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

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Abstract

Background—Studies suggested that variants in the *ABCB1* gene encoding P-glycoprotein, a xenobiotic transporter, may increase susceptibility to pesticide exposures linked to Parkinson's Disease (PD) risk.

Objectives—To investigate the joint impact of two *ABCB1* polymorphisms and pesticide exposures on PD risk.

Methods—In a population-based case control study, we genotyped *ABCB1* gene variants at rs1045642 (c.3435C/T) and rs2032582 (c.2677G/T/A) and assessed occupational exposures to organochlorine (OC) and organophosphorus (OP) pesticides based on self-reported occupational use and record-based ambient workplace exposures for 282 PD cases and 514 controls of European ancestry. We identified active ingredients in self-reported occupational use pesticides from a California database and estimated ambient workplace exposures between 1974 and 1999 employing a geographic information system together with records for state pesticide and land use. With unconditional logistic regression, we estimated marginal and joint contributions for occupational pesticide exposures and *ABCB1* variants in PD.

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Results—For occupationally exposed carriers of homozygous *ABCB1* variant genotypes, we estimated odds ratios of 1.89 [95% confidence interval (CI): (0.87, 4.07)] to 3.71 [95% CI: (1.96, 7.02)], with the highest odds ratios estimated for occupationally exposed carriers of homozygous *ABCB1* variant genotypes at both SNPs; but we found no multiplicative scale interactions.

Conclusions—This study lends support to a previous report that commonly used pesticides, specifically OCs and OPs, and variant *ABCB1* genotypes at two polymorphic sites jointly increase risk of PD.

Keywords

Pesticide; Epidemiology; Parkinson's Disease; multidrug resistance protein 1 gene (*ABCB1*); P-glycoprotein

1. INTRODUCTION

Parkinson's disease (PD), the second most common neurodegenerative disease, has a multifactorial etiology. There is extensive evidence that pesticide exposures increase risk of PD (van der Mark et al., 2012), and the risk attributable to these exposures may be modified by regulators of xenobiotic uptake and distribution across organ systems. P-glycoprotein (P-gp), encoded by the *ABCB1* gene, is a major player for the efflux of xenobiotics across the blood brain barrier (BBB) (Mahringer et al., 2011), and genetic variants may increase PD risk among pesticide exposed individuals, with a few studies suggesting gene-pesticide interactions (Dro dzik et al., 2003; Dutheil et al., 2010; Zschiedrich et al., 2009).

Certain lipophilic pesticides, such as organochlorines (OCs) can cross the blood brain barrier, and there is some evidence that the organophosphorus pesticide (OP) chlorpyrifos does as well (Corrigan et al., 2000; Escuder-Gilabert et al., 2009; Fleming et al., 1994; Parran et al., 2005). 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 (Schinkel et al., 1994). 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 (Bain et al., 1997; Lecoœur et al., 2006; Sreeramulu et al., 2007).

The *ABCB1* gene is highly polymorphic with thousands of putative single nucleotide polymorphisms; 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 (Cascorbi et al., 2001). These polymorphisms have been shown to affect P-gp function in a substrate dependent fashion (Salama et al., 2006). The mutation at rs1045642 possibly alters substrate specificity by affecting the timing of co-translational folding (Kimchi-Sarfaty et al., 2007); additionally, the homozygous TT genotype has been associated with lower P-gp expression levels (Hitzl et al., 2004), possibly through a reduction of mRNA stability (Wang et al., 2005). P-gp expression has also been lower for carriers of the TT genotype at rs2032582 (Hitzl et al., 2004).

A case-control study of French farmers reported that rs1045642 TT genotype carriers exhibited the highest PD risk when exposed to organochlorine pesticides compared with unexposed C allele carriers [odds ratio (OR) =7.2, 95% CI: (2.1, 24.8)] (Dutheil et al., 2010). Similarly for rs2032582, exposed farmers with a TT or TA genotype exhibited 7.9 times the risk of developing PD compared with unexposed G-allele carriers [95% CI: (2.2, 28.9)]. Multiplicative interaction terms were statistically significant for both case-control and case-only analyses, and a multiplicative interaction was also observed in case-only analyses for cumulative lifetime hours of organochlorine exposure and rs2032582.

