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Functional Paraoxonase 1 variants modify the risk of Parkinson's Disease due to Organophosphate Exposure

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Abstract

Background—We previously demonstrated that carriers of the "slower metabolizer" MM genotype of *paraoxonase* (*PON1)* who were also exposed to ambient organophosphate (OP) pesticides at their residences were at increased risk of developing Parkinson's disease (PD). Here, with a larger sample size, we extend our previous investigation to consider additional sources of ambient exposure and examined two additional functional *PON1* variants.

Methods—From 2001–2011, we enrolled incident cases of idiopathic PD and population controls living in central California. We genotyped three well-known functional *PON1* SNPs: two exonic polymorphisms ($PON1_{L55M}$, $PON1_{Q192R}$) and the promoter region variant ($PON1_{C\text{-}108T}$). Ambient exposures to diazinon, chlorpyrifos, and parathion at residential and workplace addresses were assessed using a validated geographic information system-based model incorporating records of agricultural pesticide applications in California.

Results—The odds ratio (OR) for Caucasians exposed to OPs at either residential or workplace addresses varied by *PON1* genotype; for exposed carriers of the "faster" metabolizer genotypes, ML or LL, we estimated lower odds ratios (range, 1.20–1.39) than for exposed carriers of the "slower" metabolizer genotype MM (range, 1.78–2.45) relative to unexposed carriers of the faster

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genotypes. We observed similarly increased ORs for exposure across *PON1*$_{Q192R}$ genotypes, but no differences across *PON1*$_{C\text{-}108T}$ genotypes. The largest ORs were estimated for exposed carriers of both *PON1*$_{192QQ}$ and *PON1*$_{55MM}$ (OR range, 2.84–3.57).

Conclusions—Several functional *PON1* variants may act together to modify PD risk for ambient OP exposures. While either *PON1*$_{L55M}$ or *PON1*$_{Q192R}$ may be sufficient to identify increased susceptibility, carriers of both slow metabolizer variants seem most susceptible to OP exposures.

Keywords

organophosphate pesticide; paraoxonase 1; Parkinson's disease

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder, and exposure to pesticides, lifestyle habits such as smoking and caffeine intake, genetic polymorphisms, and interactions between these factors are thought to contribute to disease etiology (Bekris and others 2010; Freire and Koifman 2012; van der Mark and others 2012; Wirdefeldt and others 2011). Organophosphorus pesticides (OPs) are commonly used in modern agriculture to control insects. In a few previous studies, OPs have been shown to increase the risk of developing PD (Hancock and others 2008; Kamel and others 2007; Seidler and others 1996). Many OPs are activated to their toxic analog (or oxon) by cytochrome P450 (Costa and others 2003b), and for chlorpyrifos and diazinon, two of the more common OP pesticides, the oxon is then detoxified by the paraoxonase 1 (PON1) hydrolyzing enzyme (Costa 2002). Common single nucleotide polymorphisms (SNPs) of the *PON1* gene, *PON1*$_{L55M}$, *PON1*$_{Q192R}$, and *PON1*$_{C\text{-}108T}$, have been shown to affect OP-oxon metabolism and thus may impact susceptibility to organophosphate toxicity (Costa and others 2003b). The *PON1*$_{L55M}$ polymorphism has been associated with both variability in PON1 levels and PON1 activity in plasma (Brophy and others 2001; Garin and others 1997; Mackness and others 1998), whereas the *PON1*$_{Q192R}$ polymorphism has been shown to affect catalytic efficiency in a substrate-dependent manner (Li and others 2000). The *PON1*$_{108T}$ allele has been associated with lower expression levels (Brophy and others 2001).

We previously demonstrated that the *PON1*$_{L55M}$ polymorphism modifies the associations between PD and ambient residential exposures to the organophosphates diazinon and chlorpyrifos, but not parathion (Manthripragada and others 2010). However, whether *PON1*$_{Q192R}$ and *PON1*$_{C\text{-}108T}$ polymorphisms also modify this relationship has not yet been examined. Furthermore, our prior work did not consider ambient exposure to these pesticides assessed by workplace addresses. Here we present an extension of our previous report by investigating the contributions of two additional *PON1* functional polymorphisms (*PON1*$_{C\text{-}108T}$ and *PON1*$_{Q192R}$) to gene-pesticide effects in PD. In addition, we improve the statistical power of our study by adding new control subjects and by expanding our exposure assessment to encompass both ambient OP exposures at workplace and residential addresses.

