Title

Aldehyde dehydrogenase variation enhances effect of pesticides associated with Parkinson disease



Journal

Neurology, 82(5)

ISSN

0028-3878

Authors

Fitzmaurice, Arthur G

Rhodes, Shannon L

Cockburn, Myles

et al.

Publication Date

2014-02-04

DOI

10.1212/wnl.0000000000000083

Peer reviewed

# Aldehyde dehydrogenase variation enhances effect of pesticides associated with Parkinson disease

Arthur G. Fitzmaurice, PhD*
Shannon L. Rhodes, PhD*
Myles Cockburn, PhD
Beate Ritz, MD, PhD
Jeff M. Bronstein, MD, PhD

Correspondence to Dr. Bronstein: jbronste@mednet.ucla.edu

## ABSTRACT

**Objective:** The objective of this study was to determine whether environmental and genetic alterations of neuronal aldehyde dehydrogenase (ALDH) enzymes were associated with increased Parkinson disease (PD) risk in an epidemiologic study.

**Methods:** A novel ex vivo assay was developed to identify pesticides that can inhibit neuronal ALDH activity. These were investigated for PD associations in a population-based case-control study, the Parkinson's Environment & Genes (PEG) Study. Common variants in the mitochondrial *ALDH2* gene were genotyped to assess effect measure modification (statistical interaction) of the pesticide effects by genetic variation.

**Results:** All of the metal-coordinating dithiocarbamates tested (e.g., maneb, ziram), 2 imidazoles (benomyl, triflumizole), 2 dicarboxymides (captan, folpet), and 1 organochlorine (dieldrin) inhibited ALDH activity, potentially via metabolic byproducts (e.g., carbon disulfide, thiophosgene). Fifteen screened pesticides did not inhibit ALDH. Exposures to ALDH-inhibiting pesticides were associated with 2- to 6-fold increases in PD risk; genetic variation in *ALDH2* exacerbated PD risk in subjects exposed to ALDH-inhibiting pesticides.

**Conclusion:** ALDH inhibition appears to be an important mechanism through which environmental toxicants contribute to PD pathogenesis, especially in genetically vulnerable individuals, suggesting several potential interventions to reduce PD occurrence or slow or reverse its progression.

***Neurology*® 2014;82:419–426**

## GLOSSARY

**ALDH** = aldehyde dehydrogenase; ***ALDH2*** = aldehyde dehydrogenase 2 family (mitochondrial); **CI** = confidence interval; **DOPAL** = 3,4-dihydroxyphenylacetaldehyde; **PD** = Parkinson disease; **SNP** = single nucleotide polymorphism; **UCLA** = University of California, Los Angeles.

Parkinson disease (PD) is characterized primarily by death of dopaminergic neurons in the substantia nigra pars compacta,[1] although the reason(s) for this selective vulnerability remains the subject of research. Rare mendelian and high-risk genes as well as common but low-risk genetic variants detected by genome-wide association studies account for only a small percentage of cases,[2] so environmental factors almost certainly have an important role. Pesticide exposure has surfaced as a prominent environmental risk factor in PD,[3–6] and possible modifications of pesticide associations by genetic variants have been reported,[7–10] but the mechanisms through which pesticides contribute to PD pathogenesis remain to be elucidated. We recently reported that the fungicide benomyl was associated with increased PD risk and damaged dopaminergic neurons by inhibiting aldehyde dehydrogenase (ALDH) enzyme activity in vitro and in vivo.[11] Because ALDH detoxifies the dopamine metabolite 3,4-dihydroxyphenylacetaldehyde (DOPAL), its inhibition offers a potential mechanism for the preferential loss of dopaminergic neurons in PD. The present work investigates this mechanism further by identifying several ALDH-inhibiting

**Supplemental data at www.neurology.org**

*These authors contributed equally to this work.

From the Department of Neurology (A.G.F., B.R., J.M.B.), David Geffen School of Medicine at UCLA, Los Angeles; Department of Molecular Toxicology (A.G.F., B.R., J.M.B.), University of California, Los Angeles; Departments of Epidemiology (S.L.R., B.R.) and Environmental Health Sciences (B.R.), UCLA Fielding School of Public Health, Los Angeles; Department of Preventive Medicine (M.C.), Keck School of Medicine of USC, Los Angeles; and Parkinson's Disease Research, Education, and Clinical Center (J.M.B.), Greater Los Angeles Veterans Affairs Medical Center, Los Angeles, CA.

Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

pesticides and determining their associations with PD, as well as effect measure modification by genetic variation, in a population-based, case-control, epidemiologic study.

## METHODS

**Standard protocol approvals and patient consents.** All procedures using animals were approved by the University of California, Los Angeles (UCLA) Animal Research Committee. Written informed consent was obtained from all enrolled subjects; all procedures were approved by the UCLA Human Subjects Committee.

**ALDH activity assays.** Twenty-six pesticides were tested for their effects on neuronal ALDH activity. These compounds were selected to reflect the range of chemical structures of the pesticides applied in the epidemiologic study area. A concentration of 10 μM was selected because the 50% effective concentration values of pesticides against their targeted species are typically well above this concentration.[12–17] ALDH activity was measured in suspensions of neurons derived from the substantia nigra of newborn rats in the presence or absence of test compounds, as described previously.[11] Briefly, mesencephalic neurons (postnatal day 2–9) were dissociated and incubated with the test compound and Aldefluor assay. Intracellular green fluorescence was measured on channel FL1 using fluorescence-activated cell sorting (Beckman XL-MCL; Beckman Coulter, Brea, CA). ALDH inhibition was determined by comparing fluorescence in the presence or absence of test compounds—the lower the fluorescence, the lower the ALDH activity (i.e., inhibition) during incubation.