We previously reported that ambient exposures at residences and workplaces to the OCs dieldrin and endosulfan (Fitzmaurice et al., 2014; Rhodes et al., 2013), to OPs (Lee et al., 2013; Wang et al., 2014), as well as consumption of well water possibly contaminated with OPs (Gatto et al., 2009) and frequent household use of OPs (Narayan et al., 2013) increase PD risk. Several other epidemiologic and toxicologic studies have implicated OCs and OPs in PD (Brown et al., 2006), and pesticides in these chemical classes impact P-gp function. Here, we examine the influence of two *ABCB1* polymorphisms and occupational OC and OP pesticide exposures on PD risk in a Californian rural population to replicate and expand on the prior epidemiologic findings reported for French farmers (Dutheil et al., 2010).

2. MATERIALS AND 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.

2.1 Study Subject Recruitment

We conducted a population-based case-control study of Parkinson's disease, recruiting participants 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 (Kang et al., 2005) and subject recruitment (Costello et al., 2009; Wang et al., 2011).

Through local neurologists, medical groups, and public service announcements, we identified 1167 PD patients, of whom 604 were ineligible (397 diagnosed >3 years before contact, 134 did not live in target counties, 51 without 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 (Hughes et al., 1992), 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 controls from tax assessor records for residences randomly. We enrolled controls using two approaches. We first mailed letters to selected residential units and enrolled controls by mail and phone. In a second expanded approach, we recruited controls from randomly selected 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 (Costello et al., 2009; Liew et al., 2014).

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 away prior to interview; we recruited the 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 350 cases and 724 controls of all races, and 282 cases and 514 controls of European ancestry, we have available both *ABCB1* genotype and pesticide exposure information to assess effect measure modifications of PD risk from occupational OC and OP pesticides by the two functional variants of the gene.

2.2 Data Collection

Our trained staff conducted telephone interviews to collect data on demographic characteristics, lifestyle behaviors, 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) reported (1) work with an exposure 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 additional interviews targeting specific occupational exposures, 62 (21.4%) cases and 162 (26.2%) controls refused these.

Our UCLA movement disorder specialists confirmed idiopathic PD in all cases based on published criteria (Hughes et al., 1992), 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 (Newkirk et al., 2004).

2.3 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 (Liew et al., 2014). We assigned weights representing the intensity of probable pesticide exposure, multiplied by years in the job, and calculated lifetime cumulative exposure by summing over all jobs. We consider those with a lifetime cumulative exposure score above the 75th percentile of exposed controls to be highly exposed to pesticides from farming jobs.

Using our detailed occupational interview, we collected occupational pesticide use information for fungicides, herbicides, insecticides, and other pesticides, eliciting names of pesticide products 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 (California Department of Pesticide Regulation, 2012) to identify the main active ingredients (based on product weight) of self-reported pesticide products, comparing product names and purposes of use with CDPR information (names, purposes, use types (e.g. fungicides, herbicides, insecticides), and product registration dates) on products sold in the California market during years of reported 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 in products used prior to 1970 for which no data were available 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 (Pesticide Action Network, 2012) and the Compendium of Pesticide Common Names (Wood, 2012) to determine chemical classes (e.g. organophosphorus, organochlorine) of main active ingredients.

We distinguished ever/never use of each main active ingredient and ever/never use of any occupational pesticide or 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, tribufos, mevinphos, phorate, chlorpyrifos, dimethoate, acephate, disulfoton, naled, methamidophos, ethion, bensulide).

We considered participants who screened negative for 'ever regular work with fertilizers or pesticides' never users of occupational pesticides when we did not have occupational interview data. Only one case and two controls screened positive but refused the extended occupational interview and were among seven cases and 13 controls who provided insufficient information for occupational use; thus, these 20 participants were considered to have missing values for occupational pesticide use.

2.4 Ambient Exposures to Pesticides

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 a 500 meter buffer around each address, as described elsewhere (Goldberg et al., 2008; Rull and Ritz, 2003). 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 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 (Wang et al., 2014).

2.5 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) (Narayan et al., 2013). 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 classified as ‘never/infrequent users’.

2.6 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 failed genotyping) or interaction analyses. We tested for and did not detect departures from Hardy-Weinberg equilibrium in controls.