2. Materials and Methods

2.1. Subjects

Our case-control study enrolled incident idiopathic PD patients 2001 through 2007 and population-based controls 2002 through 2011 from three mostly rural agricultural counties (Kern, Tulare, Fresno) in central California. Subject recruitment methods (Costello and others 2009) and case definition criteria (Jacob and others 2010; Kang and others 2005) have been described in detail elsewhere. Briefly, we initially screened 1,167 PD patients identified through neurologists, large medical groups, and public service announcements; 604 did not meet our eligibility criteria (397 had received a PD diagnosis 3+ years prior to recruitment, 134 lived outside the tri-counties, 51 had a diagnosis other than idiopathic PD, and 22 were too ill to participate). Of 563 eligible cases, 473 were examined by a study movement disorder specialist, and of these, 94 did not meet published criteria for idiopathic PD (Hughes and others 1992; Langston and others 1992); an additional 13 were reclassified during follow-up (Ritz and others 2012), and 6 withdrew between examination and interview. Of the remaining 360 cases, 356 provided all necessary information and biologic samples. After exclusion of 69 non-Caucasian subjects, 287 PD cases were available for analysis. We excluded non-Caucasians to reduce the known heterogeneity in *PON1* genotype frequencies between race/ethnic groups (Costa and Furlong 2002) and because there were too few non-Caucasian subjects to conduct informative analyses.

Population-based controls were recruited initially from random selection of Medicare lists (2001 and later); after implementation of Health Insurance Portability and Accountability Act (HIPAA), the majority of controls were randomly selected from residential tax assessor records that listed housing units in the tri-county area. To increase enrollment success and achieve representativeness of the control population, we applied two recruitment strategies utilizing tax assessor records: 1) random selection of residential addresses contacted by mail and phone (described in detail in Costello, 2009); and 2) random selection of a cluster of households (defined as one address plus four addresses nearby), each visited in-person by study staff members up to four times to identify and recruit eligible residents.

Using the first strategy, we contacted a total of 1,212 potential controls to determine eligibility by mail/phone, and determined that 457 were ineligible, 409 of them because they were younger than 35 years. Of the 755 eligible population controls, 409 did not participate (declined, became too ill to participate, or moved out of the area after screening and prior to enrollment). Of the 346 subjects enrolled, we excluded five controls who provided only partial information and 24 who did not provide a DNA sample for genotyping. We included in our analysis 62 controls from an early mailing without additional follow-up of non-responders. Therefore, under the first strategy, we enrolled 379 population controls. Of the 4,756 individuals screened for eligibility through the second recruitment strategy, 3,515 were ineligible (88% due to age criteria). Of the 1,241 eligible population controls, 634 declined participation; while we enrolled 607 population controls, 183 completed only an abbreviated interview that did not allow us to construct a full address history. Of the remaining 424 eligible controls who completed the comprehensive interview, only 137 participants had DNA samples available at the time of genotyping. In total, 379 controls

identified by mail or phone and 137 controls identified in-person (total n=516) provided all information and biologic samples; after exclusion of 76 non-Caucasians, 440 Caucasian controls contributed to the following analysis.

Written informed consent was obtained from all subjects, and the study was approved by the University of California at Los Angeles (UCLA) Institutional Review Board.

