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

GENETIC FACTORS AND AD

Genome-wide Analysis of Genetic Loci Associated With Alzheimer Disease

Sudha Seshadri, MD; Annette L. Fitzpatrick, PhD; M. Arfan Ikram, MD, PhD; Anita L. DeStefano, PhD; Vilmundur Gudnason, MD, PhD; Merce Boada, MD, PhD; Joshua C. Bis, PhD; Albert V. Smith, PhD; Minerva M. Carrasquillo, PhD; Jean Charles Lambert, PhD; Denise Harold, PhD; Elisabeth M. C. Schrijvers, MD; Repoeo Ramirez-Lorca, PhD; Stephanie Debette, MD, PhD; W. T. Longstreth Jr, MD; A. Cecile J. W. Janssens, PhD; V. Shane Pankratz, PhD; Jean François Dartigues, PhD; Paul Hollingworth, PhD; Thor Aspelund, PhD; Isabel Hernandez, MD; Alexa Beiser, PhD; Lewis H. Kuller, MD; Peter J. Koudstaal, MD, PhD; Dennis W. Dickson, MD; Christophe Tzourio, MD; Richard Abraham, PhD; Carmen Antunez, MD; Yangchun Du, PhD; Jerome I. Rotter, MD; Yurii S. Aulchenko, PhD; Tamara B. Harris, MD; Ronald C. Petersen, MD; Claudine Berr, MD, PhD; Michael J. Owen, MB, ChB, PhD; Jesus Lopez-Arrieta, MD; Badri N. Vardarajan, MS; James T. Becker, PhD; Fernando Rivadeneira, MD, PhD; Michael A. Nalls, PhD; Neill R. Graff-Radford, MD; Dominique Campion, MD, PhD; Sanford Auerbach, MD; Kenneth Rice, PhD; Albert Hofman, MD, PhD; Palmi V. Jonsson, MD; Helena Schmidt, MD, PhD; Mark Lathrop, PhD; Thomas H. Mosley, PhD; Rhoda Au, PhD; Bruce M. Psaty, MD, PhD; Andre G. Uitterlinden, PhD; Lindsay A. Farrer, PhD; Thomas Lumley, PhD; Agustin Ruiz, MD, PhD; Julie Williams, PhD; Philippe Amouyel, MD, PhD; Steve G. Younkin, PhD; Philip A.Wolf, MD; Lenore J. Launer, PhD; Oscar L. Lopez, MD; Cornelia M. van Duijn, PhD; Monique M. B. Breteler, MD, PhD
for the CHARGE, GERAD1, and EADI1 Consortia

Context 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 ($P<10^{-5}$) 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 $P<10^{-5}$. 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 $P<1.7\times10^{-6}$. 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; $P=1.59\times10^{-11}$) and rs597668 near *EXOC3L2/BLOC1S3/MARK4* (OR, 1.18; 95% CI, 1.07-1.29; $P=6.45\times10^{-5}$). Associations of these 2 loci plus the previously identified loci *CLU* and *PICALM* with AD were confirmed in the Spanish sample ($P<.05$). However, 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 receiver 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.

JAMA. 2010;303(18):1832-1840 www.jama.com

FIU | FLORIDA INTERNATIONAL UNIVERSITY

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"

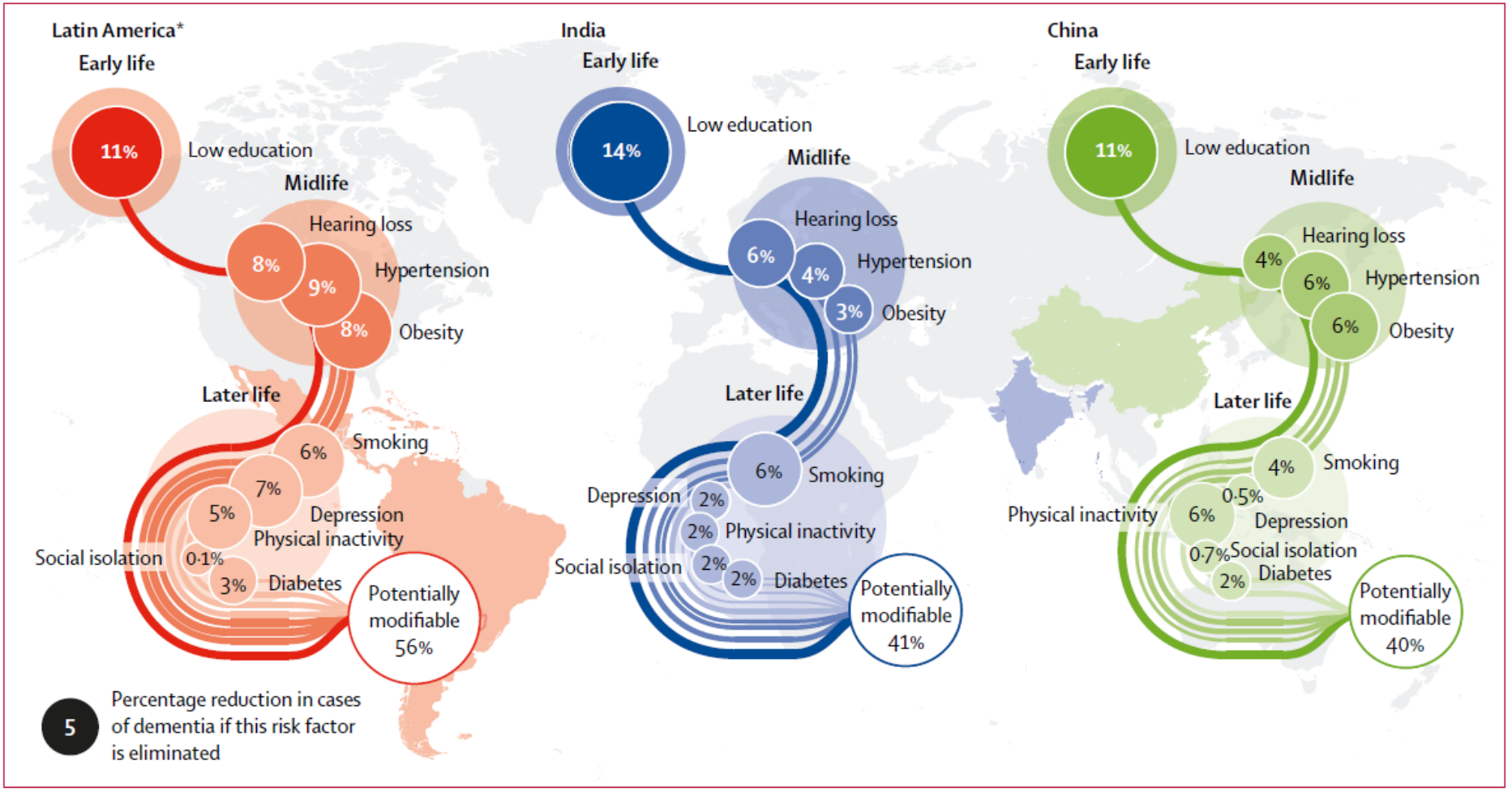


Figure: **Population attributable fractions for potentially modifiable risk factors in low-income and middle-income countries**

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

ENVIRONMENTAL EXPOSURES AS FACTORS IN AD

REVIEW

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

Miguel Santibáñez, Francisco Bolumar, Ana M García

Occup Environ Med 2007;64:723–732. doi: 10.1136/oem.2006.028209

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.

Arch Neurol. 2009;66(7):870-875

ORIGINAL CONTRIBUTION

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**).

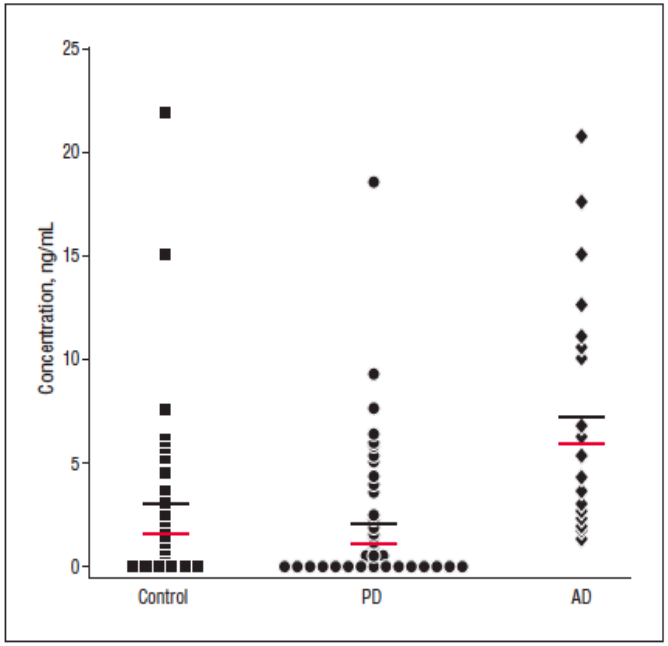
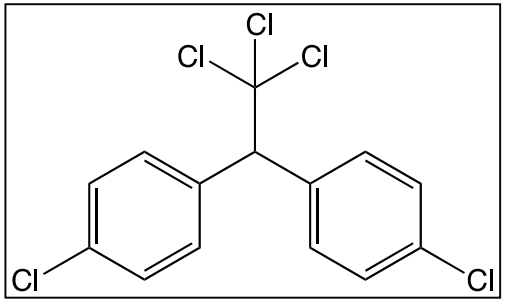


