

Of Pesticides and Men: a California Story of Genes and Environment in Parkinson's Disease

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Published online: 9 February 2016
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Abstract At the start of the postgenomics era, most Parkinson's disease (PD) etiology cannot be explained by our knowledge of genetic or environmental factors alone. For more than a decade, we have explored gene–environment (GxE) interactions possibly responsible for the heterogeneity of genetic as well as environmental results across populations. We developed three pesticide exposure measures (ambient due to agricultural applications, home and garden use, and occupational use) in a large population-based case–control study of incident PD in central California. Specifically, we assessed interactions with genes responsible for pesticide metabolism (*PONI*); transport across the blood–brain barrier (*ABCB1*); pesticides inter-

fering with or depending on dopamine transporter activity (*DAT/SLC6A3*) and dopamine metabolism (*ALDH2*); impacting mitochondrial function via oxidative/nitrosative stress (*NOS1*) or proteasome inhibition (*SKPI*); and contributing to immune dysregulation (*HLA-DR*). These studies established some specificity for pesticides' neurodegenerative actions, contributed biologic plausibility to epidemiologic findings, and identified genetically susceptible populations.

Keywords Pesticides · Gene–environment interactions · Parkinson's disease

This article is part of the Topical Collection on *Susceptibility Factors in Environmental Health*

Electronic supplementary material The online version of this article (doi:10.1007/s40572-016-0083-2) contains supplementary material, which is available to authorized users.

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Introduction

Parkinson's disease (PD) is one of the most common neurodegenerative disorders, with a complex multifactorial etiology attributed to both environmental and genetic risk factors [1, 2, 3, 4]. Pesticide exposures have received special attention ever since the neurotoxic metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was first described as causing Parkinsonism in humans [5]. And, additionally, the finding that the herbicide paraquat is not only structurally similar to this neurotoxin but also exhibits similar effects in animal models [6]. Subsequently, many studies have associated pesticides in general with PD risk, but few investigated specific chemicals [7]. Large genome-wide association studies (GWAS) have identified more than two dozen common genetic variants for PD, each with a relatively small effect size; in combination with rare Mendelian genes, genetics account for at best 10–20 % of PD [4, 8]. The remaining unexplained PD etiology has been referred to as “missing genetic heritability” and attributed to undiscovered rare genetic variants conferring larger risks [9], though early studies do not support this

explanation [10]. Nonetheless, we argue that gene–environment (GxE) interactions may have an even larger contribution that remains largely underexplored.

Success in both identifying and replicating GxE interactions remains somewhat elusive, since it depends as much on valid and reliable environmental exposure assessment as on pinpointing appropriate genetic markers. Chronic diseases of advanced age, such as PD, are especially challenging because exposures may accumulate over decades or even a lifetime. Thus, short of following humans across the lifespan, exposures in the distant past are not easily addressable and population-based case–control studies often rely on recall of exposure. Recall is more easily prone to bias and study participants are rarely able to reliably report exposures to specific chemicals.

In California, over the past decade, we were able to overcome the challenge of long-term and population-based pesticide exposure assessment by using the unique pesticide use reporting (PUR) system to explore gene–pesticide interactions [11]. The PUR system was mandated by law and initiated in 1974 in California. It provides spatially detailed information on agricultural pesticide use statewide. We developed a sophisticated geographic information system (GIS) computer model to combine pesticide use reports with land use information in combination with residential and occupational addresses to estimate ambient pesticide exposure for specific active chemical ingredients [11, 12]. Our GIS-derived method of exposure estimation has been validated for organochlorines using measurements of serum biomarkers for 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE), a metabolite of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) [13]. In addition, we identified lifelong household pesticide exposure to specific chemicals and chemical ingredients relying on self-report enhanced with the unique California Department of Pesticide Regulation product label database [14]. Finally, we combined job history information with a job exposure matrix (JEM) generating an objective measure of general occupational pesticide exposures [15].

Even though GxE studies might help explain heterogeneity of results across populations due to variation in susceptibility to pesticide neurotoxicity and contribute to biologic plausibility of associations observed in human studies, only a small number of studies have been able to test these hypotheses. Here, we will summarize, review, and put into broader context the most intriguing insights we have gained from GxE interaction analyses in a central California population enrolled for the Parkinson's Gene and Environment (PEG) study. Our studies combine well-defined, record-based exposure assessment for specific chemical agents with genetic information from a range of pathways picked for their pathophysiologic relevance to PD as well as pesticide metabolisms and transport (Table 1). The study population and methods of the PEG study are described in more detail in Supplement 1. Briefly, we conducted a population-based case–control study that enrolled

patients with incident idiopathic PD (within 3 years of diagnosis) between 2001 through the present and population controls from 2002 to 2011, from three counties in central California (Kern, Fresno, and Tulare). County resident participants were 35 years or older and had lived in California for 5 or more years prior to recruitment. Study participants were predominantly of European ancestry, over the age of 65, and did not report a family history of PD (Supplement 2).

Pesticides and PD

Paraquat is structurally similar to the neurotoxic metabolite 1-methyl-4-phenylpyridinium (MPP+) of MPTP and has become an established animal model of PD [16]. Paraquat treatment leads to incomplete dopaminergic cell death in animals but appears to act as a redox cyler and not through complex I inhibition [17]. Its toxicity is markedly enhanced by coadministration of the fungicide maneb, a manganese-containing ethylene bis-dithiocarbamate fungicide. It has been reported that maneb is toxic due to its inhibition of mitochondrial respiratory chain complex III [18]. Also, maneb toxicity may be mediated by ubiquitin-proteasome system inhibition [19, 20], aldehyde dehydrogenase inhibition [21], and disruption of protein thiol functions analogous to oxidative stress [22].