2.2.Exposure Assessment

Cases and controls completed a telephone interview to provide information on demographics, detailed lifetime residential and workplace address histories, and behavioral risk factors (including smoking and caffeine intake). Ambient residential and workplace exposures to diazinon, chlorpyrifos, and parathion were estimated for each study participant, using a validated geographic information system (GIS)-based computer model (Ritz and Costello 2006). With this approach, we combined pesticide use report data collected since 1974 by the California Department of Pesticide Regulation, land use maps created by the California Department of Water Resources, and geocoded address information for all reported residential and workplace addresses. Based on each participant's residential and workplace address, the GIS-based model estimated ambient pesticide exposure around homes or workplaces resulting from pesticide applications to agricultural crops (Goldberg and others 2008; Rull and Ritz 2003). For the period from 1974 to 1999, we estimated a year-specific average exposure for each subject and each pesticide. Specifically, we estimated pounds of pesticide applied within a 500-meter buffer around the home or workplace and weighted the total poundage by the proportion of acreage treated. We then calculated the period-specific averages, separately for diazinon, chlorpyrifos, and parathion, by summing the year-specific values and dividing by the total study period (26 years, 1974 through 1999).

Since the majority of PD patients were first diagnosed after 1999, we calculated pesticide exposure up to the year 1999 in order to restrict our exposure of interest to the time before PD patients were diagnosed. In our prior work (Manthripragada et al. 2010), we considered a three-level variable (none/low/high) to estimate marginal effect sizes for OP pesticides, but when jointly estimating gene and environmental effects, we collapsed low and high exposures. Here, we show results for the same collapsed exposure groups compared with those unexposed to all three OP pesticides in the reference category.

2.3.Genotyping Methods

DNA extraction was performed by the UCLA Biologic Sample Processing Core, and genotyping for *PON1*$_{L55M}$ (rs854560) was conducted at the UCLA Genotyping and Sequencing Core Facility, using whole blood, buccal cells, or saliva samples provided by participants. Genotyping for *PON1*$_{L55M}$ was performed using pyrosequencing technology (Manthripragada and others 2010) or TaqMan (Life Technologies, San Diego, CA). The concordance between pyrosequencing and TaqMan was 100% for 17 subjects genotyped on both systems. Genotyping for *PON1*$_{C\text{-}108T}$ and *PON1*$_{Q192R}$ SNPs was done with the Fluidigm BioMark HD system (Fluidigm Corporation, South San Francisco, CA) at the University of Washington, and was limited to Caucasians. Genotyping call rates for

PON1$_{L55M}$, *PON1*$_{C-108T}$ and *PON1*$_{Q192R}$ were 100%, 90%, and 93%, respectively. Thus, 71 and 49 of the 727 Caucasians with genotypes for *PON1*$_{C-108T}$ and *PON1*$_{Q192R}$, respectively, failed genotyping and thus did not contribute to the analyses for *PON1*$_{C-108T}$ and *PON1*$_{Q192R}$.

2.4.Statistical Methods

We assessed Hardy-Weinberg equilibrium based on chi-square tests for all three SNPs. To estimate gene-pesticide effects in PD, we employed unconditional logistic regression analyses (SAS 9.1.3, SAS Institute, Cary, NC) to calculate odds ratios (ORs) and 95% confidence intervals (CIs). All models were adjusted for age (continuous, defined as age of PD diagnosis for cases and age of enrollment for controls), gender (male/female), smoking status (ever/never), county (Fresno/Tulare/Kern), and education level (continuous, in years). We also assessed household OP pesticide use as a potential confounding variable, and presented the results in supplemental materials, Table S1.

For comparability to our prior work (Manthripragada and others 2010), we relied on a recessive inheritance model for *PON1*$_{L55M}$ comparing cases and controls carrying 2 variant alleles (i.e., MM) to those carrying one or more wild-type allele (i.e., LL and LM). Based on enzymatic activity data, individuals with the *PON1*$_{192QQ}$ genotype had the lowest serum diazoxonase activity compared to those with *PON1*$_{192QR}$ and *PON1*$_{192RR}$ genotypes (O'Leary and others 2005). Thus, for *PON1*$_{Q192R}$, we also used a recessive model and compared carriers of the QQ genotype to the combined group of those with QR and RR genotypes. Prior research has suggested that *PON1*$_{C-108T}$ heterozygotes have an intermediate phenotype (Brophy and others 2001); thus, the *PON1*$_{C-108T}$ SNP was analyzed under a log-additive genetic model, assuming that on a log scale the risk associated with 2 copies of the variant allele is double the risk associated with 1 copy of the variant allele. We examined the possible combined effects of both *PON1*$_{L55M}$ and *PON1*$_{Q192R}$ polymorphisms on PD risk by performing a diplotype analysis (MM-QQ, other combinations, LL-RR). Furthermore, we assessed risk of carrying both the MM and QQ genotypes by fitting a third model including both *PON1*$_{L55M}$ and *PON1*$_{Q192R}$ genotypes and OP exposure and compared this model - based on the log likelihood ratio test (LRT) - with the two previous ones (*PON1*$_{L55M}$ and OP exposure, or *PON1*$_{Q192R}$ and OP exposure), including only Caucasian subjects genotyped for both variants (see supplemental materials, Table S2).