2.7 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 according to 1) self-report for occupational use; 2) ambient 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) household pesticides (never/infrequent use). Thus, these reference group subjects may have still been exposed at low levels due to active occupational use or ambiently at workplaces or residences. Similar to the French farmer study (Dutheil et al., 2010) that created a separate category for gardening/home pesticide use only, we separated out frequent household pesticide users who were otherwise considered low/un-exposed to occupational sources of pesticides. Unlike the French study, our ‘occupationally OC exposed’ and ‘occupationally OP exposed’ groups included both self-reported occupational pesticide use and also high ambient exposures at workplace addresses. We also created a separate category for workplace exposure to other pesticides based on our JEM, ambient workplace exposures, and self-reported occupational use. All exposures had to have occurred prior to the index time (year of diagnosis for cases and year of interview for controls).

Using unconditional logistic regression to calculate odds ratios (ORs) and 95% confidence intervals (CIs), we estimated marginal associations for occupational OC and OP pesticides.

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 (a recessive model) as done previously (Dutheil et al., 2010) in interaction analyses. We also created an *ABCB1* risk score for 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). In addition, we present marginal associations for each *ABCB1* polymorphism by genotype.

We examined *ABCB1* polymorphisms and occupational exposures, using subjects without the risk genotype and with ‘low exposure’ as the reference group. We conducted gene-environment interaction analyses separately for rs1045642, rs2032582, and employing a ‘double recessive’ genetic model based on risk scores and added product terms to assess interactions on a multiplicative scale. We confirmed 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 with case-control analyses (Hamajima et al., 1999).

To address concerns about population stratification, we conducted analyses restricted to European ancestry participants; in sensitivity analyses we included all subjects and adjusted for race (white/nonwhite). We adjusted all analyses for age at index date (continuous, 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 $\leq$ 25/ MMSE score $>$ 25). We mutually adjusted our interaction analyses for each *ABCB1* polymorphism. Multicollinearity is unlikely to cause a problem, because the LD between the SNPs is not strong ($r^2 = 0.53$). We previously found that the *PON1* 55MM (at rs854560) variant genotype modifies PD risk from OP pesticide exposures (Lee et al., 2013), and thus, we adjusted all OP pesticide analyses for *PON1* slow metabolizer genotype status. We also adjust all OC pesticide analyses for *PON1* genotype, since many participants were occupationally exposed to both OC and OP pesticides. In separate sensitivity analyses, we adjusted for ambient residential exposures (to OPs, OCs, DTCs, and paraquat), excluded controls recruited from an unknown base of eligible subjects, excluded controls obtained with the second cluster sampling approach, did not adjust for *PON1* 55 MM variant genotype, and adjusted for education. All statistical analyses were conducted using SAS 9.3. We conducted power analyses using Quanto Version 1.2.4 (Gauderman and Morrison, 2006) with two-sided tests and an alpha level of 0.05.

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, controls were more educated than cases, and more cases than controls had a first-degree relative with PD (Table 1). *ABCB1* SNP genotype frequencies were similar in those of European ancestry and all races together (Supplemental Material, Table S1).

We observed 47–54% increases in PD risk for rs2032582 and rs1045642 using recessive genetic models for participants of European ancestry (Table 2), and similar results for all subjects (Supplemental Material, Table S2). Occupational pesticide exposures to OCs and OPs increased PD risk by 79–93% (Table 3), and doubled PD risk in all races (Supplemental Material, Table S3). 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 147 controls).

For occupational pesticide exposure and *ABCB1* SNP rs1045642, we estimated the largest ORs of 2.07–3.54 for OP or OC exposed carriers of the homozygous variant TT genotype and other pesticide exposed TT carriers compared with ‘low exposure’ non-variant genotype carriers when adjusting for the variant TT genotype at rs2032582 (Table 4). Not adjusting for genotype at rs2032582 did not appreciably alter results [ORs: 2.09–3.74]. We observed smaller ORs of 1.89–2.62 for carriers of the TT genotype for *ABCB1* SNP rs2032582 with occupational OC or OP exposures adjusting for homozygous variant genotype carrier status at rs1045642 (Table 4), though results were larger without adjustment [ORs: 2.43–3.36]. Among those occupationally exposed to OC or OP pesticides, participants with the highest risk were homozygous variant genotype carriers for both rs1045642 and rs2032582 [OR= 3.71, 95% CI: (1.96, 7.02)] (Table 4). Results were similar when adjusting for ambient residential pesticides and for all races combined (Supplemental Material, Table S4). We determined that we had at least 80% power to detect interaction ORs of 4.1–5.4 or greater as reported in the French study (Dutheil et al., 2010). However, we did not detect interactions on the multiplicative scale in case-control or case-only analyses (Supplemental Material, Tables S5 and S6).