A so-called organic pesticide, rotenone, is a very potent inhibitor of mitochondrial complex I and has become another established toxicant-based animal model of Parkinsonism [17, 23]. Unlike other pesticide models of PD, low-level rotenone exposure can produce many of the pathological features of PD. This includes the induction of alpha-synuclein misfolding leading to synuclein templating, transneuronal spread, and a widespread synucleinopathy seen in human PD [24]. While pesticide-based animal models recapitulate much of the pathology of human PD, it is impossible to model many of the important aspects of chemical exposures, such as route of entry or low level exposures spread over decades [17]. Thus, as experimental models can only capture some aspects of human PD, it remains important to investigate potential neurotoxicity of specific pesticides to susceptible humans.

A recent meta-analysis of existing human studies summarized across 39 case–control, 4 cohort, and 3 cross-sectional studies and reported an overall 62 % increase in risk for PD with ever/never pesticide exposure (OR 1.62; 95 % confidence interval (CI) 1.40–1.88) [25]. Yet, the authors strongly recommended that “future studies should focus on more objective and improved methods of pesticide exposure assessment.”

Sources of Pesticide Exposures in Humans

In the USA, the widespread use of pesticides in agriculture and homes and gardens makes it virtually impossible to completely avoid exposure via food intake, treatment of

Table 1 Genes investigated for Parkinson's disease and pesticide-related GxE interactions

Gene	Gene function	Relevant variant/s	Variant/s function
<i>DAT/SLC6A3</i>	Encodes a dopamine transporter, responsible for the reuptake of dopamine into presynaptic neurons	5' clade and 3' VNTR 9-repeat	Clade: conflicting evidence, but likely increased function/levels with clade A; VNTR: 9-repeat associated with higher striatal DAT
<i>ABCB1</i>	Encodes P-glycoprotein 1, responsible for transporting many substances in and out of cells and across the BBB	rs1045642 and rs2032582	rs1045642 has been shown to alter substrate specificity and rs2032582 alters P-gp expression
<i>PON1</i>	Encodes the paraoxonase enzyme, which detoxifies organophosphates	L55M and Q192R	L55M & Q192R influence enzyme activity, M or Q allele carriers are considered "slower" metabolizers
<i>NOS1</i>	Encodes neuronal nitric oxide synthase, which synthesizes nitric oxide	rs2682826	Unknown function; tag SNP
<i>ALDH2</i>	Encodes ALDH, which metabolizes biogenic amine-related aldehydes, including the highly toxic dopamine metabolite DOPAL	rs737280	Unknown function; tag SNP
<i>SKP1</i>	Encodes s-phase kinase-associated protein 1, a key component of E3 ubiquitin ligases	rs2284312	Unknown function; tag SNP
<i>HLA-DR</i>	Encodes HLA-DR, an MCH class II cell surface receptor responsible for presenting peptide antigens to immune cells to a elicit T-cell response	rs3129882	Unknown function; GWAS discovered tag SNP

VNTR variable number tandem repeat, *P-gp* P-glycoprotein 1, *ALDH* aldehyde dehydrogenase, *SNP* single nucleotide polymorphism, *MCH* major histocompatibility complex, *GWAS* genome-wide association study, *DOPAL* 3,4-dihydroxyphenylacetaldehyde, *BBB* blood-brain barrier

structures, or drift from large-scale applications. Insecticides are purposefully designed to be acutely neurotoxic. They may modulate the function of voltage-gated sodium channels or affect central γ -amino butyric acid, noradrenergic, dopaminergic, and cholinergic neurotransmission [26]. Other pesticide classes, such as herbicide and fungicides, do not purposefully target the nervous system but have been found to affect mechanisms relevant for neurodegeneration such as mitochondrial inhibition, oxidative stress, aldehyde dehydrogenase or proteasome inhibition, and neuroinflammation [17, 18].

Pesticides often expose agricultural communities, sometimes over substantial distances, as they travel in the air through spray drift and postapplication volatilization [27–29]. Some agricultural-use pesticides are highly persistent and can be found in treated soils and dust even decades after application, indoors [30–32] and in ambient air [33, 34]. A California central valley study assessed inhalation risks from airborne agricultural pesticides and concluded that agricultural applications of organophosphates and their oxon products may have substantial volatilization and off-field movement [35]. This was confirmed by a monitoring study conducted in a small community, where California Environmental Protection Agency (Cal EPA) placed air monitors on elementary school roofs over a 1-year period and detected 23 pesticides known to be applied in the area according to the CAPUR system [36]. Additionally, a northern California study found that crops within 1500 m of homes predicted carpet dust levels of pentachlorophenol and DDE [37]. Thus, pesticides from agricultural applications find their way into homes,

exposing residents in agricultural communities to pesticides, even those not involved in agricultural work.

Transport of Toxicants: DAT and the Pesticides Paraquat and Maneb

A recent review addressing the neurotoxicity of paraquat concluded that while evidence for paraquat being a risk factor for Parkinson's disease is accumulating from experimental, clinical, and epidemiological data, genetic variations associated with susceptibility to paraquat may be responsible for risk differences observed across populations [38]. Thus, gene-environment interactions may be crucial for resolving heterogeneity of results reported in human pesticide studies.

