GENE-ENVIRONMENT INTERACTIONS IN ALZHEIMER’S DISEASE: A PATH TO PRECISION MEDICINE AND PRECISION PUBLIC HEALTH?

JASON R RICHARDSON MS, PHD DABT ATS
PROFESSOR AND ASSOCIATE DEAN FOR RESEARCH
ROBERT STEMPEL SCHOOL OF PUBLIC HEALTH AND SOCIAL WORK
FLORIDA INTERNATIONAL UNIVERSITY
Genome-wide Analysis of Genetic Loci Associated With Alzheimer Disease

Genome-wide association studies (GWAS) have recently identified CLU, PICALM, and CR1 as novel genes for late-onset Alzheimer disease (AD).

Objectives To identify and strengthen additional loci associated with AD and confirm these in an independent sample and to examine the contribution of recently identified genes to AD risk prediction in a 3-stage analysis of new and previously published GWAS on more than 35,000 persons (8371 AD cases).

Design, Setting, and Participants In stage 1, we identified strong genetic associations \( (p < 10^{-3}) \) in a sample of 3006 AD cases and 14,642 controls by combining new data from the population-based Cohorts for Heart and Aging Research in Genomic Epidemiology consortium (1367 AD cases [973 incident]) with previously reported results from the Translational Genomics Research Institute and the Mayo AD GWAS. We identified 2708 single-nucleotide polymorphisms (SNPs) with \( p < 10^{-3} \). In stage 2, we pooled results for these SNPs with the European AD Initiative (2032 cases and 5328 controls) to identify 38 SNPs (10 loci) with \( p < 10^{-3} \). In stage 3, we combined data for these 10 loci with data from the Genetic and Environmental Risk in AD consortium (3333 cases and 6995 controls) to identify 4 SNPs with \( p < 1.7 \times 10^{-8} \). These 4 SNPs were replicated in an independent Spanish sample (1140 AD cases and 1209 controls). Genome-wide association analyses were completed in 2007-2008 and the meta-analyses and replication in 2009.

Main Outcome Measure Presence of Alzheimer disease.

Results Two loci were identified to have genome-wide significance for the first time: rs744373 near BIN1 (odds ratio [OR], 1.13; 95% confidence interval [CI], 1.06-1.21 per copy of the minor allele; \( p = 1.59 \times 10^{-11} \)) and rs597668 near EXOC3L2/BLOC153/MARK4 (OR, 1.18; 95% CI, 1.07-1.29; \( p = 6.45 \times 10^{-9} \)). Associations of these 2 loci plus the previously identified CLU and PICALM with AD were confirmed in the Spanish sample. Although CLU and PICALM were confirmed to be associated with AD in this independent sample, they did not improve the ability of a model that included age, sex, and APOE to predict incident AD (improvement in area under the operating characteristic curve from 0.847 to 0.849 in the Rotterdam Study and 0.702 to 0.705 in the Cardiovascular Health Study).

Conclusions Two genetic loci for AD were found for the first time to reach genome-wide statistical significance. These findings were replicated in an independent population. Two recently reported associations were also confirmed. These loci did not improve AD risk prediction. While not clinically useful, they may implicate biological pathways useful for future research.
Reaching the Limits of Genome-wide Significance in Alzheimer Disease
Back to the Environment

• “Clearly researchers need to pay much more attention to environmental risk and protective factors”
Figure: Population attributable fractions for potentially modifiable risk factors in low-income and middle-income countries

*a* Our data for Latin America include the data for Cuba, Dominican Republic, Mexico, Peru, Puerto Rico, and Venezuela.
REVIEW

Occupational risk factors in Alzheimer’s disease: a review assessing the quality of published epidemiological studies

Miguel Santibáñez, Francisco Bolumar, Ana M García

Main messages

- Epidemiological literature on Alzheimer’s disease and occupational exposures is, in general, scarce.
- Some agents have received most of the attention (pesticides, solvents, electromagnetic fields, lead and aluminium), mostly in case-control studies.
- In general, results are consistent with an increased risk of Alzheimer’s disease in relation to occupational exposure to pesticides.