**Human subjects.** The Parkinson's Environment & Genes Study (PEG) has been enrolling incident PD cases diagnosed no longer than 3 years before recruitment and population controls from 3 rural California counties (Fresno, Tulare, Kern) since 2001. Subject recruitment methods, case definition criteria, and clinical characteristics of the patient population have been described in detail.[5,18–21] Of 1,167 initially invited patients with PD, 563 met the eligibility criteria, of whom 473 (84%) were examined on multiple occasions by UCLA movement disorder specialists who confirmed PD diagnoses according to established criteria,[22] 56 (10%) withdrew or moved away, and 34 (6%) became too ill or died. After examination, 107 patients (24%) were excluded because of diagnoses other than idiopathic PD (e.g., essential tremor, dementia with Lewy bodies, multiple system atrophy, progressive supranuclear palsy) and 6 withdrew before interview, leaving 360 cases enrolled in the study. Of the 1,996 eligible population controls, 1,043 (52%) declined participation, were too ill, or moved, leaving 953 (48%) enrolled. All cases and 816 controls completed a telephone interview for the collection of demographic (age, sex, race/ethnicity, education), risk factor (family history of PD, smoking behavior), and detailed workplace and residential address histories.

**Pesticide exposure assessment.** Pesticide exposure estimates were calculated using a geographic information system–based computer model that has been described in detail.[5,9,23,24] This model incorporates Pesticide Use Reporting forms mandated since 1974 by the California Department of Pesticide Regulation that provide information on the location, date, and amounts of active ingredients in each commercial pesticide application. Annual ambient pesticide exposure was assumed to be proportional to the amount of pesticide applied to crop acreage within a 500-m radius surrounding the subject's address and summed over the 26-year period 1974–1999, allowing for a lag between exposure and PD diagnosis. Geocoded workplace and residential addresses were considered separately and accounted for subject mobility. A subject was considered exposed to a particular pesticide if the subject's ambient exposure estimate for that pesticide was greater than or equal to the median level of exposure observed in exposed controls. Our prior work suggested that subjects with ambient exposure to a pesticide at both workplace and residential addresses have a higher risk than subjects exposed at workplace alone or residence alone, and that subjects with exposure at workplace have a higher risk than subjects exposed at residence alone.[5] Therefore, for the individual pesticide analyses, an ordinal variable was constructed to represent this potentially increasing trend (table 1). Measures that combined exposures to any ALDH-inhibiting pesticide and exposures at either workplace or residential address were also considered. To construct the combined exposure measure, the number of pesticides to which a subject was exposed at residential addresses was summed and subjects were assigned to 1 of 3 groups: exposed to 3 or more pesticides (using an upper quartile cutoff), exposed to 1 or 2 pesticides, or unexposed to all ALDH-inhibiting pesticides at residential addresses. This process was repeated for pesticide exposure at workplace addresses, and these categories were combined into a 7-level ordinal exposure measure (table 2). The reference group for all analyses contained only subjects unexposed (less than median) at both residential and workplace addresses to all ALDH-inhibiting pesticides identified in the screen; e.g., when considering benomyl exposure, subjects unexposed to benomyl but exposed above the median to any other ALDH-inhibiting pesticide were excluded from the analysis of benomyl.

**ALDH genotyping.** Haplotypes are groups of associated single nucleotide polymorphisms (SNPs) that capture global gene structure and are identified by high linkage disequilibrium between SNPs[25,26]; a small number of SNPs can capture most of the variation in a region.[27] Haplotype-tagging SNPs for the aldehyde dehydrogenase 2 family (mitochondrial) (National Center for Biotechnology Information geneID: 217, *ALDH2*) were selected using Haploview v.4.2.[28] At the time of this study, 354 cases and 518 controls had provided DNA, which was genotyped for 5 tagSNPs in *ALDH2* (i.e., rs737280, rs968529, rs16941667, rs16941669, and rs9971942) as well as rs671 (i.e., *ALDH2*2*), a genetic variant associated with alcohol sensitivity in persons of East Asian ancestry,[29] using the SNPlex System Array (Applied Biosystems, Carlsbad, CA) and the BioMark HD System (Fluidigm Corporation, South San Francisco, CA). Overall genotyping success rate for included subjects was 97%. Haplotypes were constructed using 5 tagSNPs and clustered into 2 clades based on shared ancestry using eHap v2.0 software.[30]

**Statistical analyses.** The *t* test was used to determine whether screened pesticides significantly inhibited ALDH activity in neuronal suspensions. In epidemiologic analyses, marginal effects of pesticides, marginal effects of genetic variants, and effect measure modification analyses were performed in the subgroup of subjects with both pesticide and genetic data (354 cases, 518 controls). An analysis including all subjects with pesticide data was performed to confirm the robustness of the analyses of the subgroup (tables e-1 and e-2 on the *Neurology*® Web site at www.neurology.org). Odds ratios and 95% confidence intervals (CIs) were estimated by logistic regression, adjusting for age (at diagnosis for cases, at interview for controls), sex (male/female), and smoking status (ever/never), using SAS 9.1 (SAS Institute Inc., Cary, NC). Sensitivity analyses adjusting for home pesticide use,[31] occupational pesticide exposure as defined by a job exposure matrix,[32] and first-degree family history of PD were performed. All genetic markers were assessed for Hardy-Weinberg equilibrium in controls using a