We published the first human data showing that paraquat and maneb act synergistically in increasing PD risk [39]. Furthermore, genetic variability in the 5'A clade and the 3' VNTR 9-repeat of the human dopamine transporter (*DAT/SLC6A3*) locus was shown to increase susceptibility of humans to occupational pesticide exposure [40], especially two evolutionary clades (A and B) suspected to affect gene expression [41, 42]. Risk was strongly increased among pesticide-exposed male farmers who were carriers of multiple *DAT* susceptibility alleles (Table 2). Relying on exposure assessment most similar to the previous study, our JEM-derived occupational pesticide measure corroborated the following finding: PD risk was found to be increased among pesticide-exposed males who were carriers of *DAT/SLC6A3* susceptibility alleles (Table 2) [43]. We also observed an even larger

Table 2 Results from gene–pesticide (GxE) analyses: Parkinson’s Environment and Genes (PEG) and related GxE studies

Environmental exposure	Genetic factor					
	N cases/ controls	Adj OR (95 % CI)	N cases/ controls	Adj OR (95 % CI)	N cases/ controls	Adj OR (95 % CI)
Dopamine transporter— <i>DAT</i> / <i>SLC6A3</i> (5' A clade and 3' VNTR 9-repeat)						
Kelada et al. [41]						
Occupationally unexposed	23/37	1.00	1 susceptibility allele	49/60	1.21 (0.62, 2.36)	≥2 susceptibility alleles
Occupationally exposed ^a	7/17	1.00	0 susceptibility alleles	14/23	1.63 (0.52, 5.15)	59/87
PEG (Ritz et al. [43])						
Occupationally unexposed (JEM)	26/26	1.00	0 susceptibility alleles	1 susceptibility allele		≥2 susceptibility alleles
Occupationally exposed (JEM) ^b	13/18	1.00	1 susceptibility allele	34/46	0.70 (0.34, 1.45)	28/18
PEG (Ritz et al. [43])						
No/low ambient paraquat and maneb exposure at residences	65/80	1.00	0 susceptibility alleles	1 susceptibility allele		≥2 susceptibility alleles
High ambient paraquat and maneb exposure at residences ^c	4/5	0.88 (0.22, 3.48)	0 susceptibility alleles	99/117	0.98 (0.63, 1.52)	122/122
Multi-drug resistance protein 1— <i>ABCB1</i> (rs1045642 and rs2032582)						
Drozdziak et al. ^d [44]— <i>ABCB1</i> rs1045642	CC	–	–	CT, TT	4.9 (1.7, 14.6)	24/6
Unexposed/exposed PD cases	19/7	–	–	29/52		
Zschiedrich et al. ^d [45]— <i>ABCB1</i> rs1045642	CC	–	–	CT, TT		
Unexposed/exposed PD cases	24/2	–	–	43/17	4.74 (1.01, 22.31)	
Dutheil et al. [46]— <i>ABCB1</i> rs1045642	CC, CT	1.00	1.00	TT		
Occupationally OC unexposed	27/79	1.00	1.00	8/30	0.9 (0.3, 2.5)	
Occupationally OC exposed	27/55	1.7 (0.7, 4.3)	1.7 (0.7, 4.3)	15/16	7.2 (2.1, 24.8)	
Dutheil et al. [46]— <i>ABCB1</i> rs2032582	GG, GT, GA	1.00	1.00	TT, TA		
Occupationally OC unexposed	30/88	1.00	1.00	5/21	0.90 (0.3, 2.7)	
Occupationally OC exposed	29/60	1.90 (0.8, 4.5)	1.90 (0.8, 4.5)	13/11	7.90 (2.2, 28.9)	
PEG (Narayan et al. [47]—risk score (<i>ABCB1</i> rs1045642 & rs2032582))						
Low OC exposure	41/126	1.00	≤1 susceptibility allele	≥2 susceptibility alleles		
Frequent household OC use without occupational pesticide exposure	24/59	1.62 (0.86, 3.06)	1.62 (0.86, 3.06)	14/22	2.25 (1.01, 5.02)	
Occupational exposure to other pesticides	55/90	1.89 (1.13, 3.18)	1.89 (1.13, 3.18)	4/18	0.68 (0.21, 2.20)	
Ambient workplace OC exposure and/or self-reported occupational OC use	95/151	1.96 (1.23, 3.14)	1.96 (1.23, 3.14)	19/15	4.30 (1.89, 9.74)	
PEG (Narayan et al. [47]—risk score (<i>ABCB1</i> rs1045642 & rs2032582))						
Low OP exposure	41/126	1.00	≤1 susceptibility allele	≥2 susceptibility alleles		
Frequent household OP use without occupational pesticide exposure	24/59	1.62 (0.86, 3.07)	1.62 (0.86, 3.07)	14/22	2.25 (1.01, 5.02)	
Occupational exposure to other pesticides	30/56	1.69 (0.92, 3.10)	1.69 (0.92, 3.10)	4/18	0.68 (0.21, 2.20)	
Ambient workplace OP exposure and/or self-reported occupational OP use	121/185	2.05 (1.30, 3.22)	2.05 (1.30, 3.22)	8/7	2.62 (0.87, 7.86)	
				36/35	3.71 (1.96, 7.02)	

Table 2 (continued)