Policy implications

- Protection and surveillance of workers exposed to pesticides should consider the potential risk of Alzheimer’s disease.
- Further research, and mostly follow-up studies, can provide more conclusive evidence about this association and other risks from occupational exposures.
Elevated Serum Pesticide Levels and Risk of Parkinson Disease

Jason R. Richardson, PhD; Stuart L. Shalat, ScD; Brian Buckley, PhD; Bozena Winnik, PhD; Padraig O’Suilleabhain, MD; Ramon Diaz-Arrastia, MD, PhD; Joan Reisch, PhD; Dwight C. German, PhD

of the patients with PD. The most frequently detected pesticide was p,p’-DDE; it was detected in 36 of 50 patients with PD (72%), in 37 of 43 controls (86%), and in all 20 patients with AD. The levels of p,p’-DDE were not the same in the 3 study groups (Kruskal-Wallis H = 21.31; P < .001), and nonparametric multiple comparison tests indicated that the pesticide level was higher in the AD group (median, 5.8 ng/mL; range, 1.29-20.74 ng/mL; mean [SEM], 7.1 [5.4] ng/mL) compared with the control group (median, 1.44 ng/mL; range, 0.2-21.85 ng/mL; mean [SEM], 2.66 [4.0] ng/mL) and the PD group (median, 1.06 ng/mL; range, 0.05-18.56 ng/mL; mean [SEM], 2.4 [4.6] ng/mL), with P < .05 for the 2 post hoc comparisons (Figure 1).

Figure 1. Serum levels of p,p’-DDE are similar in controls and patients with Parkinson disease (PD) but are significantly higher in patients with Alzheimer disease (AD). Black bars indicate the mean values; red bars, the median values.
DDT

- Organochlorine insecticide
- Lipophilic P.O.P.
- Synthesized in 1850s
- Insecticidal Properties in 1930s
<table>
<thead>
<tr>
<th>Synthesized Application 1940-1972 (U.S.)</th>
<th>Gross Global Production (1940-Present)</th>
<th>Present Annual Production</th>
<th>Banned (U.S.)</th>
<th>Environmental Half Life</th>
<th>Log $K_{ow}$</th>
<th>$LD_{50}$ (mouse, oral)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1847</td>
<td>$\sim1.2\times10^{9}$ lbs</td>
<td>$\sim3.6\times10^{10}$ lbs</td>
<td>$\sim7\times10^{6}$ lbs</td>
<td>1972</td>
<td>$\leq30$ years</td>
<td>6.91</td>
</tr>
</tbody>
</table>

- Commercial DDT, a mixture of isomers:
  - $p,p'-$DDT (77%)
  - $o,p'$-DDT (15%)
  - $p,p'$-DDE & DDD (8%)

- Insecticidal Mechanism of Action:

![Image](https://example.com/image.jpg)

Fig. 1. Postsynaptic responses recorded extracellularly from the abdominal nerve cord of the cockroach as evoked by a presynaptic stimulus applied to the cercal nerve. A, control. B, after application of 28 $\mu$M DDT. Depolarizing after-potential of individual nerve fibers is greatly prolonged. From Yamasaki and Ishii (1952).
Original Investigation

Elevated Serum Pesticide Levels and Risk for Alzheimer Disease

Jason R. Richardson, PhD; Ananya Roy, ScD; Stuart L. Shalat, ScD; Richard T. von Stein, PhD;
Muhammad M. Hossain, PhD; Brian Buckley, PhD; Marla Gearing, PhD;
Allan I. Levey, MD, PhD; Dwight C. German, PhD
DDE LEVELS ARE 4X HIGHER IN AD SAMPLES

Figure 1. Serum Levels of Dichlorodiphenyldichloroethylene (DDE)

Serum levels of DDE are elevated in Alzheimer disease (AD). Data were pooled from University of Texas Southwestern Medical Center and Emory University. Levels of DDE are significantly higher in patients with AD (mean [SEM], 2.64 [0.35]) vs control participants (mean [SEM], 0.69 [0.10]; P < .001).
OR FOR AD DIAGNOSIS INCREASED IN TOP TERTILE OF DDE LEVELS

<table>
<thead>
<tr>
<th>Variable</th>
<th>Serum DDE Level, ng/mg Cholesterol/Tertile of Distribution</th>
<th>P Value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odds (95% CI) of AD diagnosis (n = 160)</td>
<td>0.09-0.26</td>
<td>0.27-1.64</td>
</tr>
<tr>
<td>Adjusted for age, sex, race/ethnicity, and location</td>
<td>1 [Reference]</td>
<td>0.70 (0.19-2.55)</td>
</tr>
<tr>
<td>Adjusted for age, sex, race/ethnicity, location, and covariates&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1 [Reference]</td>
<td>0.54 (0.13-2.18)</td>
</tr>
</tbody>
</table>
DDE LEVELS DIFFER BY RACE/ETHNICITY
PESTICIDE LEVELS DIFFER BY RACE/ETHNICITY

WHT = Non-Hispanic white; BLK = Non-Hispanic black; Mex. Am. = Mexican American
p,p'-DDT = p,p'-dichlorodiphenyltrichloroethane; p,p'-DDE = p,p'-dichlorodiphenyldichloroethylene; OXY = oxychlordane; HPE = heptachlor epoxide;
TNA = trans-nonachlor; BHC = β-hexachlorocyclohexan