**Table 1** Effect estimates (ORs and 95% CIs) for ambient exposure to individual ALDH-inhibiting pesticides at residential and workplace addresses in association with Parkinson disease

| | Cases | | Controls | | | | | |
|---|---|---|---|---|---|---|---|---|
| Exposure[a] | No. | % | No. | % | OR[b] | OR, adjusted[c] | 95% CI | p Trend |
| Benomyl | | | | | | | | |
| Unexposed to all ALDH-inhibiting pesticides | 168 | 47.6 | 284 | 54.8 | 1.00 | 1.00 | Reference | |
| Exposed to benomyl | | | | | | | | |
| Residence only | 41 | 11.6 | 61 | 11.8 | 1.14 | 1.11 | 0.71–1.73 | |
| Workplace only | 36 | 10.2 | 46 | 8.9 | 1.32 | 1.31 | 0.80–2.12 | |
| Both locations | 33 | 9.3 | 31 | 6.0 | 1.80 | 1.65 | 0.97–2.81 | 0.0591 |
| Captan | | | | | | | | |
| Unexposed to all ALDH-inhibiting pesticides | 168 | 47.6 | 284 | 54.8 | 1.00 | 1.00 | Reference | |
| Exposed to captan | | | | | | | | |
| Residence only | 34 | 9.6 | 49 | 9.5 | 1.17 | 1.15 | 0.71–1.86 | |
| Workplace only | 46 | 13.0 | 60 | 11.6 | 1.30 | 1.25 | 0.81–1.93 | |
| Both locations | 26 | 7.4 | 22 | 4.2 | 2.00 | 1.89 | 1.03–3.47 | 0.049 |
| Dieldrin | | | | | | | | |
| Unexposed to all ALDH-inhibiting pesticides | 168 | 47.6 | 284 | 54.8 | 1.00 | 1.00 | Reference | |
| Exposed to dieldrin | | | | | | | | |
| Residence only | 12 | 3.4 | 6 | 1.2 | 3.38 | 3.08 | 1.13–8.43 | |
| Workplace only | 7 | 2.0 | 4 | 0.8 | 2.96 | 2.58 | 0.74–8.99 | |
| Both locations | 7 | 2.0 | 2 | 0.4 | 5.92 | 5.91 | 1.20–29.2 | 0.0018 |
| Mancozeb | | | | | | | | |
| Unexposed to all ALDH-inhibiting pesticides | 168 | 47.6 | 284 | 54.8 | 1.00 | 1.00 | Reference | |
| Exposed to mancozeb | | | | | | | | |
| Residence only | 22 | 6.2 | 33 | 6.4 | 1.13 | 1.08 | 0.60–1.93 | |
| Workplace only | 23 | 6.5 | 20 | 3.9 | 1.94 | 1.87 | 0.99–3.53 | |
| Both locations | 19 | 5.4 | 7 | 1.4 | 4.59 | 4.51 | 1.84–11.1 | 0.0009 |
| Maneb | | | | | | | | |
| Unexposed to all ALDH-inhibiting pesticides | 168 | 47.6 | 284 | 54.8 | 1.00 | 1.00 | Reference | |
| Exposed to maneb | | | | | | | | |
| Residence only | 31 | 8.8 | 33 | 6.4 | 1.59 | 1.57 | 0.92–2.67 | |
| Workplace only | 34 | 9.6 | 30 | 5.8 | 1.92 | 1.82 | 1.06–3.10 | |
| Both locations | 15 | 4.2 | 5 | 1.0 | 5.07 | 4.47 | 1.58–12.6 | 0.0005 |
| Triflumizole | | | | | | | | |
| Unexposed to all ALDH-inhibiting pesticides | 168 | 47.6 | 284 | 54.8 | 1.00 | 1.00 | Reference | |
| Exposed to triflumizole | | | | | | | | |
| Residence only | 8 | 2.3 | 10 | 1.9 | 1.35 | 1.21 | 0.47–3.16 | |
| Workplace only | 7 | 2.0 | 7 | 1.4 | 1.69 | 1.60 | 0.54–4.77 | |
| Both locations | 6 | 1.7 | 3 | 0.6 | 3.38 | 2.99 | 0.73–12.3 | 0.033 |
| Zineb | | | | | | | | |
| Unexposed to all ALDH-inhibiting pesticides | 168 | 47.6 | 284 | 54.8 | 1.00 | 1.00 | Reference | |
| Exposed to zineb | | | | | | | | |
| Residence only | 4 | 1.1 | 3 | 0.6 | 2.25 | 1.78 | 0.39–8.13 | |
| Workplace only | 6 | 1.7 | 5 | 1.0 | 2.03 | 1.96 | 0.58–6.56 | |
| Both locations | 5 | 1.4 | 3 | 0.6 | 2.82 | 2.57 | 0.60–11.1 | 0.0263 |

*Continued*

**Table 1** Continued

| Exposure[a] | Cases No. | Cases % | Controls No. | Controls % | OR[b] | OR, adjusted[c] | 95% CI | p Trend |
|---|---|---|---|---|---|---|---|---|
| Ziram | | | | | | | | |
| Unexposed to all ALDH-inhibiting pesticides | 168 | 47.6 | 284 | 54.8 | 1.00 | 1.00 | Reference | |
| Exposed to ziram | | | | | | | | |
| Residence only | 20 | 5.7 | 40 | 7.7 | 0.85 | 0.77 | 0.43-1.37 | |
| Workplace only | 24 | 6.8 | 24 | 4.6 | 1.69 | 1.71 | 0.93-3.16 | |
| Both locations | 25 | 7.1 | 12 | 2.3 | 3.52 | 3.33 | 1.62-6.88 | 0.0036 |

Abbreviations: ALDH = aldehyde dehydrogenase; CI = confidence interval; OR = odds ratio.
[a] A subject is considered exposed if his or her geographic information system–estimated exposure is above the median level observed in the exposed control subjects.
[b] Unadjusted logistic regression ORs.
[c] Logistic regression ORs adjusted for age (continuous), sex (male/female), smoking status (ever/never), and race (Caucasian/non-Caucasian).