Marginal and joint associations for occupational pesticide exposure and *ABCB1* genotypes were similar after we excluded controls recruited from an unknown eligibility base and controls recruited with the second approach. Results were also similar when we did not adjust for the *PON1* 55 MM variant genotype and when we adjusted for education.

4. DISCUSSION

In our population based case-control study, we attempted to replicate 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, we find that PD risk is modified by both occupational exposures to organochlorine pesticides and by variants in the *ABCB1* gene at both rs1045642 and rs2032582. We newly report that PD risk is modified by occupational organophosphorus pesticide exposures and by polymorphisms in both SNPs. Our findings also suggest that the highest PD risk occurs in carriers of the TT genotype at both *ABCB1* 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 previously (Dutheil et al., 2010), but we did not replicate interactions on a multiplicative scale. Surprisingly, when exposed to pesticides in our study, individuals with risk variants at both rs1045642 and rs2032582 show a relatively smaller increase in risk than individuals with less risk variants at these loci (Table 4), which might suggest saturation effects or be an

artifact of the small sample size in this category; moreover, this difference is not significant. Importantly, one should not interpret these results to mean that TT genotype carriers are somehow protected from pesticide exposure effects as the individuals at greatest risk are TT genotype carriers at both rs1045642 and rs2032582 who have been occupationally exposed to pesticides.

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 many substrates from cells (Mahringer et al., 2011). In human brain tissue samples, P-glycoprotein is expressed by endothelial cells in central nervous system capillaries (Cordon-Cardo et al., 1989), primarily on the luminal but also the basal side of BBB endothelial cells facing the brain interstitial fluid and on intracellular organelle membranes (Bendayan et al., 2006). The transporter is 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 (Graff and Pollack, 2005; Marzolini et al., 2004).

Several studies examining associations between PD and polymorphisms in the *ABCB1* gene in European and Asian populations, without accounting for environmental exposures, reported inconsistent results (Funke et al., 2009; Furuno et al., 2002; Lee et al., 2004; Mizuta et al., 2006; Mizuta et al., 2008; Tan et al., 2005; Tan et al., 2004; Toda et al., 2003; Westerlund et al., 2009). An Italian hospital based case control study suggested an association between the TT genotype at rs1045642 and early onset (age<45) PD (Furuno et al., 2002). 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 (Tan et al., 2004). A German clinic based case control study restricted to PD cases with increased iron content in the substantia nigra, found no association between 10 SNPs in *ABCB1* and PD (Funke et al., 2009). A Swedish hospital based case control study found no association with PD for either rs1045642 or rs2032582 (Westerlund et al., 2009). 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 1×10^{-4} and 0.05 (Nalls et al., 2014). Our genetic marginal effect estimates (Table 2) suggest an increased risk of PD for homozygous variant carriers of either SNP, but since our study contains very few subjects completely unexposed to pesticides, we cannot estimate genetic effects in a pesticide unexposed population.

Three prior studies in European populations provide evidence for gene-environment interactions between pesticides and polymorphisms in *ABCB1* (Dro dzik et al., 2003; Dutheil et al., 2010; Zschiedrich et al., 2009). Two of these studies (Dro dzik et al., 2003; Zschiedrich et al., 2009) conducted case-only analyses and did not examine specific pesticides, nor did they present evidence that pesticide exposure and the rs1045642 polymorphism were independent in controls.

A Japanese hospital based case control study found no interaction (Kiyohara et al., 2013), but unlike ours and the French study, they examined interactions for rs1045642 using a dominant genetic model. Genotype frequencies were different in these populations (CC, CT, and TT genotypes 37.4%, 45.1%, and 17.4%, in Japanese controls and 24%, 50%, and 26% in French controls).

There is the possibility that the genetic markers studied are not causal variants due to linkage disequilibrium, and 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 while adjusting for *PON1* variants, 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 constituent of cigarette smoke, has been shown to modulate P-gp expression in cell studies (Sugihara et al., 2007), and smoking is inversely associated with PD. Our *ABCB1* risk score assumes a similar effect for each of the homozygous variant genotypes, and these analyses provide a parsimonious model that is exploratory and does not imply biologic function. In addition, we might have been underestimating gene environment interaction at higher exposure levels if our low exposure reference group contains enough subjects with exposures that also interact with the gene. Hopefully additional studies will investigate this further. Possible explanations for differences between the results in our study and the French study (Dutheil et al., 2010) include a ‘winner’s curse’ phenomenon (Ioannidis, 2008), differences in additional genes and population stratification, differences in co-exposures and in pesticide exposure assessment.