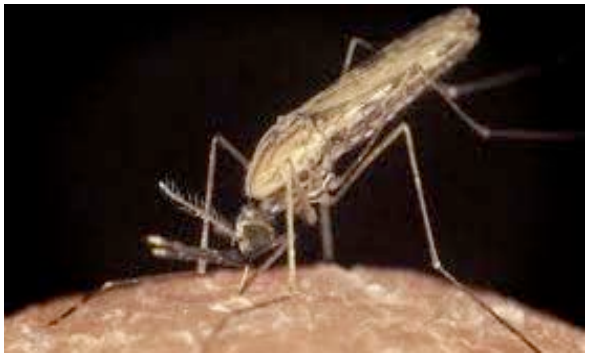
Figure 1. Serum levels of p,p′-DDE are similar in controls and patients with Parkinson disease (PD) but are significantly higher in patients with Alzheimer disease (AD). Black bars indicate the mean values; red bars, the median values.

FIU | FLORIDA INTERNATIONAL UNIVERSITY

DDT



- Organochlorine insecticide
- Lipophilic P.O.P.
- Synthesized in 1850s
- Insecticidal Properties in 1930s



FIU | FLORIDA INTERNATIONAL UNIVERSITY

Synthesized	Application 1940-1972 (U.S.)	Gross Global Production (1940-Present)	Present Annual Production	Banned (U.S.)	Environmental Half Life	Log K_{ow}	LD_{50} (mouse, oral)
1847	~1.2×10^9 lbs	~3.6×10^{10} lbs	~7×10^6 lbs	1972	≤ 30 years	6.91	150-300 mg/kg

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

- Insecticidal Mechanism of Action:

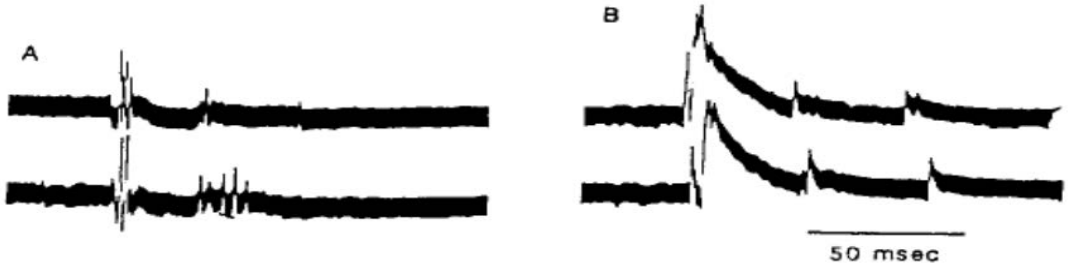


Fig. 1. Postsynaptic responses recorded extracellularly from the abdominal nerve cord of the cockroach as evoked by a presynaptic stimulus applied to the cercal nerve. A, control. B, after application of 28 μM DDT. Depolarizing after-potential of individual nerve fibers is greatly prolonged. From Yamasaki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

Flying and Biting Bugs on Jones Beach Die in a Cloud of DDT, New Insecticide

A truck-mounted fog generator squirts the poison, mixed with oil droplets, over a four-mile area of the New York City playground. Spread by Army and Navy planes and by hand sprays, DDT routed dangerous disease-bearing flies and mosquitoes on Pacific islands. Dusted on almost the entire population of Naples, it killed lice and halted a typhus epidemic. DDT has a drawback—it kills many beneficial and harmless insects, but does not kill all insect pests. Birds and fish which eat large numbers of DDT-poisoned insects may be casualties, too (page 408).

FIU | FLORIDA INTERNATIONAL UNIVERSITY

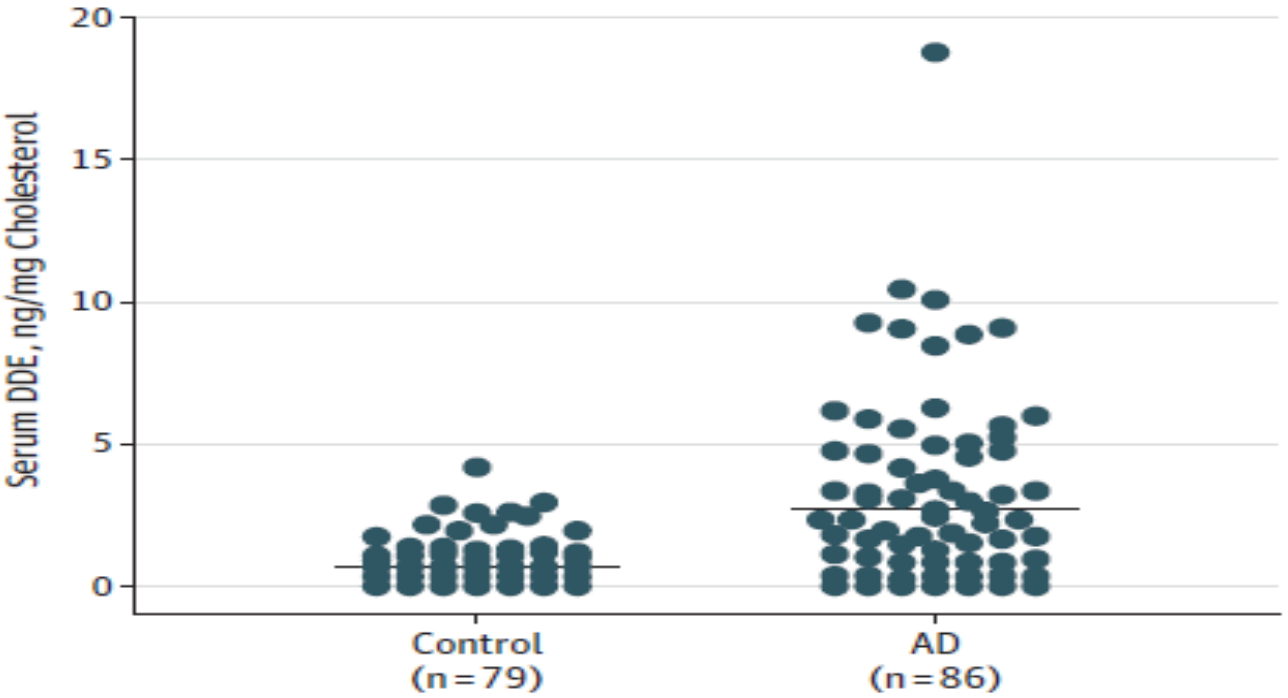
Original Investigation

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]; $P < .001$).