$\chi^2$ test. Genetic variants (6 SNPs; 1 haplotypic clade) were evaluated under an additive genetic model. Effect measure modification of ALDH-inhibiting pesticide exposure on PD risk given *ALDH2* clade type was evaluated under a dominant genetic model in a logistic regression model stratified by clade. The *p* values presented were uncorrected unless stated otherwise.

## RESULTS

**ALDH inhibitor screen.** ALDH-inhibiting pesticides fell into 4 structural classes—dithiocarbamate, imidazole, dicarboxymide, and organochlorine (table 3). All 6 of the screened dithiocarbamates that coordinate as metal complexes inhibited ALDH. Ziram was the most potent, inhibiting 20% ± 1.3% of the ALDH activity observed in neurons exposed only to vehicle. Only metam sodium, which does not exist as a coordination complex, did not inhibit ALDH. Among the imidazoles, benomyl inhibited ALDH by 30% ± 0.8% and triflumizole by 13% ± 2.4%, although thiophanate-methyl did not inhibit ALDH. The dicarboxymides captan and folpet inhibited ALDH activity by 18% ± 1.5% and 17% ± 1.9%, respectively; vinclozolin had no effect. The organochlorine dieldrin inhibited 8% ± 3.1%; endosulfan had no effect. None of the screened carbamates (i.e., aldicarb, methomyl), organophosphates (i.e., chlorpyrifos, dimethoate, methidathion, parathion, phorate), or triazines (i.e., atrazine, cyanazine/Bladex) inhibited ALDH at this concentration; neither did paraquat nor propargite.

**Pesticide exposure and PD risk.** In our population-based case-control study (table e-1), there were

**Table 2** Effect estimates (ORs and 95% CIs) for ambient exposure to aggregate ALDH-inhibiting pesticide exposures at residence and workplace addresses in association with Parkinson disease, all subjects and stratified by genetic variation in the *ALDH2* gene

| Exposure level[a] | All subjects Cases/controls | All subjects OR[b] (95% CI) | *ALDH2* clade 1/1 Cases/controls | *ALDH2* clade 1/1 OR[b] (95% CI) | *ALDH2* clade 1/2 or 2/2 Cases/controls | *ALDH2* clade 1/2 or 2/2 OR[b] (95% CI) |
|---|---|---|---|---|---|---|
| Unexposed to all ALDH-inhibiting pesticides | 168/284 | 1.00 | 76/124 | 1.00 | 92/160 | 1.00 |
| Exposed to any number of pesticides at residence but unexposed at workplace | 49/87 | 0.93 (0.62-1.40) | 29/38 | 1.17 (0.65-2.10) | 20/49 | 0.73 (0.41-1.32) |
| Exposed to any number of pesticides at workplace but unexposed at residence | 53/79 | 1.13 (0.75-1.69) | 24/33 | 1.16 (0.63-2.16) | 29/46 | 1.14 (0.66-1.95) |
| Exposed to 1 or 2 pesticides at each residence and workplace | 39/36 | 1.80 (1.09-2.98) | 22/23 | 1.58 (0.80-3.10) | 17/13 | 2.21 (1.01-4.82) |
| Exposed to ≥3 pesticides at residence but only 1 or 2 at workplace | 13/13 | 1.55 (0.70-3.46) | 5/6 | 1.21 (0.35-4.23) | 8/7 | 1.74 (0.61-5.03) |
| Exposed to ≥3 pesticides at workplace but only 1 or 2 at residence | 11/11 | 1.60 (0.67-3.85) | 4/7 | 0.92 (0.25-3.43) | 7/4 | 2.80 (0.78-10.0) |
| Exposed to ≥3 pesticides at each residence and workplace | 20/8 | 3.54 (1.51-8.30) | 9/5 | 2.47 (0.78-7.82) | 11/3 | 5.30 (1.42-19.8) |
| *p* Trend | | 0.0005 | | 0.1285 | | 0.0010 |

Abbreviations: ALDH = aldehyde dehydrogenase; *ALDH2* = aldehyde dehydrogenase 2 family (mitochondrial); CI = confidence interval; OR = odds ratio.
[a] A subject is considered exposed to a pesticide if his or her geographic information system–estimated exposure is above the median level observed in the exposed control subjects for that pesticide.
[b] Logistic regression ORs adjusted for age (continuous), sex (male/female), smoking status (ever/never), and race (Caucasian/non-Caucasian).