In this study, we relied partially on self-reported information to construct our exposure measures, although we used the CDPR database to identify whether subjects worked with OC or OP pesticides instead of asking our subjects to recall exposures to specific chemicals. The French study (Dutheil et al., 2010) enrolled participants working in agriculture or related occupations, and all OC pesticide exposed subjects professionally used these pesticides. In our study, approximately 30% of subjects worked in farming, fishing, or forestry (Liew et al., 2014), but fewer reported working directly with pesticides. Therefore, the majority of the subjects we considered occupationally exposed to OC or OP pesticides were exposed ambiently at the workplace (95–97%). 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 may be 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 (Wang et al., 2014). 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. While participant selection by *ABCB1* genotype is unlikely, it is possible that associations for pesticides are affected by differing control participation according to places of both residence and work such that the pesticide exposure measures we based on these addresses are affected. Another key strength of our study is that UCLA movement disorder specialists diagnosed PD and repeatedly evaluated most cases. A majority of our cases (86%) were interviewed within less than 3 years of first diagnosis; therefore, any differential recall is expected to be minimal.

5. CONCLUSIONS

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* and occupational exposures to organochlorines increase risk of PD. Further we find that genetic variants at rs1045642 and rs2032582 in *ABCB1* and occupational exposures to organophosphorus pesticides increase risk of PD. Together, homozygous variant genotypes at both positions appear to confer the greatest PD risk in those with both OC and OP exposures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

- The *ABCB1* gene encodes P-glycoprotein that transports xenobiotics out of the brain
- Organochlorine and organophosphorus pesticides increase Parkinson's disease risk
- PD risk is strongest in homozygous occupationally exposed ABCB1 variant carriers

Table 1

Characteristics of Study Population, Caucasians only (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)

^aThis is the age at diagnosis for cases and age at interview for controls.

^bGenotyping failed for 17 and 10 subjects, respectively, for rs2032582 and rs1045642.

Table 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)
<i>ABCB1</i> -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)
<i>ABCB1</i> -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
<i>ABCB1</i> -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)
<i>ABCB1</i> -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
<i>ABCB1</i> 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
<i>p</i> -Value for trend				0.0076	

NC: not calculated

^a Adjusted for age (continuous), sex, county, smoking (never/former/current). Results similar with additional adjustment for total MMSE score (MMSE score ≥ 25).

^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.

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 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				
<i>low exposure^b</i>	56 (19.9)	149 (28.6)	1.00	1.00
<i>frequent household pesticide use without workplace pesticide exposure</i>	28 (9.9)	78 (15.0)	0.96	1.12 (0.64, 1.97)
<i>workplace exposure to other pesticides</i>	75 (26.6)	111 (21.3)	1.80	1.84 (1.17, 2.90)
<i>ambient workplace OC exposure and/or self reported OC use^c</i>	123 (43.6)	183 (35.1)	1.79	1.79 (1.18, 2.72)
Workplace Organophosphorus Exposure				
<i>low exposure^b</i>	56 (19.8)	149 (28.6)	1.00	1.00
<i>frequent household pesticide use without workplace pesticide exposure</i>	28 (9.9)	78 (15.0)	0.96	1.13 (0.64, 1.98)
<i>workplace exposure to other pesticides</i>	38 (13.4)	66 (12.7)	1.53	1.51 (0.88, 2.59)
<i>ambient workplace OP exposure and/or self reported OP use^c</i>	161 (56.9)	228 (43.8)	1.88	1.93 (1.29, 2.87)

^a Adjusted for age (continuous), sex, county, smoking (never/former/current), total MMSE score (MMSE score ≤ 25/ MMSE score >25), and *PON1* 55MM (at rs854560) variant genotype.

^b Reference category subjects have low exposure to ambient workplace pesticides (OPs, OCs, DTCs, & paraquat; i.e. exposed below the median of exposed controls), did not use pesticides occupationally, have low exposure according to the JEM score, and are never/infrequent users of household pesticides. They may also have been exposed to ambient residential pesticides at any level.

^c Many cases (n=104) and controls (n=147) are occupationally exposed to both OC and OP pesticides.