FIU | FLORIDA INTERNATIONAL UNIVERSITY

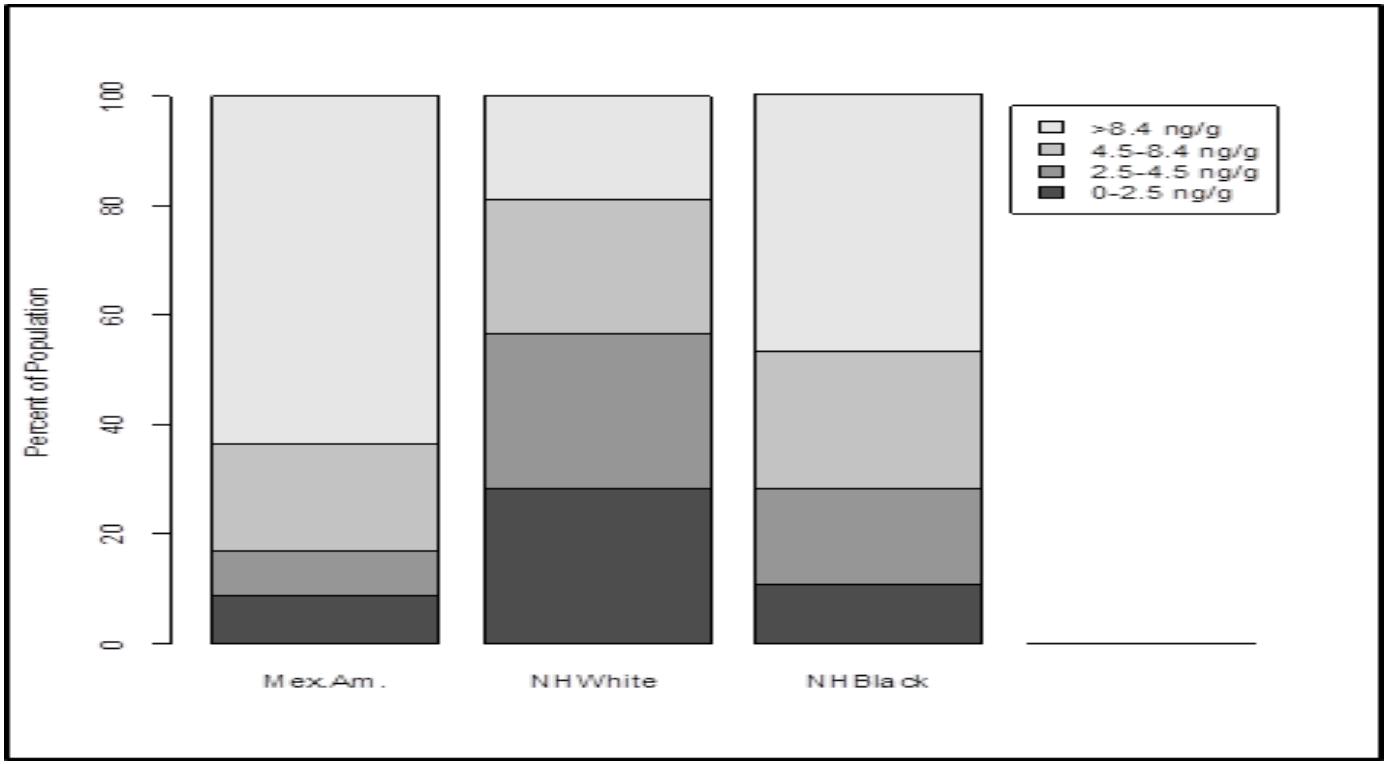
OR FOR AD DIAGNOSIS INCREASED IN TOP TERTILE OF DDE LEVELS

Table 2. Odds of AD per Tertile of DDE Distribution

Variable	Serum DDE Level, ng/mg Cholesterol/Tertile of Distribution			*P* Value[a]
	0.09-0.26	0.27-1.64	1.66-18.75	
Odds (95% CI) of AD diagnosis (n = 160)				
Adjusted for age, sex, race/ethnicity, and location	1 [Reference]	0.70 (0.19-2.55)	4.18 (2.54-5.82)	<.001
Adjusted for age, sex, race/ethnicity, location, and covariates[b]	1 [Reference]	0.54 (0.13-2.18)	3.40 (1.70-6.82)	<.001

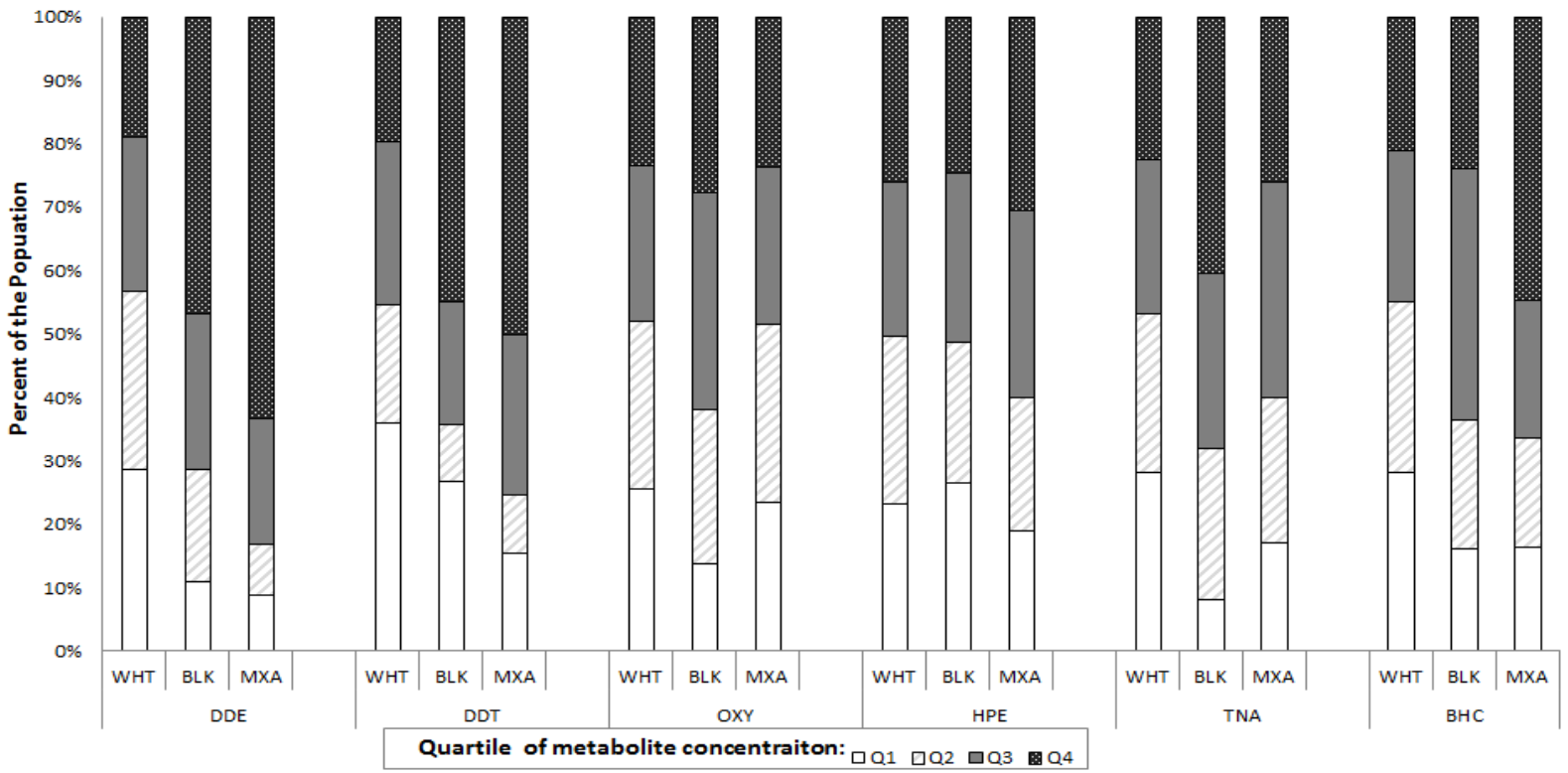
FIU FLORIDA INTERNATIONAL UNIVERSITY

DDE LEVELS DIFFER BY RACE/ETHNICITY



FIU FLORIDA INTERNATIONAL UNIVERSITY

PESTICIDE LEVELS DIFFER BY RACE/ETHNICITY



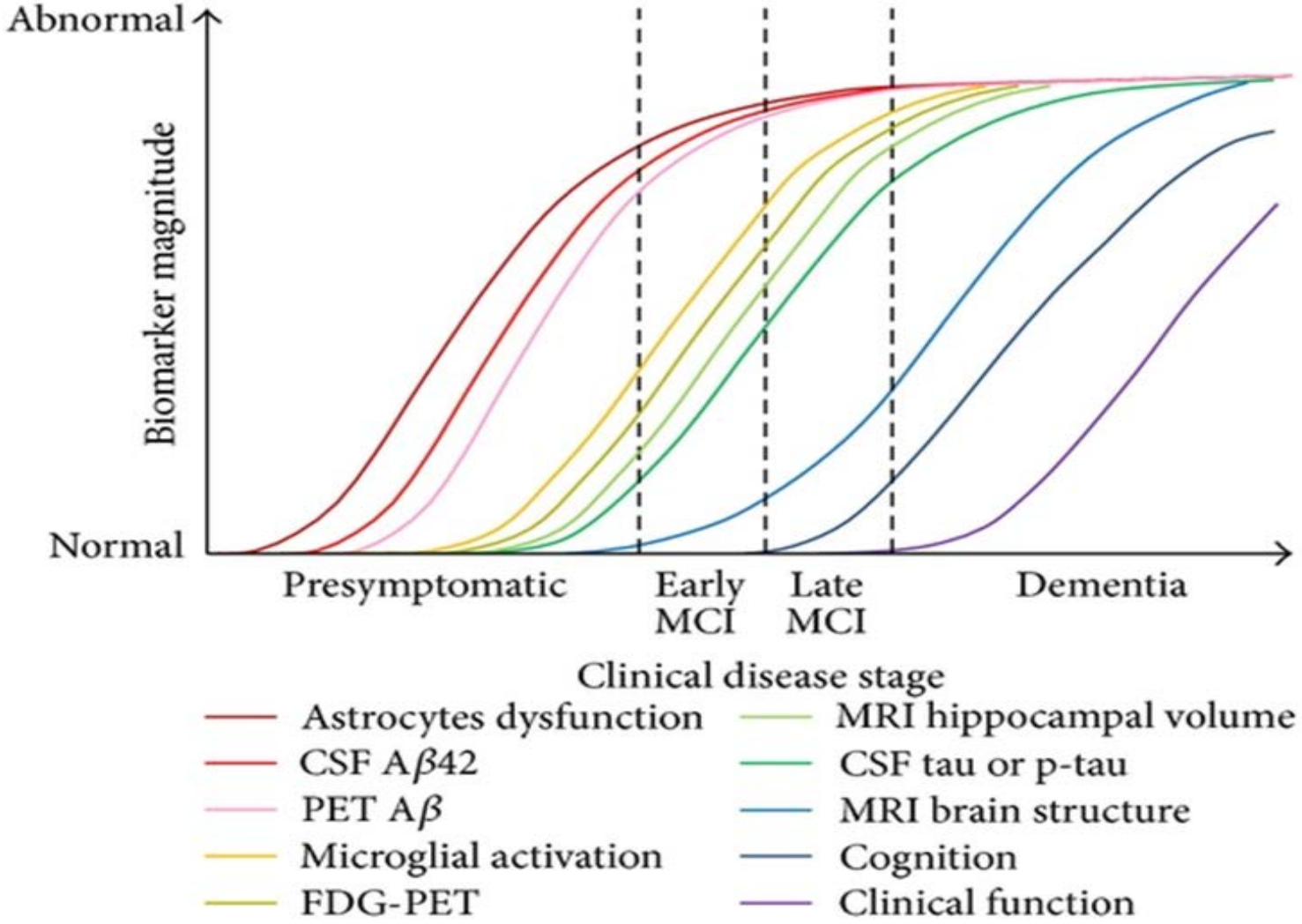
WHT= Non-Hispanic white,; BLK= Non-Hispanic black; Mex. Am. = Mexican American
p,p'-DDT = p,p'-dichlorodiphenyltrichloroethane; p,p'-DDE = p,p'- dichlorodiphenyldichloroethylene ; OXY = oxychlordane; HPE = heptachlor epoxide;
TNA = trans-nonachlor; BHC =β-hexachlorocyclohexan