**Table 3** Pesticide-induced ALDH inhibition in neurons

| Compound | ALDH activity (% of vehicle-only control ± SEM) |
|---|---|
| **Carbamate** | |
| Aldicarb | 95 ± 4.6 |
| Methomyl | 100 ± 4.0 |
| **Dicarboxymide** | |
| Captan | 82 ± 1.5[a] |
| Folpet | 81 ± 1.4[a] |
| Vinclozolin | 103 ± 3.5 |
| **Dithiocarbamate** | |
| Ferbam | 91 ± 1.9[b] |
| Mancozeb | 90 ± 1.3[b] |
| Maneb | 91 ± 4.0[b] |
| Metam sodium | 100 ± 8.1 |
| Thiram | 82 ± 7.8[a] |
| Zineb | 91 ± 2.8[b] |
| Ziram | 80 ± 1.3[a] |
| **Imidazole** | |
| Benomyl | 70 ± 1.2[a] |
| Thiophanate-methyl | 106 ± 4.8 |
| Triflumizole | 87 ± 2.4[c] |
| **Organochlorine** | |
| Dieldrin | 92 ± 3.1[c] |
| Endosulfan | 103 ± 13 |
| **Organophosphate** | |
| Chlorpyrifos | 95 ± 2.2 |
| Dimethoate | 103 ± 4.9 |
| Methidathion | 98 ± 10 |
| Parathion | 102 ± 5.3 |
| Phorate | 106 ± 5.0 |
| **Triazine** | |
| Atrazine | 106 ± 3.9 |
| Cyanazine (Bladex) | 98 ± 2.6 |
| **Other** | |
| Paraquat | 105 ± 2.9 |
| Propargite | 101 ± 8.3 |

Abbreviation: ALDH = aldehyde dehydrogenase.
[a] $p > 0.999$ compared with controls exposed only to vehicle; n = 4–12.
[b] $p > 0.95$.
[c] $p > 0.99$.

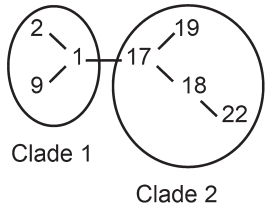
sufficient ambient workplace and residential address-based exposures to investigate individual risk factors for 8 of the 11 neuronal ALDH-inhibiting pesticides identified in the screening assay: benomyl, captan, dieldrin, mancozeb, maneb, triflumizole, zineb, and ziram (tables 1 and e-2). Ferbam, folpet, and thiram were not investigated individually in the epidemiologic study because fewer than 5 subjects were exposed to each of these agents, although these were included in the analyses of all ALDH-inhibiting pesticides combined (table 2).

Every ALDH-inhibiting pesticide identified in the screen and applied in the study area was found to be associated with increased PD risk, and all pesticides demonstrated a trend of increasing risk with increasing level of exposure as represented by the ordinal exposure variable (table 1). For most pesticides, the 95% CI excluded the null only for the highest exposure category (i.e., exposure at both workplace and residential addresses). Exposure to an ALDH-inhibiting pesticide at both workplace and residential addresses was associated with a 65% (benomyl) to 6-fold (dieldrin) increase in PD risk. Effect estimates for exposure at workplace addresses alone were smaller (odds ratio range: 1.25–2.58), and the 95% CI excluded the null only for maneb. Effect estimates for exposure at residential addresses alone were further attenuated and excluded the null only for dieldrin. These analyses considered ambient exposure estimates only; adjustments for home pesticide use or occupational exposures did not alter the results by more than 12%. Evaluating all subjects with pesticide exposure data, regardless of whether genotyping data were available, altered some effect estimates but not sufficiently to change the interpretation of the findings (tables e-1 and e-2).

When considering all 11 ALDH-inhibiting pesticides and both workplace and residential addresses, there was a trend of increasing risk with increasing exposure ($p$ value for trend = 0.0005), with a 3.5-fold increase in PD risk (95% CI: 1.51–8.30) with exposure to 3 or more pesticides (table 2). Again, adjustment for home pesticide use or occupational pesticide exposure, and inclusion of all subjects regardless of genotyping data availability, did not alter these results.

**Modification of pesticide effect by *ALDH2* genetic variation.** The haplotypes observed in this study population clustered into 1 of 2 clades, primarily determined by the genotype at rs737280 (figure 1). There were no associations between clade and PD (table e-3). The single known functional SNP in *ALDH2*, rs671, could not be assessed, because fewer than 2% of subjects carried the minor allele. The association observed between the combined pesticide exposure measure and PD risk was modified by the presence of 1 or 2 copies of the less common clade (i.e., clade 2). Specifically, there were no associations between any level of ALDH-inhibiting pesticide exposure and PD risk in subjects homozygous for clade 1, whereas there was a 2- to 5-fold increase in PD risk for subjects with greater exposure and at least 1 copy of clade 2 in *ALDH2* (table 2). To further confirm this finding, we evaluated *ALDH2* genetic

**Figure 1** *ALDH2* cladogram




| | rs737280 | rs968529 | rs16941667 | rs16941669 | rs9971942 | Prevalence (%) |
|---|---|---|---|---|---|---|
| 1 | T | C | C | T | C | 58.5 |
| 2 | T | C | C | T | T | 1.4 |
| 9 | T | T | C | T | C | 6.5 |
| 17 | C | C | C | T | C | 1.9 |
| 18 | C | C | C | T | T | 13.9 |
| 19 | C | C | C | G | C | 10.0 |
| 22 | C | C | T | T | T | 6.2 |

Haplotypes were grouped into 2 clades based on variations in 5 single nucleotide polymorphisms in the *ALDH2* gene. *ALDH2* = aldehyde dehydrogenase 2 family (mitochondrial).

variation in subjects unexposed to ALDH-inhibiting pesticides but exposed to 1 or more of 9 pesticides found in the neuronal screen to have little or no effect on ALDH activity—i.e., aldicarb, methomyl, vinclozolin, thiophanate-methyl, dimethoate, methidathion, parathion, phorate, and paraquat (table 3). Effect measure modification by *ALDH2* genetic variation in this subgroup was negligible, and no trend in increasing risk was observed for either clade stratum.

