2004 Aug;18(4):403-9.

153. Shabbir A, DiStasio S, Zhao J, Cardozo CP, Wolff MS, Caplan AJ. Differential effects of the organochlorine pesticide DDT and its metabolite p,p'-DDE on p-glycoprotein activity and expression. Toxicol Appl Pharmacol. 2005 Mar 1;203(2):91-8.

154. Lecoeur S, Videmann B, Mazallon M. Effect of organophosphate pesticide diazinon on expression and activity of intestinal P-glycoprotein. Toxicol Lett. 2006 Mar 1;161(3):200-9.

155. Sreeramulu K, Liu R, Sharom FJ. Interaction of insecticides with mammalian P-glycoprotein and their effect on its transport function. Biochim Biophys Acta. 2007 Jul;1768(7):1750-7.

156. Database of Single Nucleotide Polymorphisms (dbSNP). Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine. (dbSNP Build ID: {137}). Available from: http://www.ncbi.nlm.nih.gov/SNP/ [database on the Internet].

157. Cascorbi I, Gerloff T, Johne A, Meisel C, Hoffmeyer S, Schwab M, et al. Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1 gene in white subjects. Clin Pharmacol Ther. 2001 Mar;69(3):169-74.

158. Hitzl M, Schaeffeler E, Hocher B, Slowinski T, Halle H, Eichelbaum M, et al. Variable expression of P-glycoprotein in the human placenta and its association with mutations of the multidrug resistance 1 gene (MDR1, ABCB1). Pharmacogenetics. 2004 May;14(5):309-18.

159. Wang D, Johnson AD, Papp AC, Kroetz DL, Sadee W. Multidrug resistance polypeptide 1 (MDR1, ABCB1) variant 3435C>T affects mRNA stability. Pharmacogenet Genomics. 2005 Oct;15(10):693-704.

160. Hamajima N, Yuasa H, Matsuo K, Kurobe Y. Detection of gene-environment interaction by case-only studies. Jpn J Clin Oncol. 1999 Oct;29(10):490-3.

161. Gauderman W, Morrison J. QUANTO 1.1: A computer program for power and sample size calculations for genetic-epidemiology studies, http://hydra.usc.edu/gxe. 2006.

162. Gauderman W. Sample size requirements for association studies of gene-gene interaction. Am J Epidemiol. 2002;155:478-84.

163. Rees DC, Johnson E, Lewinson O. ABC transporters: the power to change. Nat Rev Mol Cell Biol. 2009 Mar;10(3):218-27.

164. Cordon-Cardo C, O'Brien JP, Casals D, Rittman-Grauer L, Biedler JL, Melamed MR, et al. Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. Proc Natl Acad Sci U S A. 1989 Jan;86(2):695-8.

165. Bendayan R, Ronaldson PT, Gingras D, Bendayan M. In situ localization of P-glycoprotein (ABCB1) in human and rat brain. J Histochem Cytochem. 2006 Oct;54(10):1159-67.

166. Graff CL, Pollack GM. Functional evidence for P-glycoprotein at the nose-brain barrier. Pharm Res. 2005 Jan;22(1):86-93.

167. Bartels AL. Blood-brain barrier P-glycoprotein function in neurodegenerative disease. Curr Pharm Des. 2011;17(26):2771-7.

168. Furuno T, Landi MT, Ceroni M, Caporaso N, Bernucci I, Nappi G, et al. Expression polymorphism of the blood-brain barrier component P-glycoprotein (MDR1) in relation to Parkinson's disease. Pharmacogenetics. 2002 Oct;12(7):529-34.

169. Tan EK, Drozdzik M, Bialecka M, Honczarenko K, Klodowska-Duda G, Teo YY, et al. Analysis of MDR1 haplotypes in Parkinson's disease in a white population. Neurosci Lett. 2004 Dec 6;372(3):240-4.

170. Funke C, Soehn AS, Tomiuk J, Riess O, Berg D. Genetic analysis of coding SNPs in blood-brain barrier transporter MDR1 in European Parkinson's disease patients. J Neural Transm. 2009 Apr;116(4):443-50.

171. Westerlund M, Belin AC, Anvret A, Hakansson A, Nissbrandt H, Lind C, et al. Association of a polymorphism in the ABCB1 gene with Parkinson's disease. Parkinsonism Relat Disord. 2009 Jul;15(6):422-4.

172. Nalls MA, Pankratz N, Lill CM, Do CB, Hernandez DG, Saad M, et al. Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. Nat Genet. 2014 Sep;46(9):989-93.

173. Piegorsch WW, Weinberg CR, Taylor JA. Non-hierarchical logistic models and case-only designs for assessing susceptibility in population-based case-control studies. Stat Med. 1994 Jan 30;13(2):153-62.

174. Kiyohara C, Miyake Y, Koyanagi M, Fujimoto T, Shirasawa S, Tanaka K, et al. MDR1 C3435T polymorphism and interaction with environmental factors in risk of Parkinson's disease: a case-control study in Japan. Drug Metab Pharmacokinet. 2013;28(2):138-43.

175. Sugihara N, Toyama K, Okamoto T, Kadowaki M, Terao K, Furuno K. Effects of benzo(e)pyrene and benzo(a)pyrene on P-glycoprotein-mediated transport in Caco-2 cell monolayer: a comparative approach. Toxicol In Vitro. 2007 Aug;21(5):827-34.

176. Adgate JL, Kukowski A, Stroebel C, Shubat PJ, Morrell S, Quackenboss JJ, et al. Pesticide storage and use patterns in Minnesota households with children. J Expo Anal Environ Epidemiol. 2000 Mar-Apr;10(2):159-67.

177. Rothman KJ. Causes. Am J Epidemiol. 1976 Dec;104(6):587-92.

178. Berardelli A, Wenning GK, Antonini A, Berg D, Bloem BR, Bonifati V, et al. EFNS/MDS-ES/ENS [corrected] recommendations for the diagnosis of Parkinson's disease. Eur J Neurol. 2013 Jan;20(1):16-34.

179. Dorsey ER, Constantinescu R, Thompson JP, Biglan KM, Holloway RG, Kieburtz K, et al. Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. Neurology. 2007 Jan 30;68(5):384-6.

180. Lill CM, Roehr JT, McQueen MB, Kavvoura FK, Bagade S, Schjeide BM, et al. Comprehensive research synopsis and systematic meta-analyses in Parkinson's disease genetics: The PDGene database. PLoS Genet. 2012;8(3):e1002548.