FIU | FLORIDA INTERNATIONAL UNIVERSITY

COGNITIVE DYSFUNCTION ASSOCIATED WITH PESTICIDE EXPOSURE DIFFER BY RACE/ETHNICITY

Metabolite	n	Mean change in DSST Score per 1 loge Serum Concentration (ng/g) Effect (95 % CI[1])	P-value
All Subjects[2]			
p,p'-DDE	667	1.2 (-0.0, 2.5)	0.0524
p,p'-DDT	618	**-2.2 (-4.0, -0.4)**	**0.0190**
Oxychlordane	599	-1.5 (-4.2, 1.3)	0.2827
Heptachlor epoxide	591	**-1.9 (-3.6, -0.3)**	**0.0250**
Trans-nonachlor	658	-1.3 (-4.0, 1.3)	0.3083
β-hexachlorocyclohexane	654	-0.6 (-1.7, 0.4)	0.2230
Non-Hispanic White[3]			
p,p'-DDE	396	1.3 (-0.2, 2.7)	0.0824
p,p'-DDT	360	-1.8 (-4.2, 0.7)	0.1583
Oxychlordane	348	-1.4 (-4.5, 1.8)	0.3966
Heptachlor epoxide	345	**-2.0 (-3.8, -0.1)**	**0.0339**
Trans-nonachlor	392	-1.4 (-4.3, 1.5)	0.3395
β-hexachlorocyclohexane	390	-0.7 (-1.9, 0.5)	0.2719
Non-Hispanic Black[3]			
p,p'-DDE	87	**3.5 (-0.1, 7.1)**	**0.0505**
p,p'-DDT	79	-0.8 (-3.4, 1.9)	0.5648
Oxychlordane	76	-1.9 (-7.2, 3.5)	0.4835
Heptachlor epoxide	74	-1.9 (-5.5, 1.7)	0.2863
Trans-nonachlor	85	-1.6 (-6.7, 3.5)	0.5313
β-hexachlorocyclohexane	84	-0.9 (-4.4, 2.5)	0.5947
Mexican American[3]			
p,p'-DDE	142	**-1.9 (-3.8, -0.1)**	**0.0358**
p,p'-DDT	141	**-3.3 (-5.5, -1.2)**	**0.0021**
Oxychlordane	138	-0.4 (-4.7, 3.9)	0.8500
Heptachlor epoxide	135	**-3.1 (-5.6, -0.5)**	**0.0168**
Trans-nonachlor	140	0.5 (-4.0, 4.9)	0.8359

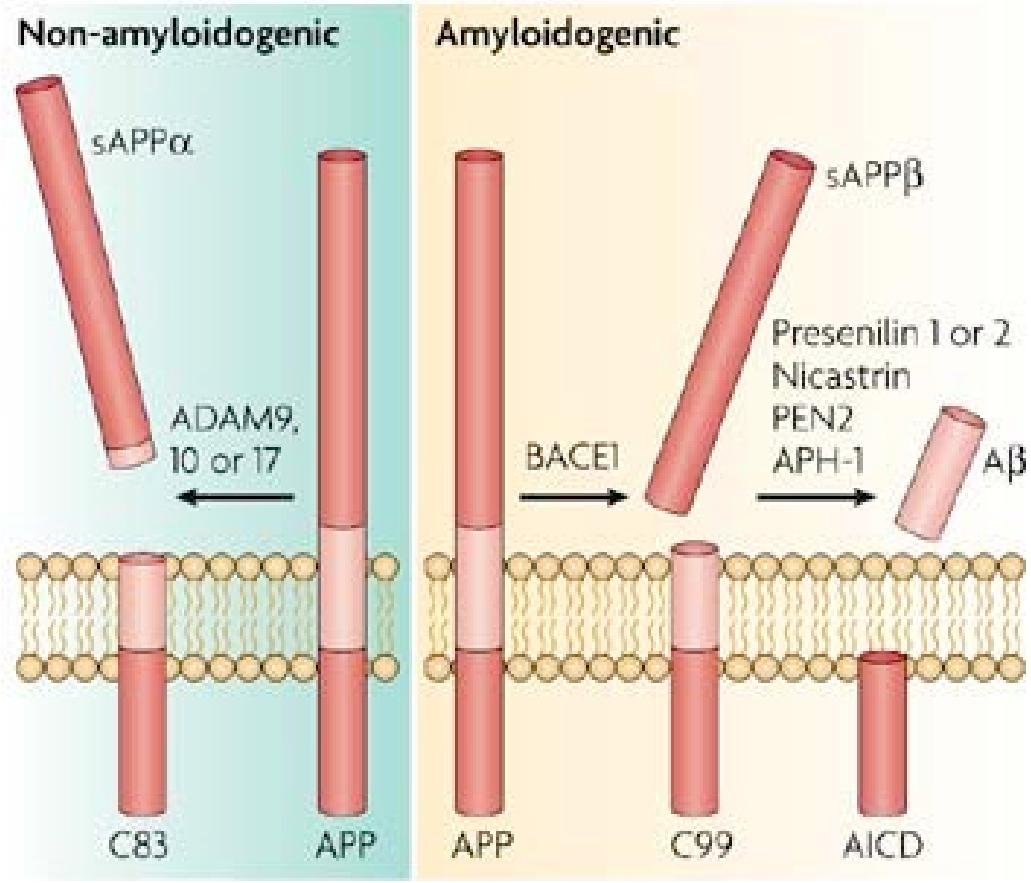
FIU | FLORIDA INTERNATIONAL UNIVERSITY

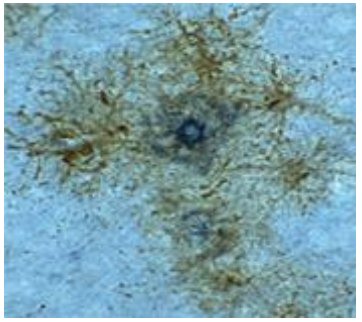


Leclerc and Alburob, 2013

FIU FLORIDA INTERNATIONAL UNIVERSITY

APP proteolysis →Aβ





Amyloid plaques

ApolipoproteinE
(ApoE)