**DISCUSSION** This epidemiologic study is unique in our ability to test for associations between PD incidence and specific pesticide exposures assessed with Pesticide Use Reporting records and lifetime address histories. Here, we report that several pesticides inhibited ALDH activity experimentally in neuronal suspensions, and exposures to these same pesticides were associated with increased risks of developing PD in a human population. Although it would be intriguing to attribute causation to one or more specific ALDH-inhibiting pesticides, this would be overreaching because very few subjects were exposed to only one ALDH-inhibiting pesticide, and exposures to the pesticides in our epidemiologic study were highly correlated (e.g., $r^2 > 0.2$ for exposure above the median to benomyl and mancozeb at residential addresses). Thus, we combined all ALDH-inhibiting pesticides into a single measure that attempted to account for the possibility of smaller increases in risk of exposure at residential addresses, larger increases in risk of exposure at workplace addresses, and cumulative effects of exposures to multiple pesticides at multiple addresses. We observed an exposure-dependent trend of increasing PD risk with this combined exposure measure, using as our reference those subjects who were not exposed to any of these mechanism-associated pesticides. This finding is consistent with a prior study in which we observed no association between exposure to paraquat (i.e., not an ALDH inhibitor; table 3) and PD occurrence unless subjects were also exposed to the ALDH-inhibiting pesticides ziram or maneb.[5]

We observed associations between PD and genetic variation in *ALDH2* only when an environmental contribution was also considered. This finding was predictable because of the likely heterogeneous nature of PD etiology, which demonstrates the need for gene-environment studies to elucidate PD pathogenesis.[33] Intriguingly, PD risks associated with ALDH-inhibiting pesticide exposures were strongly potentiated by genetic variation in the *ALDH2* gene. While a "negative control" in the experimental sense is not possible in epidemiology—because some of the pesticides that do not inhibit ALDH activity could have an impact on PD etiology through a different mechanism—our observed effect measure modification with genetic variation in *ALDH2* for ALDH-inhibiting pesticides and lack thereof for noninhibiting pesticides together provide additional support for ALDH inhibition in PD etiology.

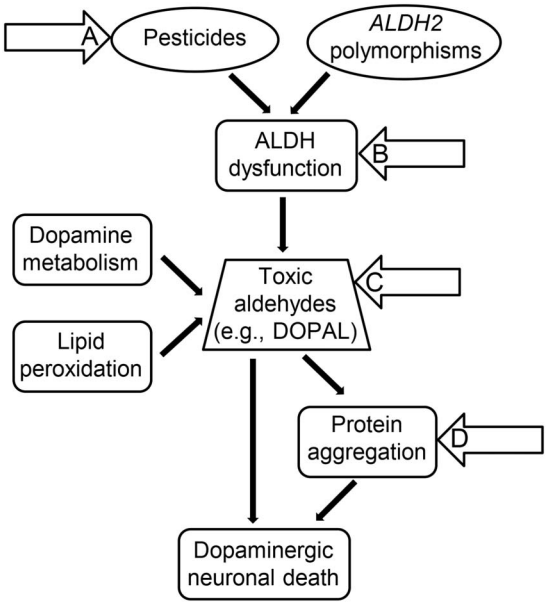
There are no prior studies of *ALDH2* genetic variation in PD, and little is known about biologically relevant variants beyond the loss-of-function variant at rs671, which was not present in this study population, so we used cladistic analysis in an attempt to capture the general variation present in the entirety of the *ALDH2* gene as well as 10 kb of the promoter region. It is possible that the effect measure modification was a result of linkage disequilibrium with a genetic variant of a neighboring gene, for example, acyl-CoA dehydrogenase family, member 10 (*ACAD10*). This gene encodes an enzyme involved in β-oxidation of fatty acids in the mitochondria, for example, to 4-hydroxy-2-nonenal, which is both a substrate for ALDH and has been reported to inhibit ALDH.[34,35] This presents potential interactions to explain the effect measure modification, although this is speculative.

The chemical structures of ALDH-inhibiting pesticides suggest additional toxic mechanisms at play. We previously reported that benomyl inhibits ALDH activity but not via its benzimidazole moiety, also known as carbendazim.[11] Similarly, another carbendazim precursor, thiophanate-methyl, did not inhibit ALDH, providing additional evidence that the imidazole ring does not confer ALDH inhibitory ability. Benomyl inhibits ALDH after being metabolized into thiocarbamate compounds.[36] Consistently, all of the coordinating dithiocarbamates inhibited ALDH activity, suggesting a common mechanism. The carbamates aldicarb and methomyl did not inhibit ALDH, so the thiol group is likely responsible for the inhibitory function of the dithiocarbamates,[37] although these compounds can also evolve carbon disulfide gas, which has been shown to cross-link proteins such as ALDH.[38] Because metam sodium

did not inhibit ALDH, it is likely that the metal complexes stabilize the binding of the dithiocarbamates to ALDH, enabling inhibition. The inhibiting dicarboxymides suggest an additional mechanism. Captan and folpet contain a sulfur-dichloromethyl moiety that can react with glutathione to form thiophosgene (i.e., $CSCl_2$), which inhibits dehydrogenase enzymes.[39] We previously reported that pesticide-induced ALDH inhibition can lead to accumulation of toxic aldehydes (e.g., DOPAL) and result in dopaminergic cell death.[11] Taken together, these findings suggest several mechanisms through which pesticides might contribute to PD pathogenesis via ALDH inhibition, revealing multiple possible therapeutic targets to reverse disease progression.