Environmental exposure	Genetic factor					
	N cases/ controls	Adj OR (95 % CI)	N cases/ controls	Adj OR (95 % CI)	N cases/ controls	Adj OR (95 % CI)
Paraoxonase— <i>PONI</i> (L55M and Q192R)						
PEG (Lee et al. [48])— <i>PONI</i> L55M-Q192R	55LL-192RR		55LM-192QR		55MM-192QQ	
No ambient diazinon exposure	6/14	1.00	53/100	1.16 (0.41, 3.27)	6/14	1.16 (0.29, 4.62)
Low/high ambient diazinon exposure ^e	19/33	1.10 (0.35, 3.47)	133/175	1.47 (0.53, 4.05)	28/23	2.43 (0.78, 7.56)
PEG (Lee et al. [48])— <i>PONI</i> L55M-Q192R	55LL-192RR		55LM-192QR		55MM-192QQ	
No ambient chlorpyrifos exposure	6/14	1.00	53/100	1.18 (0.42, 3.33)	6/14	1.14 (0.29, 4.58)
Low/high ambient chlorpyrifos exposure ^e	15/28	1.19 (0.36, 3.92)	115/137	1.77 (0.64, 4.92)	26/17	3.28 (1.02, 10.58)
PEG (Lee et al. [48])— <i>PONI</i> L55M-Q192R	55LL-192RR		55LM-192QR		55MM-192QQ	
No ambient parathion exposure	6/14	1.00	53/100	1.16 (0.41, 3.28)	6/14	1.14 (0.28, 4.55)
Low/high ambient parathion exposure ^e	15/29	0.99 (0.30, 3.25)	117/146	1.57 (0.56, 4.37)	22/16	2.56 (0.78, 8.39)
PEG (Narayan et al. [14])— <i>PONI</i> Q192R	RR+RQ		QQ			
Occasional household OP use	74/133	1.00	75/120	1.09 (0.72, 1.65)		
Frequent household OP use	28/48	1.03 (0.58, 1.82)	37/24	2.62 (1.42, 4.83)		
Nitric oxide synthase— <i>NOS1</i> (rs2682826)						
Hancock et al. ^f [49]						
Unexposed	CC		CT+TT			
Exposed	N/A	1.00	N/A	1.00		
PEG (Paul et al. [50])	N/A	3.52 (1.78, 6.95)	N/A	1.23 (0.63, 2.40)		
Occasional household OP use	CC		CT+TT			
Frequent household OP use	81/109	1.00	70/104	0.88 (0.58, 1.39)		
PEG (Paul et al. [50])	32/39	1.30 (0.72, 2.34)	41/22	2.84 (1.49, 5.40)		
No/low ambient exposure	CC		CT+TT			
High ambient OP exposure ^g	117/169	1.00	116/162	0.99 (0.70, 1.40)		
Aldehyde dehydrogenase— <i>ALDH2</i> (clade 1/2 primarily determined by rs737280)	33/18	2.42 (1.27, 4.61)	43/12	4.83 (2.39, 9.73)		
PEG (Fitzmaurice et al. [21])						
Unexposed to all ALDH-inhibiting pesticides ^h	Clade 1/1		Clade 1/2 or 2/2			
Exposed to any number of pesticides at residences, unexposed at workplace addresses	76/124	1.00	92/160	1.00		
Exposed to any number of pesticides at residences, unexposed at workplace addresses	29/38	1.17 (0.65, 2.10)	20/49	0.73 (0.41, 1.32)		
Exposed to any number of pesticides at workplace addresses, unexposed at residences	24/33	1.16 (0.63, 2.16)	29/46	1.14 (0.66, 1.95)		
Exposed to 1 or 2 pesticides at residences and workplace addresses	22/23	1.58 (0.80, 3.10)	17/13	2.21 (1.01, 4.82)		
Exposed to ≥3 pesticides at residences and 1 to 2 pesticides at workplace addresses	5/6	1.21 (0.35, 4.23)	8/7	1.74 (0.61, 5.03)		

Table 2 (continued)

Environmental exposure	Genetic factor					
	N cases/ controls	Adj OR (95 % CI)	N cases/ controls	Adj OR (95 % CI)	N cases/ controls	Adj OR (95 % CI)
Exposed to ≥ 3 pesticides at workplace addresses and 1 to 2 pesticides at residences	4/7	0.92 (0.25, 3.43)	7/4	2.80 (0.78, 10.0)		
Exposed to ≥ 3 pesticides at residences and workplace addresses	9/5	2.47 (0.78, 7.82)	11/3	5.30 (1.42, 19.8)		
S-phase kinase-associated protein 1— <i>SKP1</i> (rs2284312)						
PEG (Rhodes et al. [51])	CC		CT/TT			
Low/no ambient exposure to UPS inhibiting pesticides ⁱ	79/148	1.00	29/52	1.01 (0.59, 1.73)		
High exposure at either workplace addresses or residences	68/103	1.25 (0.83, 1.90)	33/37	1.65 (0.95, 2.85)		
High exposure at both workplace addresses and residences	43/50	1.62 (0.98, 2.66)	29/7	7.57 (3.14, 18.3)		
Major histocompatibility complex class II region:						
<i>HLA-DR</i> (rs3129882)						
PEG (Kannarkat et al. [52])— additive genetic model	AA		AG		GG	
No ambient pyrethroid exposure	95/117	1.00	172/161	0.91 (0.73, 1.13)	50/81	0.82 (0.53, 1.27)
Ambient exposure to at least 1 pyrethroid ⁱ	47/58	0.83 (0.53, 1.28)	71/66	1.25 (0.88, 1.78)	30/14	1.87 (1.08, 3.35)
PEG (Kannarkat et al. [52])— model includes homozygous genotypes only	AA		GG			
No ambient pyrethroid exposure	95/117	1.00	50/81	0.73 (0.47, 1.14)		
Ambient exposure to at least 1 pyrethroid ⁱ	47/58	1.04 (0.65, 1.67)	30/14	2.48 (1.24, 4.97)		

OR odds ratio, OP organophosphates, OC organochlorine pesticides, JEM job exposure matrix, N/A not available

^a Subjects considered exposed if reported occupation for 6+ months involved pesticide exposure

^b JEM based occupational pesticide exposure (males only, N = 347)

^c Ambient exposure at residential addresses

^d Case-only analysis

^e Ambient exposure at residential and workplace addresses

^f Family-based study, self-report pesticide exposure

^g Ambient OP exposure at residential and/or workplace addresses

^h Ambient exposure at residential and workplace addresses to ALDH-inhibiting pesticides (benomyl, mancozeb, ziram, zineb, triflumizole, captan, dieldrin)

ⁱ Ambient exposure at residential and workplace addresses to UPS-inhibiting pesticides (benomyl, cyanazine, dieldrin, endosulfan, ferbam, metam, propargite, rotenone, triflumizole, ziram)

^j Ambient exposure at residential and/or workplace addresses to pyrethroid pesticides (fenvalerate, permethrin, phenothrin, resmethrin, flucythrinate, cypermethrin, (S)-cypermethrin, tau-fluvalinate, fenpropathrin, lamda-cyhalothrin, bifenthrin, esfenvalerate, and tralomethrin)

increase in risk for those with high ambient residential exposure to both maneb and paraquat and two or more *DAT/SLC6A3* susceptibility alleles (Table 2).