To assess the robustness of our results, we conducted several sensitivity analyses; i.e., excluding: 1) 37 controls who came from an unknown base of eligible subjects and thus may be different from all other population controls; and 2) 15 randomly selected controls from residential clusters in which multiple controls had lived at the time of enrollment for 2 or more years prior to 1999; thus possibly introducing co-linearity due to correlated exposures. Multiple comparison correction was not implemented, as the rationale for examining the three *PON1* SNPs is based on previous research with prior strong support for the impact of the *PON1*$_{L55M}$ and *PON1*$_{Q192R}$ SNPs on metabolism for the selected OP pesticides. To allow for comparison to our previous *PON1*$_{L55M}$ analyses (Manthripragada and others 2010), we also present results for this variant for all race/ethnicity groups (see supplemental materials, Table S3) while adjusting for race (Caucasian/non-Caucasian).

3. Results

Participants with idiopathic PD were slightly older, more likely to be male, less likely to report ever having smoked cigarettes, and more likely to be exposed to ambient OP pesticides at residential or workplace addresses than population controls (Table 1). Genotype frequencies for the three *PON1* SNPs examined among controls did not significantly differ from Hardy-Weinberg equilibrium ($p>0.05$). When estimating exposures and genotypes jointly, we observed an odds ratio (OR) of 2.45 for PD (95% CI=1.24–4.83; Table 2) for those with ambient exposure to chlorpyrifos at their home or workplace addresses and carrying the MM genotype, as compared to those without exposure and carrying the LM or LL genotypes. The effect estimates for PD among subjects exposed to diazinon or parathion who carried the $PON1_{55MM}$ genotype were 1.84 and 1.78, respectively (Table 2). These effect estimates were similar when our analyses also included non-Caucasian subjects (see supplemental materials, Table S3).

We estimated an OR of 1.95 (95% CI=1.13–3.37; Table 3) for ambient exposure to chlorpyrifos in carriers of the $PON1_{192QQ}$ genotype, as compared to unexposed subjects with the QR or RR genotypes. We also observed ORs of 1.53 and 1.65 for PD with diazinon or parathion exposure in $PON1_{192QQ}$ carriers, but the 95% CIs of these effect estimates included the null (Table 3). We observed the strongest associations for OPs with PD for those exposed to OPs and carrying both the $PON1_{55MM}$ and $PON1_{192QQ}$ genotypes; e.g., for chlorpyrifos-exposed carriers of the MM-QQ diplotypes, OR=3.28 (95% CI=1.02–10.58) compared to those unexposed with a LL-RR diplotype (Table 4). When we compared models including both $PON1_{L55M}$ and $PON1_{Q192R}$ SNPs to those for each *PON1* SNP alone, using LRTs, we observed no improvement for models with both SNPs compared to models with one SNP alone (see supplemental materials, Table S2).

No notable differences in OP exposure estimates were observed for the $PON1_{C\text{-}108T}$ variant (supplemental materials, Table S4). The estimates for the joint effects of pesticides and *PON1* variants did not change notably when we: 1) adjusted for household use of OP pesticides (supplemental materials, Table S1); 2) excluded the 37 controls with an unknown base population (e.g., chlorpyrifos exposure in MM genotype carriers, OR= 2.19 (95% CI=1.13–4.23)); 3) or excluded 15 controls from the same residential cluster (e.g., chlorpyrifos exposure in MM genotype carriers, OR=2.56 (95% CI=1.29–5.08)). For all gene-pesticide analyses, we used the same reference group of participants - those unexposed to all three OPs - to allow for comparison of effect estimates across individual exposures. The results were almost identical when we defined the reference group as those not exposed to each OP pesticide separately (results not shown).