The potential importance of this work is illustrated in figure 2: risks associated with pesticide exposures and *ALDH2* genetic variation suggest that ALDH dysfunction might contribute to PD pathogenesis through gene-environment interactions. The arrows identify several potential targets for lowering risk: (A) reduction of pesticide use or protection from exposures; (B) enhancement of ALDH function perhaps by pharmacologic interference with one or more of the mechanisms described in the previous paragraph; (C) removal of toxic aldehydes such as DOPAL; and (D) degradation or prevention of protein aggregation such as through enhanced proteasomal or autophagic activities or other pharmacologic intervention.[40] This report provides evidence for the relevance of ALDH inhibition in PD pathogenesis, identifies pesticides that should be avoided to reduce the risk of developing PD, and suggests that therapies modulating ALDH enzyme activity or otherwise eliminating toxic aldehydes should be developed and tested to potentially reduce PD occurrence or slow or reverse its progression particularly for patients exposed to pesticides.

**Figure 2** **Parkinson pathogenesis from aldehyde dehydrogenase inhibition via gene-environment interactions**




This schematic illustrates how pesticide exposures and genetic variation might explain dopaminergic neuronal death and contribute to Parkinson disease risk via aldehyde dehydrogenase (ALDH) inhibition. The arrows identify several potential targets for lowering risk: (A) reduction of pesticide use or exposures; (B) enhancement of ALDH function; (C) removal of toxic aldehydes; and (D) degradation or prevention of protein aggregation. *ALDH2* = aldehyde dehydrogenase 2 family (mitochondrial); DOPAL = 3,4-dihydroxyphenylacetaldehyde.

## AUTHOR CONTRIBUTIONS

A.G.F. and S.L.R. drafted/revised the manuscript, designed the study, analyzed/interpreted data. M.C. designed the study. B.R. and J.M.B. drafted/revised the manuscript and designed the study.


## STUDY FUNDING

This work was funded in part by the National Institute of Environmental Health Sciences (grants P01ES016732, R01ES010544, 5R21ES16446-2, and U54ES012078), the National Institute of Neurologic Disorders and Stroke (grant NS038367), the Veterans Administration Healthcare System (SW PADRECC), the Michael J. Fox Foundation, the Levine Foundation, and the Parkinson Alliance. A.G.F. was supported in part by the National Defense Science & Engineering Graduate Fellowship and the USHHS Ruth L. Kirschstein Institutional National Research Service Award (T32ES015457) in Molecular Toxicology to UCLA.


## DISCLOSURE

The authors report no disclosures relevant to the manuscript. Go to Neurology.org for full disclosures.


*Received June 27, 2013. Accepted in final form October 25, 2013.*


## REFERENCES


1. Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 2003;24:197–211.
2. Nalls MA, Plagnol V, Hernandez DG, et al. Imputation of sequence variants for identification of genetic risks for Parkinson's disease: a meta-analysis of genome-wide association studies. Lancet 2011;377:641–649.
3. Priyadarshi A, Khuder SA, Schaub EA, Priyadarshi SS. Environmental risk factors and Parkinson's disease: a metaanalysis. Environ Res 2001;86:122–127.
4. Kamel F, Tanner C, Umbach D, et al. Pesticide exposure and self-reported Parkinson's disease in the agricultural health study. Am J Epidemiol 2007;165:364–374.
5. Wang A, Costello S, Cockburn M, Zhang X, Bronstein J, Ritz B. Parkinson's disease risk from ambient exposure to pesticides. Eur J Epidemiol 2011;26:547–555.
6. Pezzoli G, Cereda E. Exposure to pesticides or solvents and risk of Parkinson disease. Neurology 2013;80:2035–2041.
7. Elbaz A, Levecque C, Clavel J, et al. CYP2D6 polymorphism, pesticide exposure, and Parkinson's disease. Ann Neurol 2004;55:430–434.
8. Hancock DB, Martin ER, Vance JM, Scott WK. Nitric oxide synthase genes and their interactions with environmental factors in Parkinson's disease. Neurogenetics 2008;9:249–262.
9. Ritz BR, Manthripragada AD, Costello S, et al. Dopamine transporter genetic variants and pesticides in Parkinson's disease. Environ Health Perspect 2009;117:964–969.
10. Manthripragada AD, Costello S, Cockburn MG, Bronstein JM, Ritz B. Paraoxonase 1, agricultural organophosphate exposure, and Parkinson disease. Epidemiology 2010;21:87–94.