Quartile of metabolite concentration:
- O1
- O2
- O3
- O4
COGNITIVE DYSFUNCTION ASSOCIATED WITH PESTICIDE EXPOSURE DIFFER BY RACE/ETHNICITY

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>n</th>
<th>Effect (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All Subjects</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p,p'-DDE</td>
<td>667</td>
<td>1.2 (-0.0, 2.5)</td>
<td>0.0524</td>
</tr>
<tr>
<td>p,p'-DDT</td>
<td>618</td>
<td>-2.2 (-4.0, -0.4)</td>
<td>0.0190</td>
</tr>
<tr>
<td>Oxychlordane</td>
<td>599</td>
<td>-1.5 (-4.2, 1.3)</td>
<td>0.2827</td>
</tr>
<tr>
<td>Heptachlor epoxide</td>
<td>591</td>
<td>-1.9 (-3.6, -0.3)</td>
<td>0.0250</td>
</tr>
<tr>
<td>Trans-nonachlor</td>
<td>658</td>
<td>-1.3 (-4.0, 1.3)</td>
<td>0.3083</td>
</tr>
<tr>
<td>β-hexachlorocyclohexane</td>
<td>654</td>
<td>-0.6 (-1.7, 0.4)</td>
<td>0.2230</td>
</tr>
<tr>
<td><strong>Non-Hispanic White</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p,p'-DDE</td>
<td>396</td>
<td>1.3 (-0.2, 2.7)</td>
<td>0.0824</td>
</tr>
<tr>
<td>p,p'-DDT</td>
<td>360</td>
<td>-1.8 (-4.2, 0.7)</td>
<td>0.1583</td>
</tr>
<tr>
<td>Oxychlordane</td>
<td>348</td>
<td>-1.4 (-4.5, 1.8)</td>
<td>0.3966</td>
</tr>
<tr>
<td>Heptachlor epoxide</td>
<td>345</td>
<td>-2.0 (-3.8, -0.1)</td>
<td>0.0339</td>
</tr>
<tr>
<td>Trans-nonachlor</td>
<td>392</td>
<td>-1.4 (-4.3, 1.5)</td>
<td>0.3395</td>
</tr>
<tr>
<td>β-hexachlorocyclohexane</td>
<td>390</td>
<td>-0.7 (-1.9, 0.5)</td>
<td>0.2719</td>
</tr>
<tr>
<td><strong>Non-Hispanic Black</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p,p'-DDE</td>
<td>87</td>
<td>3.5 (-0.1, 7.1)</td>
<td>0.0505</td>
</tr>
<tr>
<td>p,p'-DDT</td>
<td>79</td>
<td>-0.8 (-3.4, 1.9)</td>
<td>0.5648</td>
</tr>
<tr>
<td>Oxychlordane</td>
<td>76</td>
<td>-1.9 (-7.2, 3.5)</td>
<td>0.4835</td>
</tr>
<tr>
<td>Heptachlor epoxide</td>
<td>74</td>
<td>-1.9 (-5.5, 1.7)</td>
<td>0.2863</td>
</tr>
<tr>
<td>Trans-nonachlor</td>
<td>85</td>
<td>-1.6 (-6.7, 3.5)</td>
<td>0.5313</td>
</tr>
<tr>
<td>β-hexachlorocyclohexane</td>
<td>84</td>
<td>-0.9 (-4.4, 2.5)</td>
<td>0.5947</td>
</tr>
<tr>
<td><strong>Mexican American</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p,p'-DDE</td>
<td>142</td>
<td>-1.9 (-3.8, 0.1)</td>
<td>0.0358</td>
</tr>
<tr>
<td>p,p'-DDT</td>
<td>141</td>
<td>-3.3 (-5.5, -1.2)</td>
<td>0.0021</td>
</tr>
<tr>
<td>Oxychlordane</td>
<td>138</td>
<td>-0.4 (-4.7, 3.9)</td>
<td>0.8500</td>
</tr>
<tr>
<td>Heptachlor epoxide</td>
<td>135</td>
<td>-3.1 (-5.6, -0.5)</td>
<td>0.0168</td>
</tr>
<tr>
<td>Trans-nonachlor</td>
<td>140</td>
<td>0.5 (-4.0, 4.9)</td>
<td>0.8359</td>
</tr>
</tbody>
</table>
APP proteolysis $\rightarrow$ Aβ

Nature Reviews Neuroscience 8, 499-509 (July 2007)

DDT INCREASES APP AND AB SECRETION

A. SHSY5Y Human Neuroblastoma Cells

B.  

C. C57BL6J Mouse Hippocampal Primary Neurons

D.  