4. Discussion

We had previously shown that $PON1_{L55M}$ variants associated with slower OP detoxification need to be considered when estimating the odds of developing PD from OP exposures. Here, we were able to corroborate our previous findings with a larger sample size, due to additional population control recruitment and after expanding exposure assessment to include ambient workplace OP exposures. In addition, we were able to enhance our

investigations of the importance of *PON1* for OP detoxification when assessing PD risk to include two additional functional polymorphisms, $PON1_{C\text{-}108T}$ and $PON1_{Q192R}$. Our results suggest that both the 192QQ and the 55MM genotypes but not the C-108T variant contribute to PD risk in subjects exposed to the three Ops: diazinon, chlorpyrifos, and parathion. It is unlikely that the influences of these SNPs on PD risk are due to linkage disequilibrium, since the pairwise R^2s for the three examined SNPs were all below 0.22. We also assessed the combined effects of both $PON1_{L55M}$ and $PON1_{Q192R}$ polymorphisms on PD risk and observed the strongest associations with PD for those exposed to OPs and carrying both the $PON1_{55MM}$ and $PON1_{192QQ}$ genotypes compared to unexposed carriers of a LL-RR diplotype. Furthermore, when we investigated whether accounting for both the $PON1_{L55M}$ and $PON1_{Q192R}$ polymorphisms in the same model improved model fit over inclusion of only one or the other SNP, we did not find any improvement based on likelihood ratio tests. Thus, although several functional *PON1* variants may act together to modify PD risk due to OP exposure, either $PON1_{L55M}$ or $PON1_{Q192R}$ alone may suffice to assess susceptibility to OP toxicity based on genotype. Due to sample size limitations, we were not able to examine three-way gene by gene by pesticide interactions.

PON1 is a protein component of high-density lipoprotein (HDL), with the key function of detoxifying organophosphorous compounds and protecting both low- and high-density lipoproteins from oxidation (Aviram and others 1998; Mackness and others 1993; Watson and others 1995). It is unlikely that any *PON1* polymorphism is an independent risk factor for PD; i.e. in the absence of OP pesticide exposure (Belin and others 2012; Liu and others 2012; Zintzaras and Hadjigeorgiou 2004). However, we speculated that genetic variation in *PON1* modifies the risk of PD, given exposure to OP pesticides, due to the known detoxification activity and the demonstrated functionality of these specific genetic variants. Thus, our gene-pesticide analyses targeted a biologic interaction and did not attempt to assess statistical interaction (e.g., testing the statistical significance of a product term in the model). Although we cannot statistically rule out a model where *PON1* and OP pesticides are acting independently, we argue that most likely there is a biological role for *PON1* in metabolizing OP pesticides, especially since the latest meta-analyses of *PON1* and PD did not corroborate an independent effect for the gene (Liu et al. 2012). Indeed, both our present and previous *PON1* and OP results (Manthripragada and others 2010) suggest ORs from 1.20 to 1.48 for subjects exposed to OPs who carry the variants related to higher metabolic function of the protein as compared to ORs of 1.53 to 2.45 for subjects exposed to OPs who carried the genetic variants with low metabolic function.

Previous studies examining the relationship of *PON1* polymorphisms and pesticide exposures with PD have been limited by small sample sizes, with most studies including few subjects exposed to pesticides among carriers of specific genetic variants in *PON1*. For example, an Australian study of 214 subjects that examined the $PON1_{Q192R}$-pesticide association included only 9 exposed subjects (4 cases, 5 controls) with the $PON1_{192RR}$ genotype (Taylor and others 2000). No study to date has examined associations between pesticide exposure, the $PON1_{C\text{-}108T}$ polymorphism, and PD. Although the $PON1_{C\text{-}108T}$ polymorphism has been shown to influence PON1 expression levels, we did not observe any effect of the $PON1_{C\text{-}108T}$ genetic variants on PD risk when controlling for OP pesticide exposure in our study. However, this may be due to insufficient sample size to detect such

associations; e.g., based on 287 cases and 440 controls, we have approximately 77% power to detect a genetic association of OR of 1.5 or greater for allele frequencies of 30% or less.

