UCSF
University of California
San Francisco

The Search for Gene*Environment Interactions in Parkinson's Disease

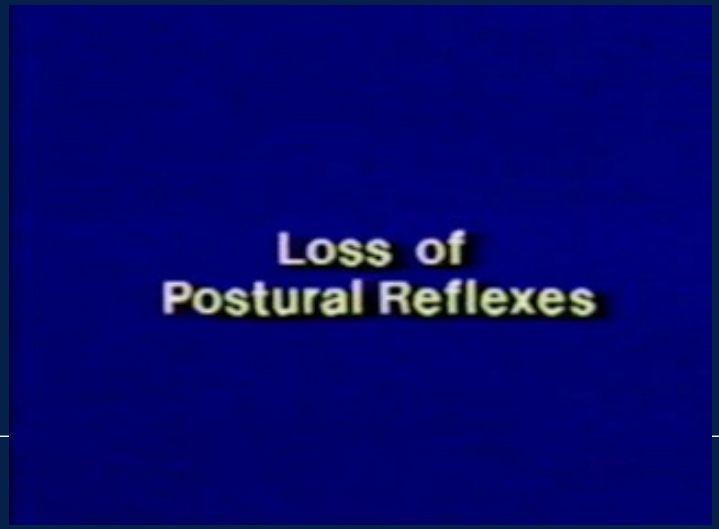
Samuel M. Goldman, MD, MPH
Emeritus Professor
Division of Occupational, Environmental, and Climate Medicine
& Department of Neurology
University of California San Francisco

Disclosures: None

Parkinson Disease

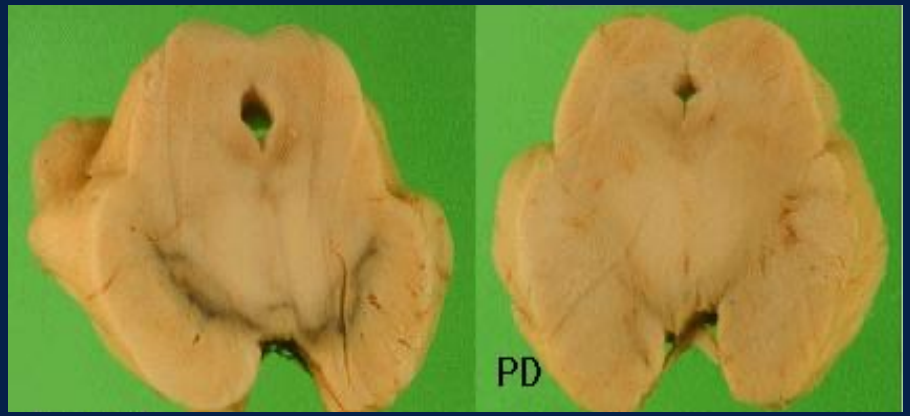
Clinical Syndrome: *Parkinsonism*
Bradykinesia, resting tremor, rigidity, postural reflex impairment

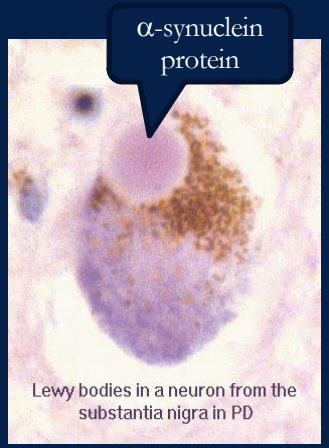
Neural substrate: Nigrostriatal system, disrupted dopaminergic transmission



UCSF

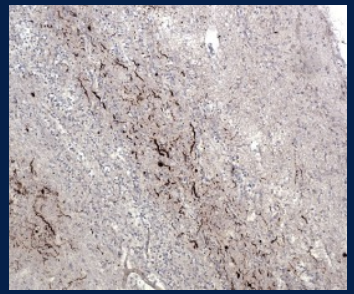
PD Pathology: Loss of Pigmented Dopaminergic Neurons in the *substantia nigra pars compacta*