FIU FLORIDA INTERNATIONAL UNIVERSITY
DDT INCREASES AMYLOID–RELATED GENE EXPRESSION AND PROTEIN IN VIVO
DDT INCREASES AB42 IN 3XTG MICE

A. Hippocampus

B. Cortex

C. 

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>DDT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subbiculum</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Male

Female
AD BRAIN TISSUE WITH HIGH DDE LEVELS EXHIBIT INCREASED 4G8 STAINING IN THE FRONTAL CORTEX

Low DDE (17.6 ng/g)
Female 68 years old

High DDE (43.7 ng/g)
Female 56 years old
GENE X ENVIRONMENT INTERACTIONS?
APOE GENOTYPE MODIFIES COGNITIVE EFFECTS OF DDE IN AD

Table 3. APOE4 Polymorphism Modifies the Association Between DDE and MMSE Scores

<table>
<thead>
<tr>
<th>MMSE</th>
<th>β (95% CI)</th>
<th>P Value</th>
<th>P Value for Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Independent effects in main effects model</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DDE (3rd tertile vs 1st tertile)</td>
<td>-0.84 (-1.60 to -0.08)</td>
<td>.03</td>
<td></td>
</tr>
<tr>
<td>APOE4</td>
<td>-3.56 (-4.59 to -2.54)</td>
<td>&lt;.0001</td>
<td></td>
</tr>
<tr>
<td>Effect of DDE by APOE genotype-stratified model</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APOE4</td>
<td>-1.70 (-3.29 to -0.11)</td>
<td>.04</td>
<td></td>
</tr>
<tr>
<td>APOE2/E3</td>
<td>-0.53 (-0.62 to -0.43)</td>
<td>&lt;.0001</td>
<td>.04</td>
</tr>
<tr>
<td>Interaction model</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APOE4</td>
<td>-1.80 (-2.30 to -1.28)</td>
<td>&lt;.0001</td>
<td></td>
</tr>
<tr>
<td>APOE2/3</td>
<td>-1.75 (-3.40 to -0.11)</td>
<td>.04</td>
<td></td>
</tr>
</tbody>
</table>

a Effect estimate based on linear regression model.
DDT-APOE4 PRODUCES GREATER Aβ
GENOTYPE-SPECIFIC IPSC TO STUDY MECHANISMS

Control

DDT 500 nM

Control

DDE 500 nM

APOE 4/4

APOE 3/3
RNASEQ ANALYSIS REVEALS APOE-DDT INTERACTIONS ON COGNITION AND CELLULAR SIGNALING

Male

APOE4 DDT vs APOE3 DDT

Downregulated
Heterogeneous Disease Population

Patient Demographics
- Age
- Sex
- Education

Family History
- Genetics (Early-Onset)
- Shared Environment

Genetic Screening
- Variants: APOE, CD33, ABCA7, CR1, and more from IGAP

Environmental Exposure
- Particulate Matter
- Pesticide Exposure/DDT

Lifestyle Questionnaire
- Smoking, Drinking, CVD, TBI

Identification of Contributing Risk Factors

Biomarker Screen
- Neuroimaging: PET, MRI
- Serum Biomarkers

Modification of Risks
- Remove exposure
- Alter lifestyle

Personalized Therapeutic Intervention

Identify subsets from the heterogeneous population which respond to specific therapeutics

Aβ Lowering

Tau Mediated

Combination

Inflammation based

Other Targets

Eid, Mhatre and Richardson, 2019 Pharmacol Ther in press
FRAMEWORK USING DDT AS A MODEL

- Genetic Sequencing
- Blood/CSF Biomarkers
- Environmental Exposures
  - History
  - Measurements
- Neuroimaging
  - Amyloid
  - Tau

APOE4
Others?

Early Identification
Removal of DDT/DDE?

Prediction

- High DDE
  - ↑ APP
  - ↑ Aβ

BACE1 Inhibitor?
Aβ antibody?
OVERALL CONCLUSIONS

• Environmental Factors Play a Role in Neurological Disease and Dysfunction

• Interplay between Genetic Susceptibility and Environment is Likely a Key Mechanism

• Identification of Genetic Susceptibility and Environmental Contributors may Lead to Early Identification

• Understanding Mechanisms May Lead to a Personalized Approach to Prevention/Treatment
ACKNOWLEDGEMENTS

- Dwight German, PhD
- Stuart Shalat ScD
- Brian Buckley PhD
- Allan Levey MD, PhD
- Marla Gearing PhD
- Ananya Roy ScD
- Muhammad Hossain PhD
- Richard von Stein PhD
- Ron Hart PhD
- Angela Tiethof MS
- Judith Graber PhD
- Gerry Harris PhD
- Aseel Eid PhD

Supported by NIEHS, NINDS, and the Michael J Fox Foundation for Parkinson’s Disease Research