Most previous studies of pesticide exposure and PD or pesticide-*PON1* effects and PD relied on self-reported pesticide exposure and evaluated only broadly defined categories of exposure, such as “any pesticide exposure” or exposure to a specific pesticide class. A major strength of our study is the availability of our record-based GIS modeling system to assess long-term ambient pesticide exposure at residential and workplace addresses and the ability of our model to assign exposure to the specific pesticides, diazinon, chlorpyrifos, and parathion, using California pesticide records. This exposure assessment tool avoids differential recall bias for historical exposure; however, non-differential misclassification of exposure cannot be ruled out, due to geocoding errors and uncertainties concerning pesticide drift. Although our new analyses included two additional functional SNPs ($PON1_{Q192R}$ and $PON1_{C\text{-}108T}$) that have been shown to account for variations in serum PON1 activity (Costa and others 2005), we do not have information on participants’ PON1 serum activity levels. In a given population, serum PON1 activity can vary 40-fold (Davies and others 1996; Eckerson and others 1983; Mueller and others 1983; Richter and Furlong 1999), and up to 13-fold variations in enzyme levels are present in samples of persons with the same $PON1_{Q192R}$ genotype (Costa and others 2003a; Davies and others 1996). Although a two-substrate enzyme analysis provides both an individual’s serum PON1 activity and $PON1_{Q192R}$ genotype information (Richter and Furlong 1999), this analysis was not feasible in our study because it would have required serum or plasma samples rather than DNA from all participants.

Our data suggest increased risk of PD in our study participants exposed to parathion among carriers of the common genetic variants in $PON1_{L55M}$ and $PON1_{Q192R}$. However, previous animal studies observed that both $PON1_{192R}$ and $PON1_{192Q}$ alleles have similar protection against diazoxon, and the $PON1_{Q192R}$ variant provides no protection against paraoxon exposure *in vivo* due to insufficient efficiency of PON1 to hydrolyze paraoxon (Li and others 2000; Richter and others 2009). Therefore, we would not expect the size of the estimated risk for parathion and PD to depend on variant carrier status. The observed difference in effect size may indeed be due to co-exposures to chlorpyrifos and diazinon in parathion-exposed subjects (90% of parathion-exposed subjects were also exposed to chlorpyrifos or diazanon). In fact, we cannot clearly attribute these risk increases to either chlorpyrifos or diazinon because of high co-exposures of participants to these three pesticides (71% of subjects exposed to diazinon were also exposed to chlorpyrifos, and 86% of subjects exposed to chlorpyrifos were also exposed to diazinon). Thus, since very few study participants were exposed to only one of the three specific OP, we are unable to draw conclusions regarding any single OP pesticide to the exclusion of the others.

The worldwide prevalence of Parkinson’s disease varies, and is reported to be slightly lower in Asia than in Western countries (Muangpaisan and others 2009; von Campenhausen and others 2005). Whether this is attributable to genetics or environmental causes, or is simply due to differences in case ascertainment, diagnostic criteria, and case-finding strategies is not known. Our study targeted an area with high agricultural pesticide use in central California that provided sufficient exposure prevalence and, thus, statistical power, to

examine gene-pesticide interactions in PD, but whether our results are generalizable to other populations depends on factors that determine baseline risk of PD.