11. Fitzmaurice AG, Rhodes SL, Lulla A, et al. Aldehyde dehydrogenase inhibition as a pathogenic mechanism in Parkinson disease. Proc Natl Acad Sci USA 2013;110:636–641.
12. Kataria HR, Grover RK. Effect of benomyl and thiophanate-methyl on metabolic activities of Rhizoctonia solani Kuhn. Ann Microbiol 1976;127A:297–306.
13. LaMondia JA, Douglas SM. Sensitivity of Botrytis cinerea from Connecticut greenhouses to benzimidazole and dicarboximide fungicides. Plant Dis 1997;81:729–732.
14. Hutchinson CM, McGiffen ME Jr, Sims JJ, Becker JO. Fumigant combinations for Cyperus esculentum L control. Pest Manag Sci 2004;60:369–374.
15. Potocnik I, Vukojevic J, Stajic M, et al. In vitro toxicity of selected fungicides from the groups of benzimidazoles and demethylation inhibitors to Cladobotryum dendroides and Agaricus bisporus. J Environ Sci Health B 2009;44:365–370.
16. Deepak SA, Basavaraju P, Chaluvaraju G, Shetty NP, Oros G, Shekar Shetty H. Developmental stage response of pearl millet downy mildew (Sclerospora graminicola) to fungicides. Appl Ecol Environ Res 2006;4:125–149.
17. Prom LK. Laboratory, greenhouse, and field assessment of fourteen fungicides for activity against Claviceps africana, causal agent of sorghum ergot. Plant Dis 2003;87 252–258.
18. Kang GA, Bronstein JM, Masterman DL, Redelings M, Crum JA, Ritz B. Clinical characteristics in early Parkinson's disease in a central California population-based study. Mov Disord 2005;20:1133–1142.
19. Bordelon YM, Hays RD, Vassar SD, Diaz N, Bronstein J, Vickrey BG. Medication responsiveness of motor symptoms in a population-based study of Parkinson disease. Parkinsons Dis 2011;2011:967839.
20. Ritz B, Rhodes SL, Bordelon Y, Bronstein J. Alpha-synuclein genetic variants predict faster motor symptom progression in idiopathic Parkinson disease. PLoS One 2012;7:e36199.
21. Vassar SD, Bordelon YM, Hays RD, et al. Confirmatory factor analysis of the motor unified Parkinson's disease rating scale. Parkinsons Dis 2012;2012:719167.
22. Hughes AJ, Ben-Shlomo Y, Daniel SE, Lees AJ. What features improve the accuracy of clinical diagnosis in Parkinson's disease: a clinicopathologic study. 1992. Neurology 2001;57(suppl 3):S34–S38.
23. Goldberg DW, Wilson JP, Knoblock CA, Ritz B, Cockburn MG. An effective and efficient approach for manually improving geocoded data. Int J Health Geogr 2008;7:1–20.
24. Rull RP, Ritz B. Historical pesticide exposure in California using pesticide use reports and land-use surveys: an assessment of misclassification error and bias. Environ Health Perspect 2003;111:1582–1589.
25. Daly MJ, Rioux JD, Schaffner SF, Hudson TJ, Lander ES. High-resolution haplotype structure in the human genome. Nat Genet 2001;29:229–232.
26. Wall JD, Pritchard JK. Assessing the performance of the haplotype block model of linkage disequilibrium. Am J Hum Genet 2003;73:502–515.
27. Crawford DC, Nickerson DA. Definition and clinical importance of haplotypes. Annu Rev Med 2005;56:303–320.
28. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263–265.
29. Takeuchi F, Isono M, Nabika T, et al. Confirmation of ALDH2 as a major locus of drinking behavior and of its variants regulating multiple metabolic phenotypes in a Japanese population. Circ J 2011;75:911–918.
30. Seltman H, Roeder K, Devlin B. Evolutionary-based association analysis using haplotype data. Genet Epidemiol 2003;25:48–58.
31. Narayan S, Liew Z, Paul K, et al. Household organophosphorus pesticide use and Parkinson's disease. Int J Epidemiol 2013;42:1476–1485.
32. Liew Z, Wang A, Bronstein J, Ritz B. Job exposure matrix (JEM) derived estimates of lifetime occupational pesticide exposure and the risk of Parkinson's disease. Arch Environ Occup Health 2013 March 18. Available at: http://www.tandfonline.com/doi/full/10.1080/19338244.2013.778808#.UrIrPSiv1To. Accessed March 18, 2013.
33. Vance JM, Ali S, Bradley WG, Singer C, Di Monte DA. Gene-environment interactions in Parkinson's disease and other forms of parkinsonism. Neurotoxicology 2010;31:598–602.
34. Mitchell DY, Petersen DR. Inhibition of rat hepatic mitochondrial aldehyde dehydrogenase-mediated acetaldehyde oxidation by trans-4-hydroxy-2-nonenal. Hepatology 1991;13:728–734.
35. Doorn JA, Hurley TD, Petersen DR. Inhibition of human mitochondrial aldehyde dehydrogenase by 4-hydroxynon-2-enal and 4-oxonon-2-enal. Chem Res Toxicol 2006;19:102–110.
36. Staub RE, Quistad GB, Casida JE. Mechanism for benomyl action as a mitochondrial aldehyde dehydrogenase inhibitor in mice. Chem Res Toxicol 1998;11:535–543.
37. Staub RE, Sparks SE, Quistad GB, Casida JE. S-methylation as a bioactivation mechanism for mono- and dithiocarbamate pesticides as aldehyde dehydrogenase inhibitors. Chem Res Toxicol 1995;8:1063–1069.
38. Valentine WM, Amarnath V, Amarnath K, Rimmele F, Graham DG. Carbon disulfide mediated protein cross-linking by N,N-diethyldithiocarbamate. Chem Res Toxicol 1995;8:96–102.
39. Sparks SE, Quistad GB, Li W, Casida JE. Chloropicrin dechlorination in relation to toxic action. J Biochem Mol Toxicol 2000;14:26–32.
40. Prabhudesai S, Sinha S, Attar A, et al. A novel "molecular tweezer" inhibitor of alpha-synuclein neurotoxicity in vitro and in vivo. Neurotherapeutics 2012;9:464–476.