But….PD is a <u>systemic</u> disease

Synuclein pathology in myenteric plexus



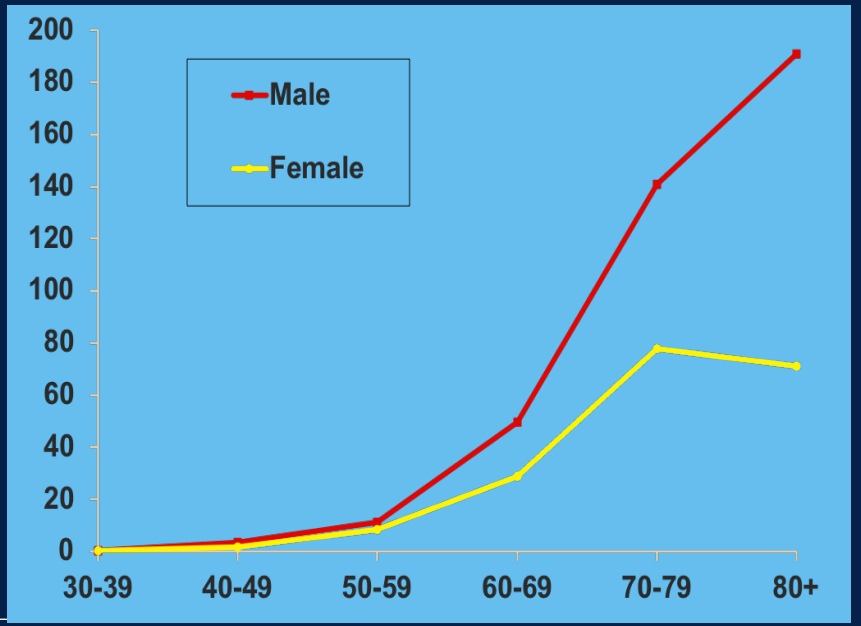
Synuclein pathology in olfactory bulb

UCSF

Parkinson's Disease: Overview

- Most common cause of parkinsonism
- Depicted in ancient texts:
  - Maimonides 1200s Spain
  - "kampavata" in Indian Ayurveda
- Described by James Parkinson 1817
- Affects ~ 1 million in U.S.
- ~ 2% prevalence > 65 years of age
- Men > Women
- Whites > Blacks