5. Conclusions

Despite these caveats, overall our study shows that both *PON1*$_{L55M}$ and *PON1*$_{Q192R}$ SNPs modify the effects of OP pesticides on PD risk and that these variants may determine susceptibility to these toxins. Our results should alert researchers that the estimates of marginal effects for ambient OP pesticides in studies of PD that ignore polymorphisms in the *PON1* gene can be misleading, particularly in studies that collect data from a number of ethnic groups where allele frequencies for these loci and functionality of the proteins can vary substantially. Finally, our study further strengthens the evidence that the *PON1* gene plays a mediating role in the etiology of PD among subjects exposed to pesticides detoxified by the paraoxonase enzyme. Either *PON1*$_{L55M}$ or *PON1*$_{Q192R}$ may be sufficient to identify those with an increased susceptibility to OP exposures due to slow metabolizer genotypes, but carriers of both slow metabolizer variants (diplotype carriers) seem most susceptible to OP exposures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

PD	Parkinson’s disease
OPs	organophosphorus pesticides
PON1	paraoxonase 1
SNPs	single nucleotide polymorphisms
HIPAA	Health Insurance Portability and Accountability Act
GIS	geographic information system
UCLA	University of California at Los Angeles
ORs	odds ratios
LRT	likelihood ratio test
HDL	high-density lipoprotein

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Highlights

- both *PON1*$_{L55M}$ and *PON1*$_{Q192R}$ SNPs modify the effects of OP pesticides on PD risk
- *PON1* gene plays a role in the etiology of PD among subjects exposed to pesticides
- *PON1*$_{L55M}$ or *PON1*$_{Q192R}$ alone may suffice to assess susceptibility to OP toxicity

Table 1

Demographic characteristics of the study population by PD status, Caucasians only

	Cases N=287	Controls N=440
Age (years)[a], mean (SD)	69.0 (10.5)	67.6 (11.9)
Education level (years), mean (SD)	14.1 (3.4)	15.0 (3.2)
Gender, n (%)		
Female	126 (43.9)	223 (50.7)
Male	161 (56.1)	217 (49.3)
County, n (%)		
Fresno	134 (46.7)	194 (44.0)
Kern	101 (35.2)	174 (39.6)
Tulare	52 (18.1)	72 (16.4)
Smoking status, n (%)		
Never	158 (55.1)	206 (46.8)
Ever	129 (44.9)	234 (53.2)
1st degree relative with PD, n (%)		
Yes	41 (14.3)	41 (9.3)
No	246 (85.7)	399 (90.7)
***PON1*$_{L55M}$ genotype**, n (%)		
LL	120 (41.8)	195 (44.3)
LM	125 (43.6)	196 (44.6)
MM	42 (14.6)	49 (11.1)
***PON1*$_{Q192R}$ genotype**[b], n (%)		
RR	27 (9.6)	54 (13.6)
QR	110 (39.1)	163 (41.1)
QQ	144 (51.3)	180 (45.3)
***PON1*$_{C-108T}$ genotype**[b], n (%)		
GG	77 (28.6)	104 (26.9)
GA	121 (45.0)	187 (48.3)
AA	71 (26.4)	96 (24.8)
Exposed to ambient organophosphate pesticide at residences or workplaces		
Diazinon, n (%)		
Zero Exposure	103 (35.9)	181 (41.1)
Low/High Exposure	184 (64.1)	259 (58.9)
Chlorpyrifos, n (%)		
Zero Exposure	127 (44.3)	231 (52.5)
Low/High Exposure	160 (55.7)	209 (47.5)
Parathion, n (%)		
Zero Exposure	131 (45.6)	225 (51.1)
Low/High Exposure	156 (54.4)	215 (48.9)

[a] age at diagnosis for PD patients and age at interview for controls

[b] failed genotype: *PON1*Q192R (N=49); *PON1*C-108T (N=71);

Table 2

Effect estimates (ORs and 95% CI) for ambient residential or workplace exposure to diazinon, chlorpyrifos and parathion and Parkinson's disease by *PON1*$_{L55M}$ genotype, Caucasians only