In organotypic midbrain culture, the coadministration of a DAT inhibitor prevents dopaminergic cell death caused by paraquat treatment [53]. While paraquat in its native divalent cation state (paraquat²⁺ N,N'-dimethyl-4,4'-bipyridinium) is not a substrate for DAT [54], microglia can convert it to the monovalent cation paraquat(+), which is a substrate for DAT and induces oxidative stress and cytotoxicity [55]. Also, impaired DAT function in cultured cells and mutant mice attenuates paraquat neurotoxicity [55]. *DAT/SLC6A3* polymorphisms may generate a selective vulnerability to paraquat in dopaminergic neurons. A human imaging study measured striatal DAT availability with [123I]β-CIT SPECT in 79 healthy young adults and genotyped both the 40-base-pair *VNTR* in the 3' region of the *DAT/SLC6A3* gene as well as the single nucleotide polymorphisms rs2652511 and rs2937639 in the 5' end of the gene [56]. *DAT VNTR* 9-repeat carriers exhibited higher striatal DAT availability than 10-repeat homozygotes. The haplotype T-A-9 repeat (rs2652511-rs2937639-*VNTR*) was associated with higher striatal DAT expression than other haplotypes [56]. Thus, a combination of polymorphisms in both the 3' and the 5' ends of the *DAT/SLC6A3* gene seems to be associated with in vivo striatal DAT expression in the same direction expected by our GxE results.

Our DAT pesticide results are in agreement with Kelada et al. [40]. They suggest that the gene encoding the dopamine transporter might increase susceptibility for pesticides such as paraquat. Our GIS-derived, record-based residential pesticide exposure measures added specificity to the human exposure assessment. Instead of relying solely on a general occupational measure, we were able to examine the chemical of most interest based on the putative role the dopamine transporter plays in paraquat neurotoxicity.

Transport of Toxicants: the Multidrug Resistance Protein 1 (ABCB1) and Organophosphorus and Organochlorine Pesticides

The P-glycoprotein, encoded by the multidrug resistance protein 1 (*MDR1* or *ABCB1*) gene, is thought to protect the brain against neurotoxicants. *ABCB1* genetic variants known to affect transporter function might influence PD risk. Animal and cell studies have suggested that some pesticides are removed from blood–brain barrier endothelial cells by P-glycoprotein [57]. Specifically, many lipophilic and amphipathic xenobiotic compounds, including several organochlorines and organophosphates, are P-glycoprotein substrates and they may stimulate or inhibit its transporter activity or modulate P-glycoprotein expression [58–60]. Genetic variants of importance for transporter function that have been studied most are

the following two SNPs: a synonymous mutation in rs1045642—possibly altering substrate specificity [61]—and a missense mutation in rs2032582 [62]. Homozygous TT genotypes at rs1045642 and rs2032582 have been related to lower protein expression [63].

For our population, we created a genetic risk score that included the *ABCB1* SNPs rs1045642 and rs2032582 and counted the number of variant or “susceptibility” alleles. Ambient work place and residential address-derived organophosphorus (OP) and organochlorine (OC) pesticide exposure as well as household or occupational OP or OC pesticide use increased PD risk as much as twofold in participants with one or less susceptibility alleles, but three to fourfold in OP or OC exposed carriers of at least two susceptibility alleles (Table 2) [47].

Three prior studies in European populations reported on interactions between pesticides and polymorphisms in *ABCB1* and PD [44–46]. While the first two studies used a case-only design and reported over fourfold increased risks in pesticide exposed T allele carriers of rs1045642, both studies were small and only able to generically assess pesticide exposure (Table 2) [44, 45]. The third study [46] focused on an agricultural population mainly with OC exposure from professional farming-related use and found those exposed to OC pesticides in combination with being carriers of the rs1045642 TT genotype to be at highest risk of PD compared with unexposed C allele carriers (OR = 7.2 (95 % CI 2.1, 24.8) [46]. Also, exposed farmers with a TT or TA genotype in rs2032582 exhibited 7.9 times the risk of PD compared with unexposed G allele carriers (95 % CI 2.2, 28.9) (Table 2). In conclusion, experimental data described P-glycoprotein's role in xenobiotic transport across the blood–brain barrier. Our results support several prior studies that showed that pesticide exposures contributed to PD risk in combination with variants in *ABCB1* that may increase susceptibility to xenobiotics and suggest a role specifically for OC or OP pesticides.

Metabolism of Toxicants: the Paraoxonase Enzyme (PON1) Detoxifies Organophosphorus Pesticides

We have previously reported that OP pesticides increase PD risk [64]. The paraoxonase enzyme (PON1) detoxifies organophosphorus compounds and, importantly, enzyme serum activity varies up to 40-fold in populations [65, 66]. This variation has been attributed to a few important genetic polymorphic sites, including the *PON1* 192QQ, the 55MM, and the C-108T genotypes [67]. Based on functional research, those with an MM genotype at L55M and QQ or QR at Q192R are considered “slower” metabolizers [68]. Polymorphisms in this gene are not marginally associated with PD [69].