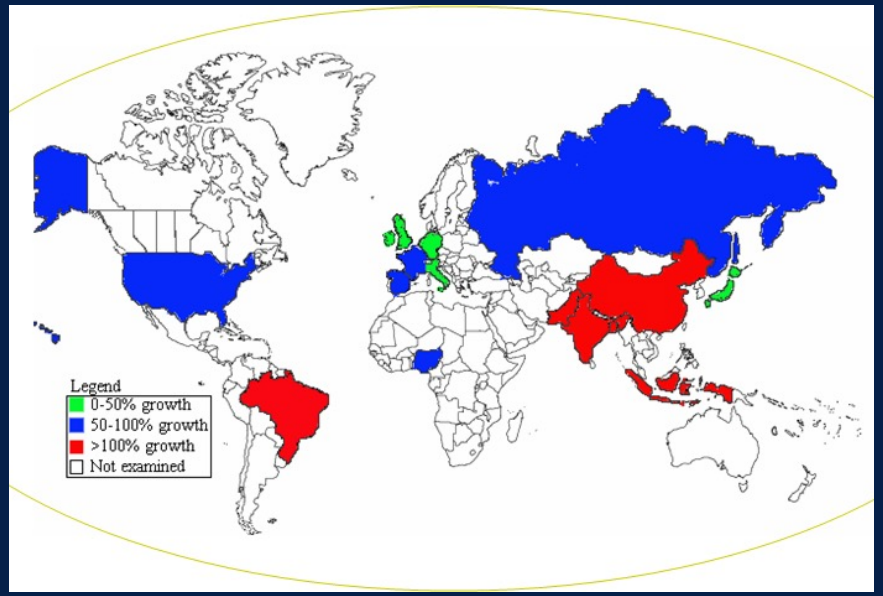
Van den Eeden et al, Am J Epi, 2003



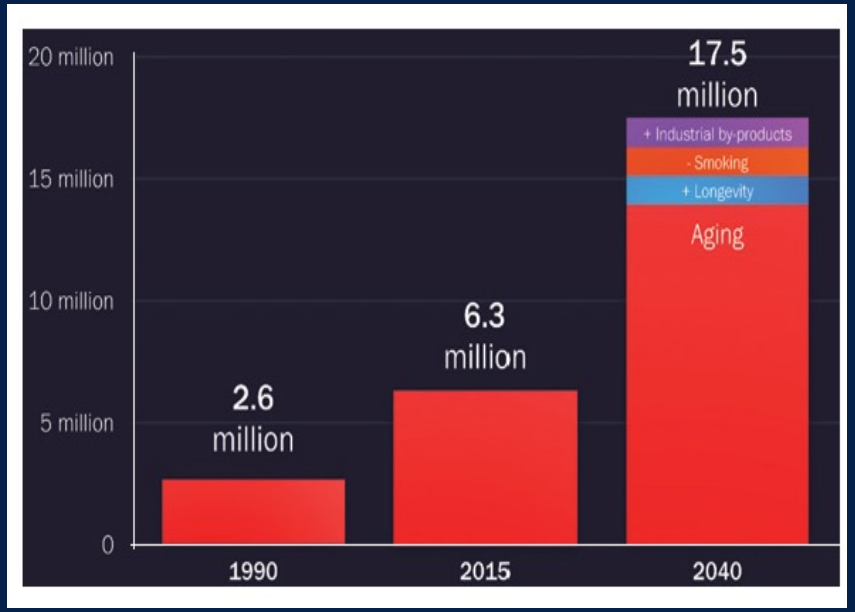
UCSF

The Global Burden of PD: Projected Increases

Change in number of people with PD in the world's most populous nations from 2005 to 2030



Projected global burden of PD



Dorsey et al, Neurology 2007;68:384-6;
Dorsey et al, JPD 2018;S3-S8.

UCSF

PD Genetics vs. Environment Debate

"…paralysis agitans is not a family disease"

Charcot, 1877

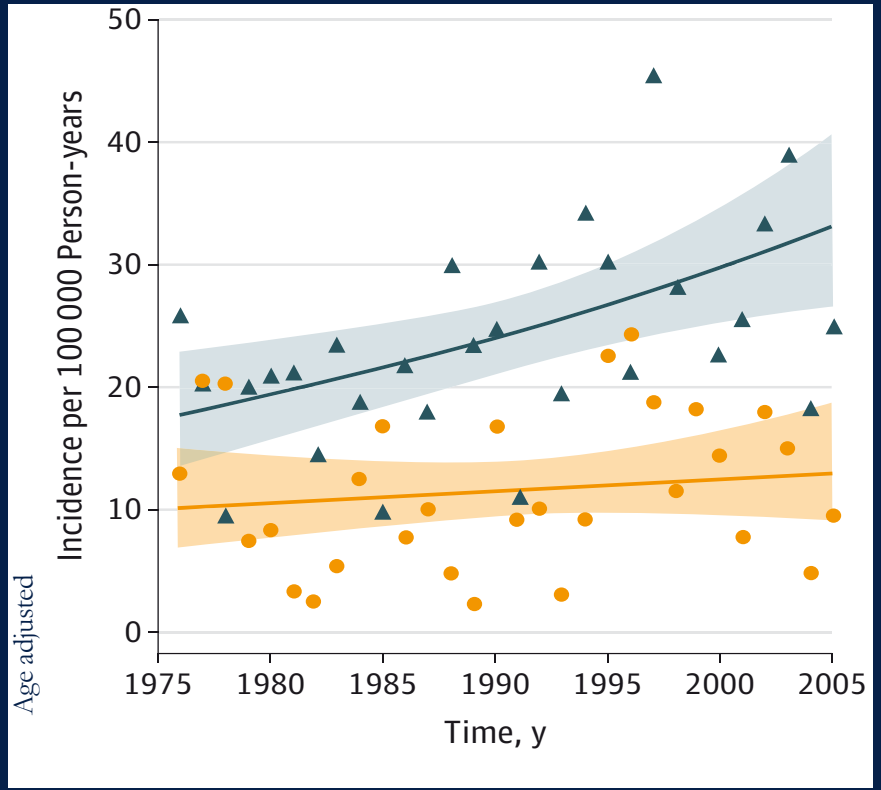
"Many patients with the disease have a strong family history…"