Exposure	*PON1-55* LL+LM			*PON1-55* MM		
	No. cases/controls	Crude OR	Adjusted OR (95% CI)[a]	No. cases/controls	Crude OR	Adjusted OR (95% CI)[a]
Ambient Residential or Workplace Exposure[b]						
Diazinon						
Zero Exposure	61/121	1.00	1.00	6/16	0.74	0.91 (0.33–2.52)
Low/High Exposure	156/231	1.34	1.20 (0.80–1.82)	28/28	1.98	1.84 (0.97–3.47)
Chlorpyrifos						
Zero Exposure	61/121	1.00	1.00	6/16	0.74	0.91 (0.33–2.50)
Low/High Exposure	134/188	1.41	1.39 (0.91–2.12)	26/21	2.46	2.45 (1.24–4.83)
Parathion						
Zero Exposure	61/121	1.00	1.00	6/16	0.74	0.90 (0.33–2.48)
Low/High Exposure	134/194	1.37	1.22 (0.80–1.87)	22/21	2.08	1.78 (0.89–3.60)

[a] adjusted for age, gender, smoking status, county, and education level

[b] reference group of participants is for those unexposed to all three OPs

Table 3

Effect estimates (ORs and 95% CI) for ambient residential or workplace exposure to diazinon, chlorpyrifos and parathion and Parkinson's disease by *PON1*$_{Q192R}$ genotype, Caucasians only

Exposure	*PON1-192* RR+QR			*PON1-192* QQ		
	No. cases/controls	Crude OR	Adjusted OR (95% CI)[a]	No. cases/controls	Crude OR	Adjusted OR (95% CI)[a]
Ambient Residential or Workplace Exposure[b]						
Diazinon						
Zero Exposure	33/69	1.00	1.00	32/59	1.13	1.14 (0.62–2.09)
Low/High Exposure	89/124	1.50	1.33 (0.78–2.26)	91/107	1.78	1.53 (0.90–2.61)
Chlorpyrifos						
Zero Exposure	33/69	1.00	1.00	32/59	1.13	1.14 (0.62–2.09)
Low/High Exposure	73/100	1.53	1.48 (0.86–2.56)	83/82	2.12	1.95 (1.13–3.37)
Parathion						
Zero Exposure	33/69	1.00	1.00	32/59	1.13	1.13 (0.62–2.08)
Low/High Exposure	77/109	1.48	1.34 (0.78–2.32)	77/82	1.96	1.65 (0.95–2.89)

[a] adjusted for age, gender, smoking status, county, and education level

[b] reference group of participants is for those unexposed to all three OPs

Table 4

Effect estimates (ORs and 95% CI) for ambient residential or workplace exposure to diazinon, chlorpyrifos and parathion and Parkinson's disease by *PON1*$_{L55M}$ and *PON1*$_{Q192R}$ diplotypes, Caucasians only

Exposure	*PON1*$_{55LL\text{-}192RR}$			*PON1*$_{55LM\text{-}192QR}$			*PON1*$_{55MM\text{-}192QQ}$		
	No. cases /controls	Crude OR	Adjusted OR (95% CI)[a]	No. cases/ controls	Crude OR	Adjusted OR (95% CI)[a]	No. cases/ controls	Crude OR	Adjusted OR (95% CI)[a]
Ambient Residential or Workplace Exposure[b]									
Diazinon									
Zero Exposure	6/14	1.0	1.0	53/100	1.24	1.16 (0.41–3.27)	6/14	1.00	1.16 (0.29–4.62)
Low/High Exposure	19/33	1.34	1.10 (0.35–3.47)	133/175	1.77	1.47 (0.53–4.05)	28/23	2.84	2.43 (0.78–7.56)
Chlorpyrifos									
Zero Exposure	6/14	1.0	1.0	53/100	1.24	1.18 (0.42–3.33)	6/14	1.00	1.14 (0.29–4.58)
Low/High Exposure	15/28	1.25	1.19 (0.36–3.92)	115/137	1.96	1.77 (0.64–4.92)	26/17	3.57	3.28 (1.02–10.58)
Parathion									
Zero Exposure	6/14	1.0	1.0	53/100	1.24	1.16 (0.41–3.28)	6/14	1.00	1.14 (0.28–4.55)
Low/High Exposure	15/29	1.21	0.99 (0.30–3.25)	117/146	1.87	1.57 (0.56–4.37)	22/16	3.21	2.56 (0.78–8.39)

[a] adjusted for age, gender, smoking status, county, and education level

[b] reference group of participants is for those unexposed to all three OPs