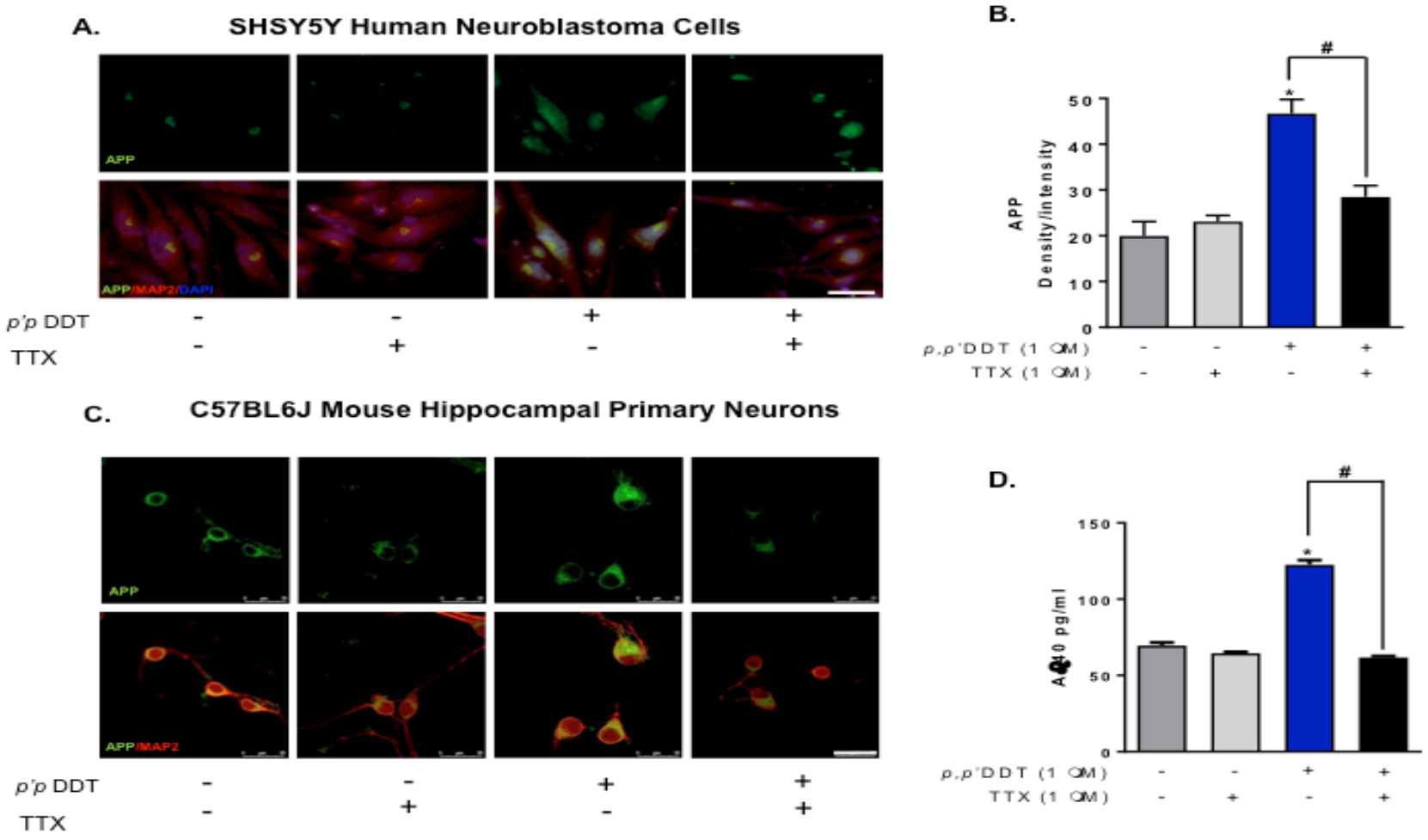
Neprilysin
(NEP)