In our population, we assessed whether the three *PON1* SNPs modified PD risk from three commonly used organophosphate pesticides: diazinon, chlorpyrifos, and parathion. Assessing the risk by genotype separately or jointly using

the L55M/Q192R diplotype showed an increased risk for PD with ambient exposures in homozygous variant genotype carriers (slow metabolizers) as compared with fast metabolizer diplotype carriers (OR = 2.43, 95 % CI = 0.78–7.56 to OR = 3.28, 95 % CI = 1.02–10.58) (Table 2) [48]. Without OP exposure, slow metabolizer diplotype carriers were not at increased risk of developing PD. Furthermore, we saw the same for exposures to household pesticide products that contain OPs as active ingredients, i.e., carriers of the *PONI* 55MM-192QQ diplotype using OPs in homes or gardens were at the highest risk of developing PD relative to low/unexposed non-carriers (Table 2) [14].

Two studies in populations of European ancestry previously investigated pesticide interactions with *PONI* SNPs in PD. The first identified only nine pesticide-exposed subjects with the *PONI*192RR genotype and found no interaction [70]. The second was a large European multicenter study [71]. They did not find interactions between *PONI* and occupational pesticide use. However, the mostly clinic-based enrollment of prevalent PD cases in this study might have introduced survival bias in cases and controls. Importantly, including participants from different European countries increased the heterogeneity of the pesticide measure and occupational practices. Thus, no previous studies have investigated OP pesticide–*PONI* interactions sufficiently and to date, ours is the only study to suggest that *PONI* variants identifying slow metabolizers of OP pesticides contribute to PD risk in OP-exposed subjects.

Toxicants Interacting with Biologic Pathways Relevant for PD: Nitric Oxide Synthase (NOS1) and Organophosphorus Pesticides

The nitric oxide synthase (NOS) enzymes catalyze reactions that generate free radicals including nitric oxide (NO), a chemical messenger used in neurotransmission that can also contribute to oxidative/nitrosative stress and damage dopaminergic neurons [72]. Further, NO readily reacts with superoxides to form peroxynitrite (NO₃⁻), a potent toxin able to irreversibly inhibit mitochondrial respiration [72–74]. The *NOS1* gene encodes neuronal NOS expressed in the brain [75]. While polymorphisms in *NOS1* have been associated with PD risk in some studies [49, 76–79], GWAS did not identify a role for the gene in PD [4]. Nevertheless, experimental evidence strongly supports a neurotoxic role for NOS since inhibition or absence of neuronal NOS prevents MPTP-induced Parkinsonism [80, 81]. Furthermore, in the nigrostriatal region of postmortem human PD brains, NO levels were found to be increased [82].

An early study investigated GxE interactions with pesticides relying on a family-based design and described interactions between self-reported home pesticide use and a *NOS1* rs2682826 variant for PD [49]. We identified interactions

between pesticides and *NOS1* rs2682826, though in the opposite direction to what was previously reported. We not only found interactions with household use of OPs but also based on our PUR-derived ambient exposures from agriculturally applied OPs. Concerning household OP use, frequent users carrying the T allele were at highest risk (OR = 2.84, 95 % CI = 1.49–5.40; Table 2); high ambient OP exposures at both residential or workplace addresses increased risk most strongly in T allele carriers (Table 2) [50]. Furthermore, three additional *NOS1* SNPs, rs1047735, rs1816353, and 3741480, also showed similar interactions with all of our OP exposure measures (data not shown) [50].

Our results suggest that reactive oxygen or nitrogen species (ROS/RNS) from multiple sources may potentiate each other, increasing the stress in dopamine neurons beyond their capacity to cope. We identified gene–pesticide interactions and estimated strong effects for exposure to common home and agricultural use OP pesticides and PD in genetically susceptible subjects carrying *NOS1* variant alleles. This human data supports the hypothesis that oxidative/nitrosative stress-inducing mechanisms may be involved in OP pesticide effects on PD [83].

Toxicants Interacting with Biologic Pathways Relevant for PD: ALDH2 and ALDH-Inhibiting Pesticides

Aldehyde dehydrogenase (ALDH) metabolizes biogenic amine-related aldehydes, including the highly toxic dopamine metabolite 3,4-dihydroxyphenylacetaldehyde (DOPAL), protecting neurons against aldehyde- and oxidative stress-related neurotoxicity. Thus, inhibition or lower function of this enzyme may be a mechanism for dopaminergic neuron loss in PD. ALDH acts in the inner mitochondrial membrane and the enzymatic activity of mitochondrial ALDH was found to be increased in postmortem brains of PD patients [84]. The pesticide rotenone was recently shown to decrease intracellular ALDH [85], while on the other hand, activation of ALDH was neuroprotective in rotenone-induced cell and animal models of Parkinsonism [86]. Cellular ALDH enzymes are sensitive to oxidative stress and lipid peroxidation, and we previously reported that the fungicide benomyl damages dopaminergic neurons by inhibiting ALDH enzyme activity in vitro and in vivo and is associated with increased PD risk [87].

In a laboratory screen, we identified multiple neuronal ALDH-inhibiting pesticides: benomyl, captan, dieldrin, mancozeb, maneb, triflumizole, zineb, and ziram to which our PEG study participants were exposed [21]. We found a trend of increasing risk with ambient exposures at both workplace and residential addresses with a 3.5-fold increase in PD risk (95 % CI 1.51–8.30) for exposure to three or more of these pesticides [21]. The *ALDH2* gene haplotypes in our study population clustered into two clades, primarily determined by the genotype at rs737280. The presence of one or

two copies of the less common clade (clade 2) modified the association between exposure to ALDH-inhibiting pesticides and PD; there was a 2- to 5-fold increase in PD risk for subjects with high exposure who carried at least one copy of clade 2 in *ALDH2* compared with unexposed subjects homozygous for clade 1 (Table 2). This provides the very first evidence for the relevance of ALDH inhibition in PD pathogenesis and identifies new GxE pesticide interactions with relevance for PD.