ABCB1 polymorphisms and Exposure to Workplace Organochlorine & Organophosphorus Pesticides in Association With Parkinson Disease, participants with European ancestry.

Table 4

ABCB1-rs2032582							
	GG+GT			TT			Product p-value
	N case/control	Crude OR	Adjusted ^a OR (95% CI)	N case/control	Crude OR	Adjusted ^a OR (95% CI)	
Workplace Organochlorine Exposure							
low exposure ^b	41/124	1.00	1.00	14/24	1.76	1.49 (0.61, 3.65)	-
frequent household pesticide use without workplace pesticide exposure	22/60	1.11	1.45 (0.76, 2.78)	6/18	1.01	0.76 (0.25, 2.30)	0.13
workplace exposure to other pesticides (E2)	53/89	1.80	1.77 (1.05, 2.98)	21/17	3.74	3.31 (1.37, 8.04)	0.69
ambient workplace OC exposure and/or self reported OC use	94/147	1.93	1.95 (1.21, 3.14)	26/32	2.46	1.89 (0.87, 4.07)	0.39
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	30/54	1.68	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	118/182	1.96	1.96 (1.24, 3.10)	39/40	2.95	2.62 (1.26, 5.42)	0.80
ABCB1-rs1045642							
	CC+CT			TT			
N case/control	Crude OR	Adjusted ^a OR (95% CI)	N case/control	Crude OR	Adjusted ^a OR (95% CI)	Adjusted ^a Interaction OR (95% CI)	Product p-value

Workplace Organochlorine Exposure								
low exposure ^b	38/119	1.00	1.00	18/30	1.88	1.98 (0.86, 4.54)	-	-
frequent household pesticide use without workplace pesticide exposure	21/55	1.20	1.60 (0.81, 3.13)	7/22	1.00	0.94 (0.33, 2.69)	0.30 (0.08, 1.06)	0.06
workplace exposure to other pesticides (E2)	45/82	1.72	1.85 (1.06, 3.22)	30/26	3.61	3.54 (1.65, 7.61)	0.97 (0.35, 2.65)	0.95
ambient workplace OC exposure and/or self reported OC use	84/131	2.01	2.07 (1.26, 3.41)	38/49	2.43	2.43 (1.21, 4.87)	0.59 (0.24, 1.47)	0.26
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)	0.30 (0.08, 1.06)	0.06
workplace exposure to other pesticides	28/52	1.69	1.81 (0.96, 3.42)	10/13	2.41	2.07 (0.73, 5.84)	0.58 (0.17, 2.00)	0.39
ambient workplace OP exposure and/or self reported OP use	102/161	1.98	2.08 (1.29, 3.37)	58/62	2.93	3.00 (1.58, 5.72)	0.73 (0.31, 1.75)	0.49
ABCBI risk score ^c								
1				2				
Workplace Organochlorine 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, 3.06)	4/18	0.68	0.68 (0.21, 2.20)	0.19 (0.04, 0.81)	0.03
workplace exposure to other pesticides (E2)	55/90	1.88	1.89 (1.13, 3.18)	19/15	3.89	4.30 (1.89, 9.74)	1.01 (0.32, 3.16)	0.99
ambient workplace OC exposure and/or self reported OC use	95/151	1.93	1.96 (1.23, 3.14)	25/27	2.85	2.98 (1.48, 6.00)	0.67 (0.24, 1.88)	0.45

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)	0.19 (0.04, 0.81)	0.02
workplace exposure to other pesticides	30/56	1.65	1.69 (0.92, 3.10)	8/7	3.51	2.62 (0.87, 7.86)	0.69 (0.17, 2.77)	0.60
ambient workplace OP exposure and/or self reported OP use	121/185	2.01	2.05 (1.30, 3.22)	36/35	3.16	3.71 (1.96, 7.02)	0.81 (0.30, 2.15)	0.66

^aAdjusted for age(continuous), sex, county, smoking (never/former/current), total MMSE score (MMSE score ≥ 25), and *PONI* 55MM (at rs854560) variant genotype. 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.

^bReference category subjects have low exposure to ambient workplace pesticides (OPs, OCs, DTCs, & paraquat; i.e. exposed below the median of exposed controls), did not use pesticides occupationally, have low exposure according to the JEM score, and are never/infrequent users of household pesticides. They may also have been exposed to ambient residential pesticides at any level.

^cWe 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).