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

Metzger, J. Neuropath Mol. Neurol, 1998

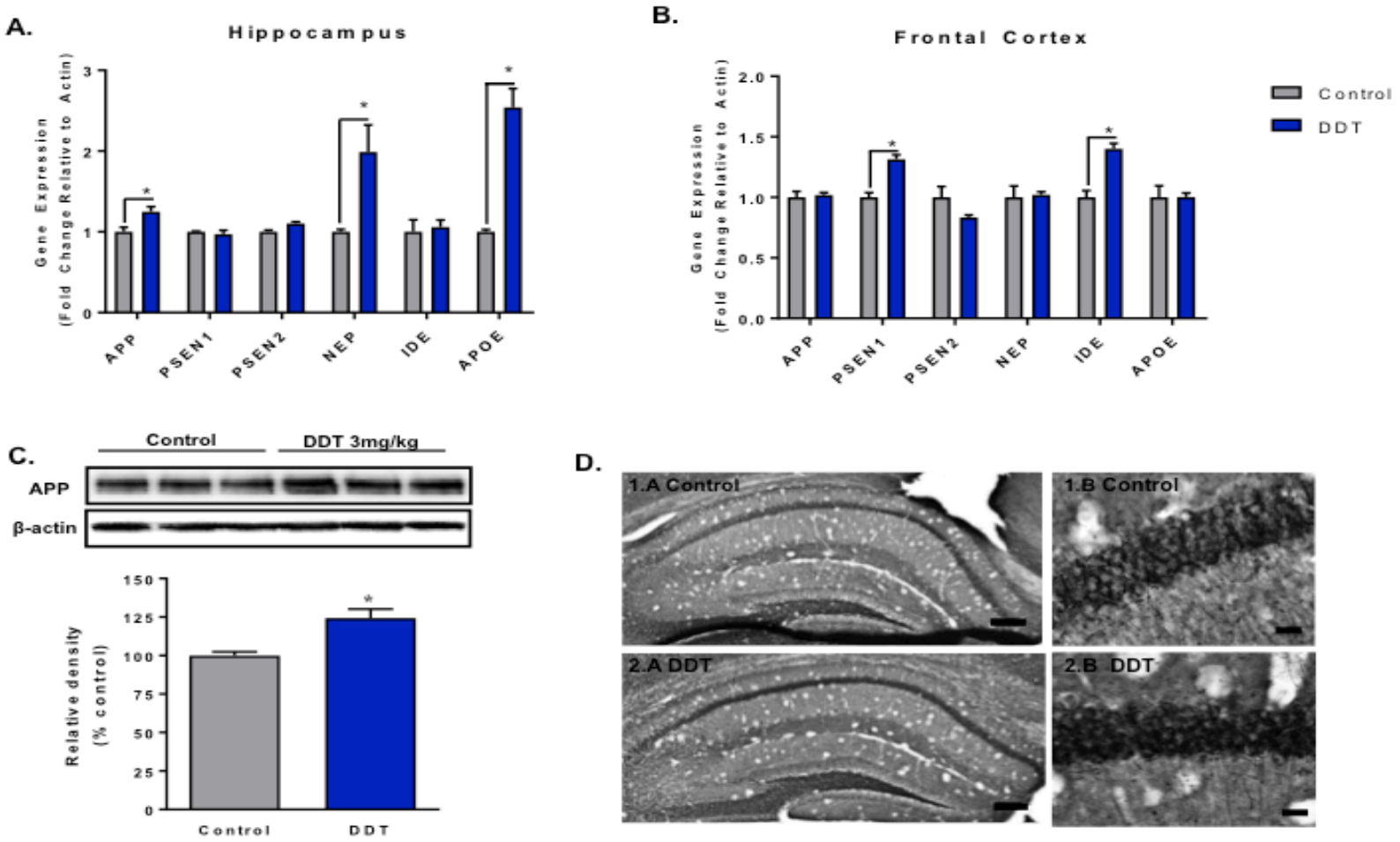
FIU FLORIDA INTERNATIONAL UNIVERSITY

DDT INCREASES APP AND AB SECRETION



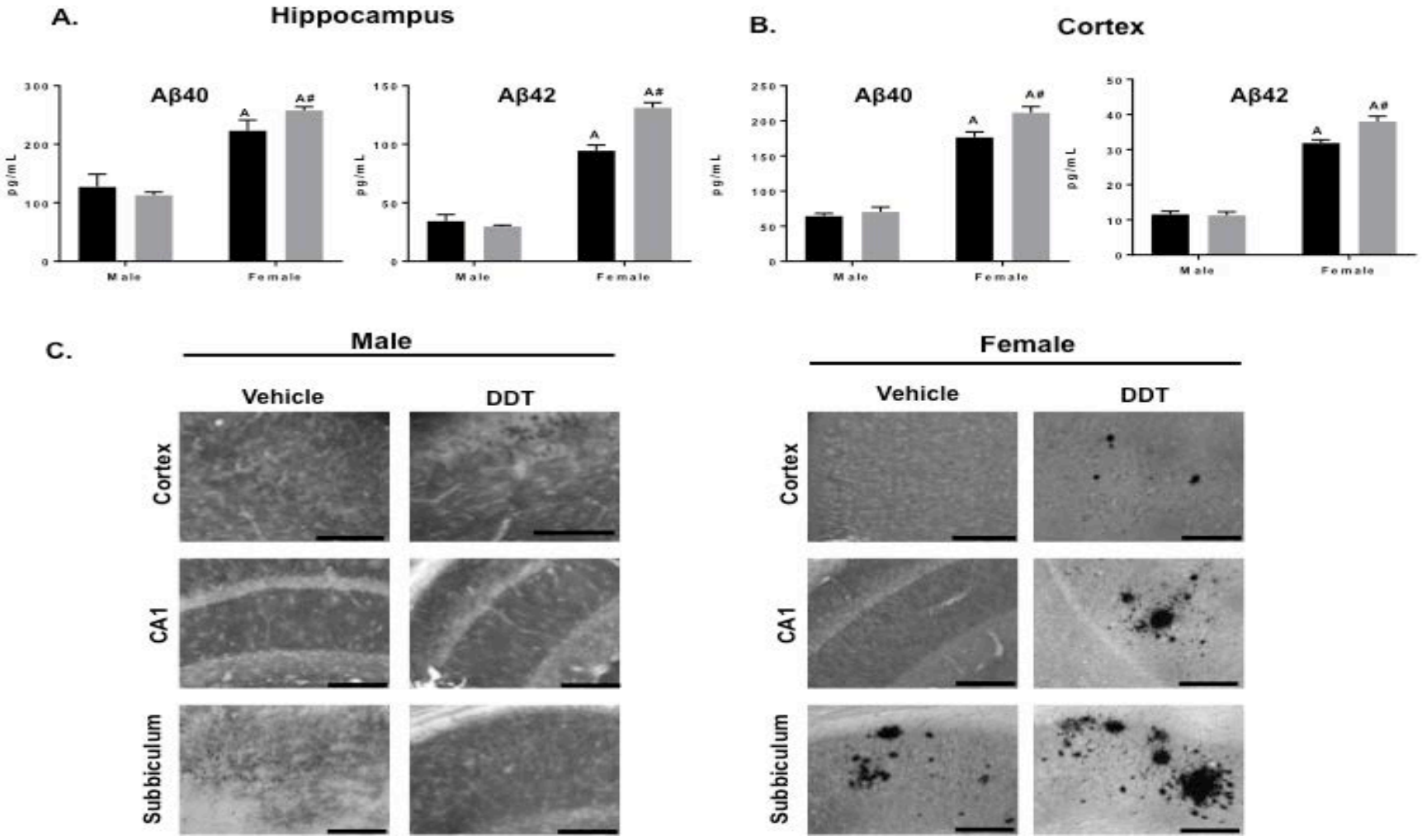
FIU FLORIDA INTERNATIONAL UNIVERSITY

DDT INCREASES AMYLOID–RELATED GENE EXPRESSION AND PROTEIN *IN VIVO*



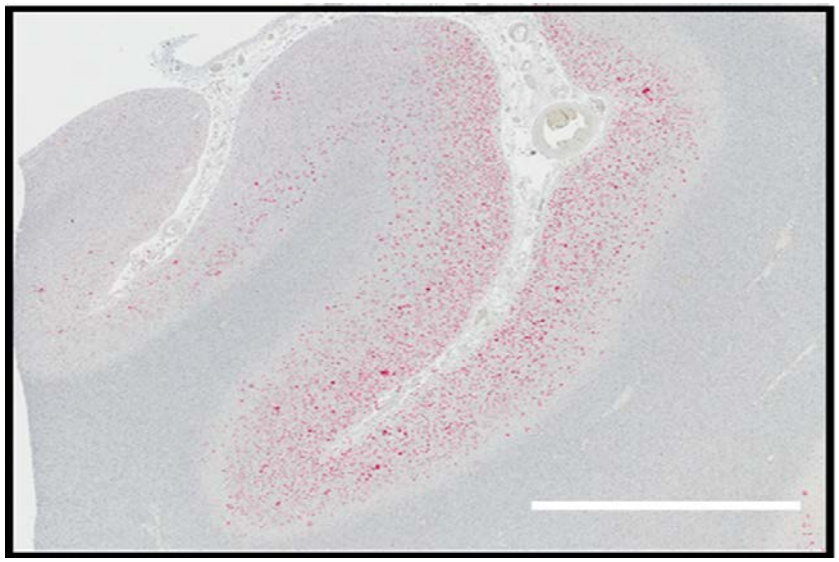
FIU FLORIDA INTERNATIONAL UNIVERSITY

DDT INCREASES AB42 IN 3XTG MICE

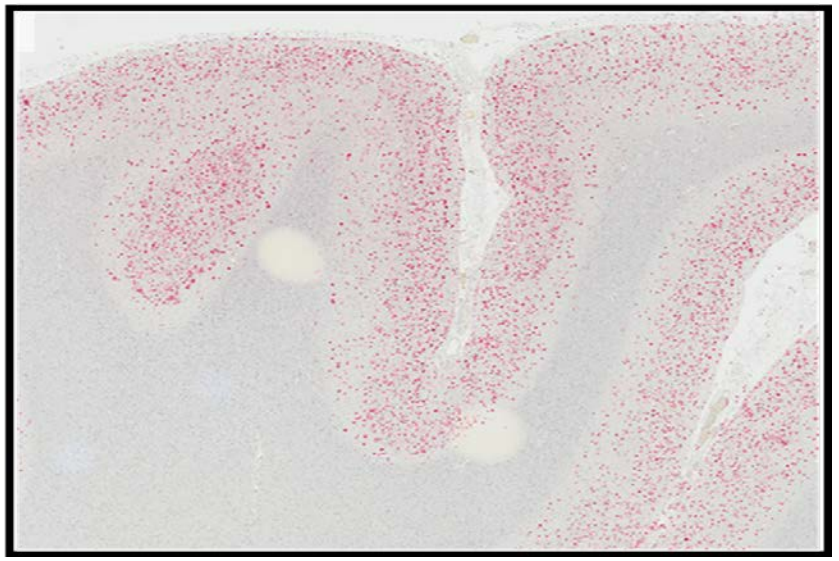


FIU FLORIDA INTERNATIONAL UNIVERSITY

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



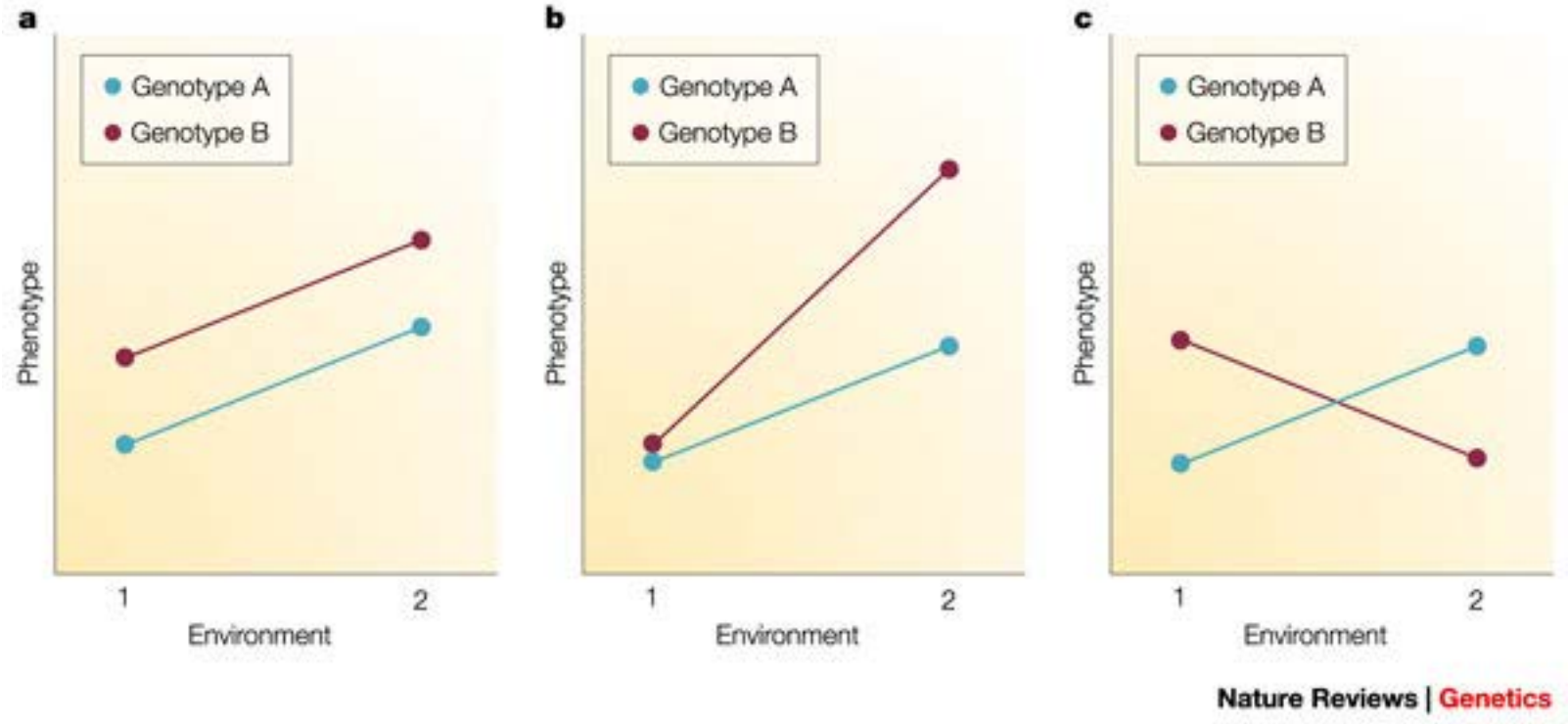
Low DDE (17.6 ng/g)
Female 68 years old



High DDE (43.7 ng/g)
Female 56 years old

FIU | FLORIDA INTERNATIONAL UNIVERSITY

GENE X ENVIRONMENT INTERACTIONS?