Toxicants Interacting with Biologic Pathways Relevant for PD: SKP1 Gene and UPS-Inhibiting Pesticides

Parkinson's disease shares protein misfolding, aggregation, and deposition as a disease mechanism with other neurodegenerative diseases. One of the elaborate pathways neurons use to remove damaged protein is the ubiquitin-proteasome system (UPS). Ever since rare familial PD has been linked to genetic defects in the E3 ubiquitin ligase gene *parkin* (*PARK2*) and in *ubiquitin carboxyl-terminal hydrolase L1* (*UCHL1*) [88], the UPS has been a candidate pathway for PD etiology. This hypothesis gained further support from experimental studies in cultured cells and animals as well as human post-mortem brain tissue [89]. In Parkinson's, Lewy bodies are cytoplasmic accumulations of damaged proteins that contain not only α -synuclein protein, but also ubiquitin and UCHL1 [90] as well as other damaged proteins not removed by the UPS or autophagy [91]. Permanently raised levels of α -synuclein impair the UPS, which in turn leads to further α -synuclein accumulation [92]. Chemical agents that induce oxidative stress and damage proteins may contribute to this process.

The UPS breaks down proteins in multiple steps starting with the activation of ubiquitin by E1 enzyme, followed by conjugation of activated ubiquitin to an E2 enzyme, ubiquitination of proteins via an E3 ligase, breaking down of ubiquitinated proteins in the 26S proteasome, and finally, the recycling of ubiquitin by deubiquitinating enzymes such as the *UCHL1* [93]. Previous studies also identified pesticides that inhibit the proteasome in model systems [19, 94–96].

Thus, we investigated interactions between UPS-inhibiting pesticides our study participants were exposed to in PEG (propargite, cyanazine, dieldrin, endosulfan, benomyl, carbendazim, triflumizole, ferbam, metam, ziram) and six candidate genes in the UPS pathway and found evidence for interaction with *s-phase kinase-associated protein 1* (*SKP1*). Specifically, we found that the combination of high ambient exposure in carriers of at least one T allele in *SKP1* rs2284312 resulted in a considerably larger risk of PD (OR of 7.57; Table 2) [51]. The function of rs2284312 is unknown, but the *SKP1* gene encodes an E3 enzyme involved in target identification for ubiquitination [97] and expression of *SKP1* in PD brains has been reported to be downregulated [98]. In addition, a cell

model of murine substantia nigra found a decrease in *SKP1* after treatment with MPTP [99].

The increase in PD risk with exposures at both residential and workplace addresses for the UPS-inhibiting pesticides and their interaction with the polymorphism in the *SKP1* gene that encodes an important enzyme in the UPS pathway is intriguing but needs to be further investigated.

Toxicants Interacting with Biologic Pathways Relevant for PD: HLA-DR Influenced Immune Response and Pyrethroid Exposures

Inflammation has widely been suspected to contribute to PD etiology, with increased expression of inflammatory cytokines and altered composition of peripheral immune cells found in serum and spinal fluid of PD patients, as well as microglial activation and lymphocyte invasion found in areas of degeneration in postmortem PD brains [52]. The major histocompatibility complex class II (MHC-II) region, specifically *HLA-DR* rs3129882, implicated in idiopathic PD in multiple populations and in GWAS [2, 4], is responsible for antigen presentation to the adaptive immune system. It has, however, also been suggested that associations between MCH-II and PD may depend on environmental exposures such as pesticides. Environmental exposures have been found to influence inflammation, for example, by impacting antigen presentation [100].

Thus, we examined interactions between immunomodulatory pesticides, including the OPs, OCs, and pyrethroids, and rs3129882 of *HLA-DR* in PD. We found positive interactions when comparing groups homozygous at the SNP (AA versus GG; Table 2) and when we used an additive genetic model to include heterozygous individuals among those exposed to pyrethroids. Interestingly, neither the genotype nor pyrethroid exposure alone influenced PD risk, but in those exposed to pyrethroids and carrying the GG genotype, PD risk was increased two and a half fold compared with unexposed AA carriers (Table 2). This pesticide–gene interaction suggests that variation in this MHC-II region together with exposure to pyrethroids, pesticides shown to modulate the immune system, influence PD risk. However, these associations need to be reexamined in other populations.

Conclusions

A review of 38 epidemiologic studies on pesticides and PD published almost a decade ago concluded that while general pesticide–PD association were reported consistently, evidence was lacking for causal relationships with any particular pesticide compound or for combinations of pesticide exposures in humans [7]. This conclusion was recently repeated in several reviews stating that although classes of pesticides have been

linked to PD, attention still needs to be directed to identify specific chemicals in human studies [25•]. Further recommendations were that detailed quantitative exposure data for humans should be accompanied by determinations of genetic polymorphisms [101], especially those that increase genetic susceptibility in metabolism, elimination, and transport of pesticides [102]. The epidemiologic studies we conducted in California in the past decade in combination with evidence from other human GxE studies and experimental studies are starting to move us closer to reaching these goals.