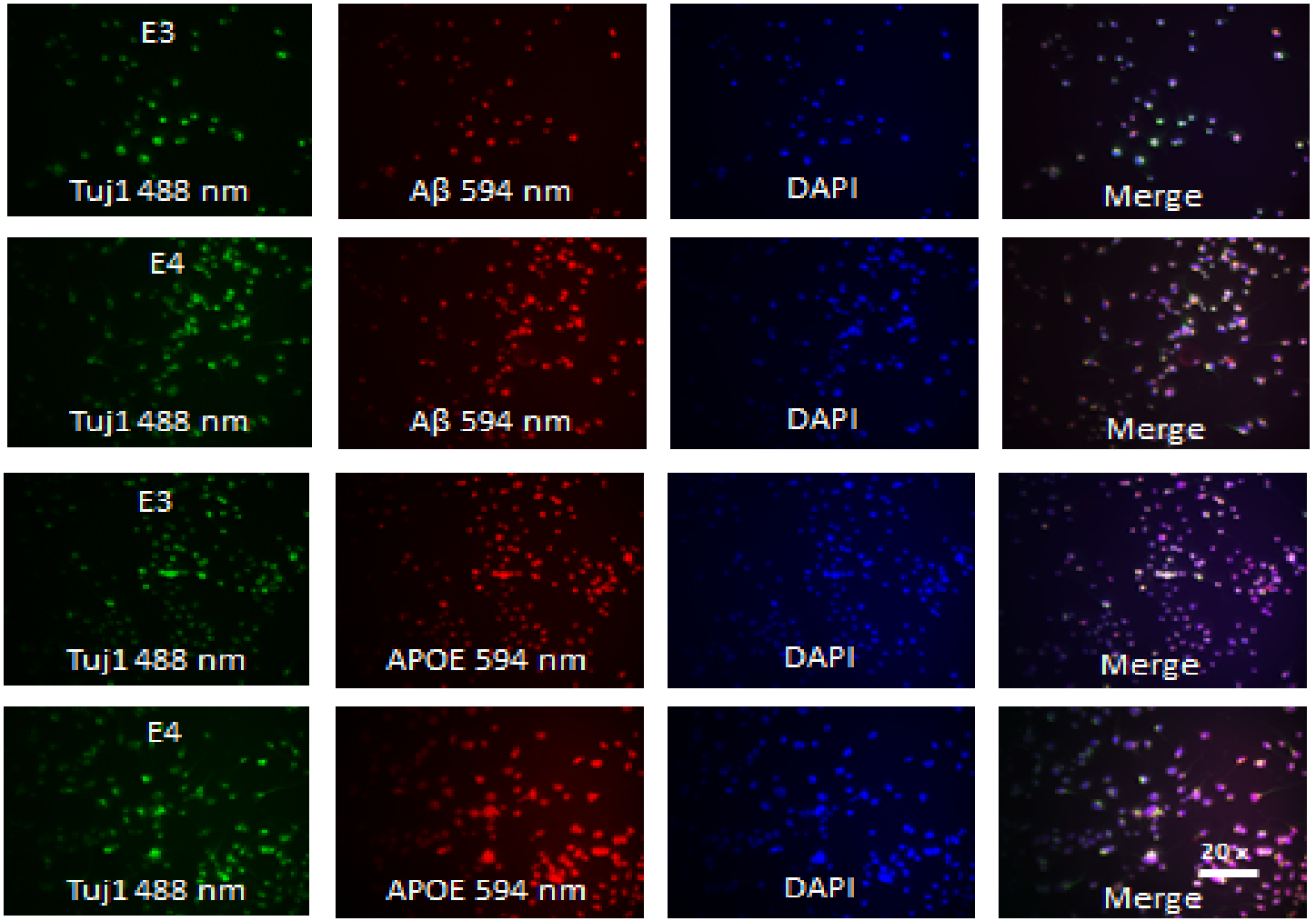
APOE GENOTYPE MODIFIES COGNITIVE EFFECTS OF DDE IN AD

Table 3. *APOE4* Polymorphism Modifies the Association Between DDE and MMSE Scores[a]