Different from most previous epidemiologic studies of PD, all of our diagnoses were clinically confirmed in one or more examination(s) by movement disorder specialists. Thus, we expect disease misclassification to be minimal. Additionally, population controls were drawn from the same region as the cases, likely providing adequate representativeness of the source population. We estimated pesticide exposure from multiple sources—household, occupational use, and ambient from agricultural uses—and our GxE results mutually corroborated each other when using these various exposure assessment methods. We were able to assess exposures to specific chemicals based on our GIS approach that used the unique CA-PUR records and examined GxE interactions taking existing knowledge about biologic/toxicological pathways into account (Fig. 1). We employed pathway-specific

knowledge for pesticides that cause Parkinsonism in animal models and linked *DAT/SLC6A3* variants likely affecting transport of specific chemicals into dopaminergic neurons to an increased risk from paraquat and maneb exposures. We learned that maneb is a UPS-inhibiting chemical and that genetic vulnerability in this pathway together with chemicals affecting the UPS contributes to PD risk. We confirmed previous reports that the metabolizing enzyme PON1 increases susceptibility to PD for OP-pesticide exposed individuals who are considered slow metabolizers. We found vulnerability for OP exposures in carriers of *NOS1* gene variants that may contribute to the nitrosative stress pathway and—finally—corroborated a previous report that OC effects on PD risk may depend on *ABCB1* gene variants that impact blood–brain barrier transport of chemicals. We were able to identify a group of ALDH-inhibiting pesticides and to show associations with PD for some common *ALDH2* gene variants that increased susceptibility to these specific agents. Finally, for the latest chemicals to come into widespread agricultural and in-home use, pyrethroid insecticides, we found that they may be responsible for increased immune response among *HLA-DR* variant carriers putting them at an increased risk of developing PD.

Exploring additional gene–pesticide interactions with candidate genes in pathomechanistically relevant pathways for

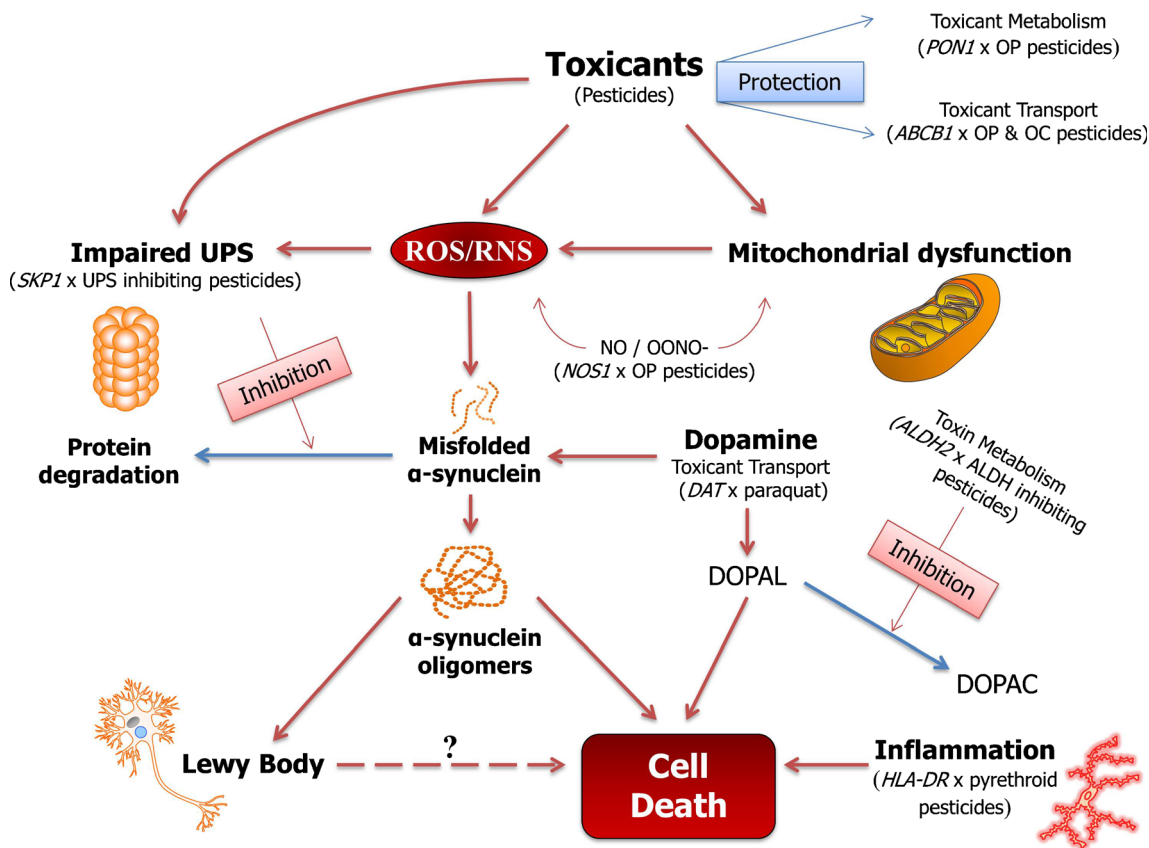


Fig. 1 Proposed Parkinson’s disease pathological mechanisms involving discussed GxE reports

PD and pesticides is possible and needed. However, the past decade of research illustrates that it is unlikely that a single pathway/mechanism or a single pesticide is responsible for the complex etiology of neurodegeneration. The constant change in potentially neurotoxic agents used for pest control worldwide requires continued research and surveillance to protect humans from the long-term effects of neurotoxic agents.

Acknowledgments This work was funded in part by NIEHS Grants R01-ES010544, U54-ES012078, P01ES016732; the Michael J. Fox Foundation; The Parkinson Alliance, and the American Parkinson Disease Association. The funding organizations had no role in the design, conduct, interpretation, or publication of this work. Kim Paul also reports support from the Burroughs Wellcome Fund.

Compliance with Ethical Standards

Conflict of Interest BR Ritz, KC Paul, and JM Bronstein declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent *Standard Protocol Approvals and Patient Consents*

Written informed consent was obtained from all enrolled subjects. All procedures using animals were approved by the UCLA Human Subjects Committee.

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