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

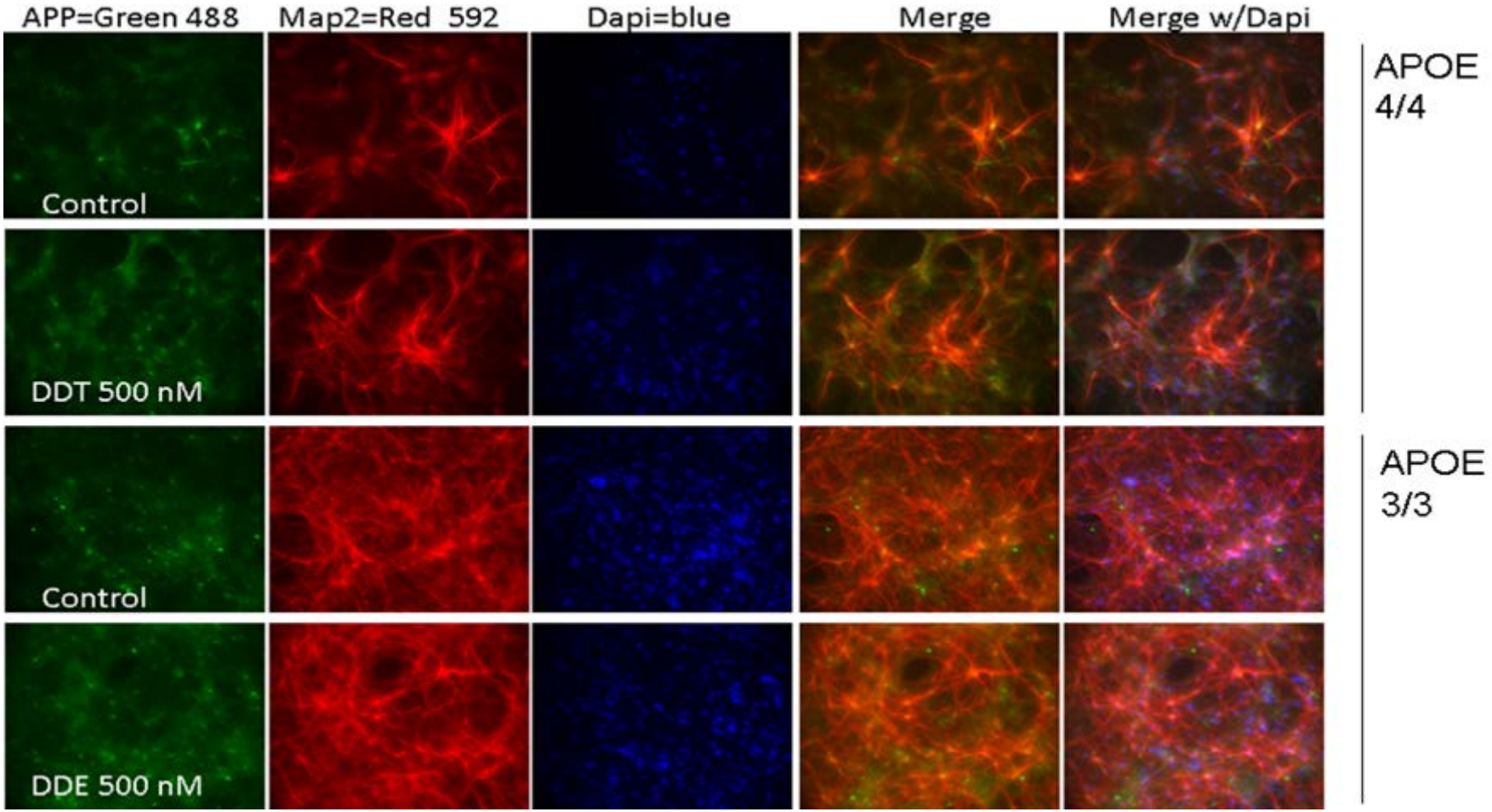
FIU | FLORIDA INTERNATIONAL UNIVERSITY

DDT-APOE4 PRODUCES GREATER AB



FIU | FLORIDA INTERNATIONAL UNIVERSITY

GENOTYPE-SPECIFIC IPSC TO STUDY MECHANISMS



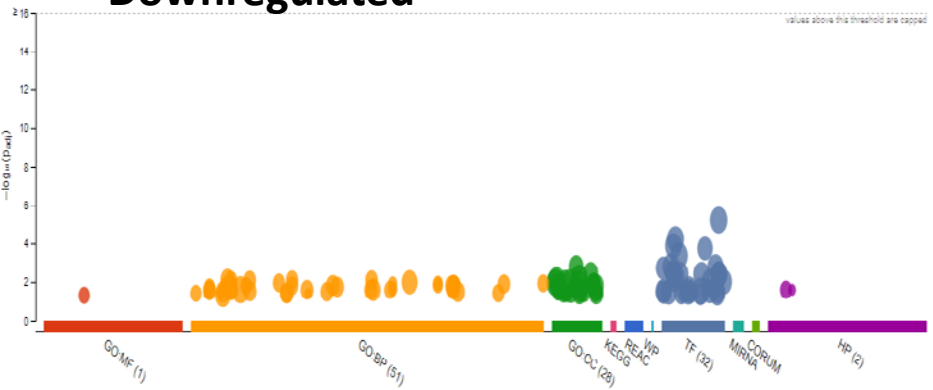
FIU FLORIDA INTERNATIONAL UNIVERSITY

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

Male

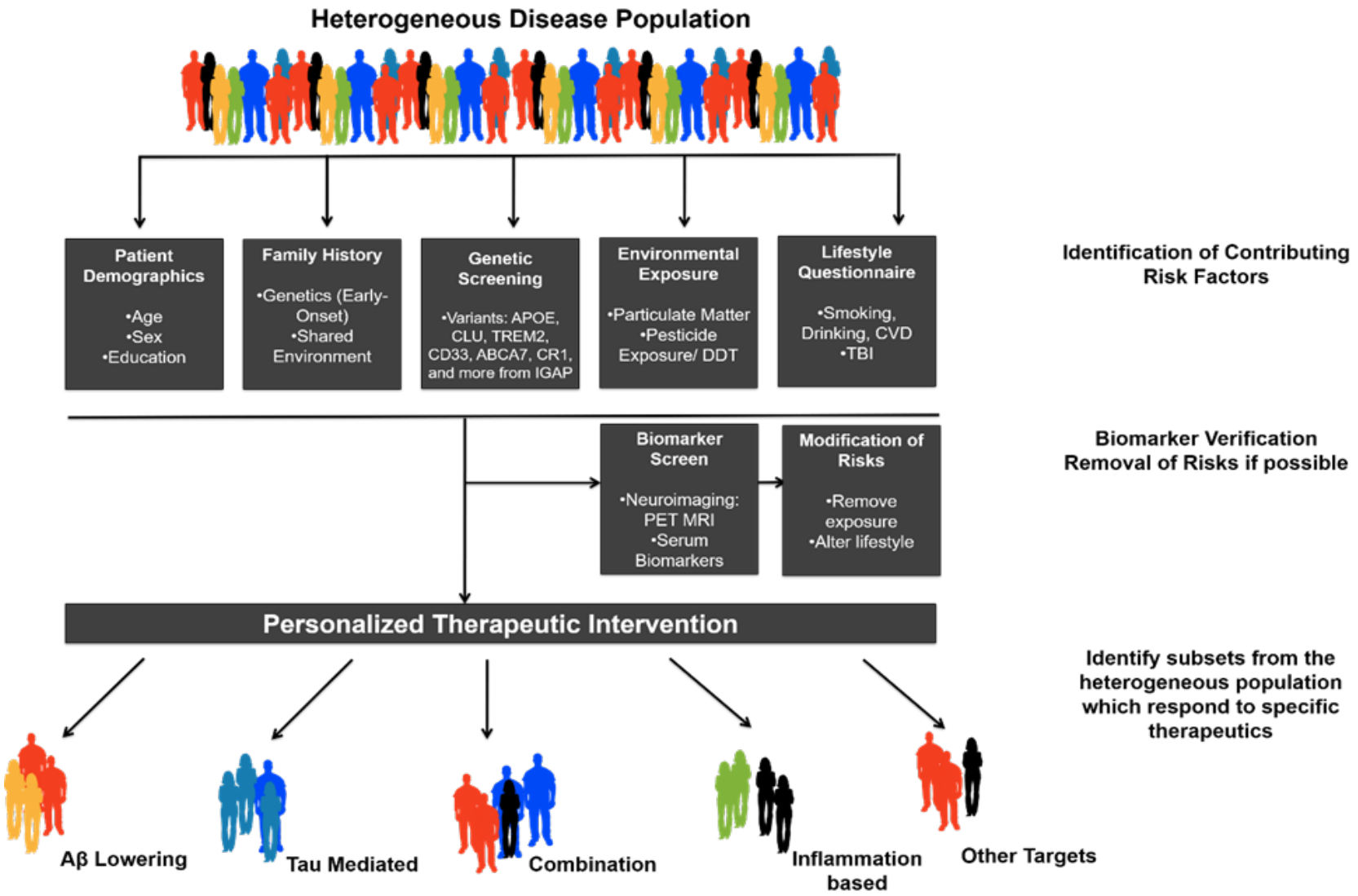
APOE4 DDT vs APOE3 DDT

Downregulated



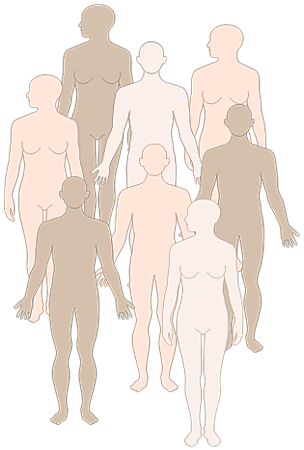
GO:BP		stats	
Term name	Term ID	p_{adj}	$-\log_{10}(p_{adj})$
cognition	GO:0050890	7.853×10^{-3}	
regulation of transporter activity	GO:0032409	7.853×10^{-3}	
cell-cell signaling	GO:0007267	7.853×10^{-3}	
learning or memory	GO:0007611	8.483×10^{-3}	
regulation of metal ion transport	GO:0010959	8.483×10^{-3}	
regulation of biological quality	GO:0065008	1.039×10^{-2}	
regulation of transmembrane transporter activity	GO:0022898	1.118×10^{-2}	
regulation of ventricular cardiac muscle cell membrane rep...	GO:0060307	1.182×10^{-2}	
regulation of cation channel activity	GO:2001257	1.182×10^{-2}	
membrane repolarization	GO:0086009	1.182×10^{-2}	
ventricular cardiac muscle cell membrane repolarization	GO:0099625	1.293×10^{-2}	
regulation of cation transmembrane transport	GO:1904062	1.293×10^{-2}	
chemical synaptic transmission	GO:0007268	1.526×10^{-2}	
anterograde trans-synaptic signaling	GO:0098916	1.526×10^{-2}	
synaptic signaling	GO:0099536	1.526×10^{-2}	
trans-synaptic signaling	GO:0099537	1.526×10^{-2}	
regulation of cardiac muscle cell membrane repolarization	GO:0099623	1.526×10^{-2}	
cardiac muscle cell action potential	GO:0086001	1.526×10^{-2}	
regulation of ion transport	GO:0043269	1.526×10^{-2}	
regulation of heart contraction	GO:0008016	1.526×10^{-2}	
positive regulation of epithelial cell migration	GO:0010634	1.615×10^{-2}	
regulation of ion transmembrane transporter activity	GO:0032412	1.615×10^{-2}	
regulation of cardiac muscle cell action potential	GO:0098901	1.709×10^{-2}	
regulation of action potential	GO:0098900	1.742×10^{-2}	
cardiac muscle cell membrane repolarization	GO:0099622	1.771×10^{-2}	
regulation of system process	GO:0044057	1.809×10^{-2}	
renal system process	GO:0003014	1.809×10^{-2}	
regulation of membrane repolarization	GO:0060306	2.212×10^{-2}	
regulation of transport	GO:0051049	2.593×10^{-2}	
regulation of renal system process	GO:0098801	2.593×10^{-2}	
modulation of chemical synaptic transmission	GO:0050804	2.593×10^{-2}	
regulation of trans-synaptic signaling	GO:0099177	2.593×10^{-2}	
regulation of ion transmembrane transport	GO:0034765	2.593×10^{-2}	
behavior	GO:0007610	2.593×10^{-2}	
blood circulation	GO:0008015	2.593×10^{-2}	
cellular process	GO:0009987	2.593×10^{-2}	
heart contraction	GO:0060047	2.593×10^{-2}	
circulatory system process	GO:0003013	2.693×10^{-2}	
heart process	GO:0003015	2.898×10^{-2}	
regulation of neuron projection development	GO:0010975	3.078×10^{-2}	
phosphatidylcholine biosynthetic process	GO:0006656	3.227×10^{-2}	
cation transport	GO:0006812	3.227×10^{-2}	
regulation of membrane potential	GO:0042391	3.273×10^{-2}	
regulation of plasma membrane bounded cell projection or...	GO:0120035	3.313×10^{-2}	
regulation of cell projection organization	GO:0031344	3.793×10^{-2}	
regulation of blood circulation	GO:1903522	3.894×10^{-2}	
action potential	GO:0001508	3.970×10^{-2}	
negative regulation of cytokine secretion involved in immun...	GO:0002740	3.989×10^{-2}	
positive regulation of cell projection organization	GO:0031346	3.989×10^{-2}	
operant conditioning	GO:0035106	3.989×10^{-2}	
phosphate-containing compound metabolic process	GO:0006796	4.966×10^{-2}	

FIU | FLORIDA INTERNATIONAL UNIVERSITY



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?

Prediction

Early Identification

Removal of DDT/DDE?

High DDE
↑ APP
↑ Aβ

BACE1 Inhibitor?
Aβ antibody?

FIU | FLORIDA INTERNATIONAL UNIVERSITY

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

FIU | FLORIDA INTERNATIONAL UNIVERSITY