Gowers, 1888

PD Age-Adjusted Incidence Is Increasing

Parkinson's disease incidence
in Olmstead County, MN
1976-2005

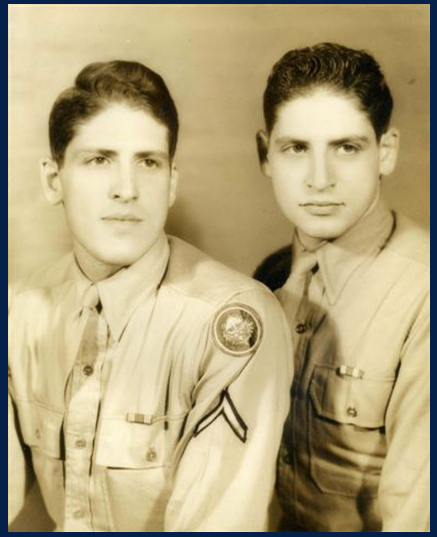
Increase *per decade*
Men: 26%
Women: 12%



Savica et al, JAMA Neurol, 2016

Twins: Nature's Controlled Study

Tanner, et al, JAMA 1999; Goldman, et al, Ann Neurol 2019



NAS/NRC WWII
VETERAN TWINS ROSTER
31,848 TWINS BORN 1917 - 1927

- Identical twins share 100% of genes
- Fraternal twins share ~ 50% of genes

Hypothesis: If PD is primarily genetic, MZ concordance should be >> DZ concordance

Results: Similar concordance in MZ & DZ twin pairs
80% Heritability ≤ age 50
19% Heritability > age 50

Conclusion:

- Environment is the major contributor to typical PD
- 13% concordance in DZ twins is 3x higher than typical sibs: shared early environment!

UCSF

Name This Disease

Mechanism of MPTP Toxicity

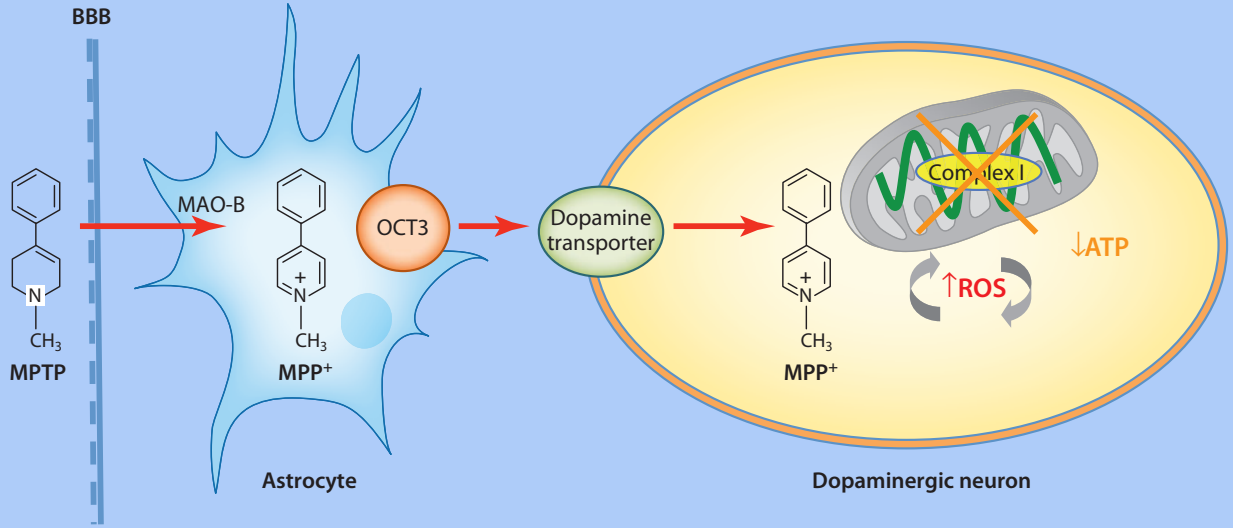


Figure 1

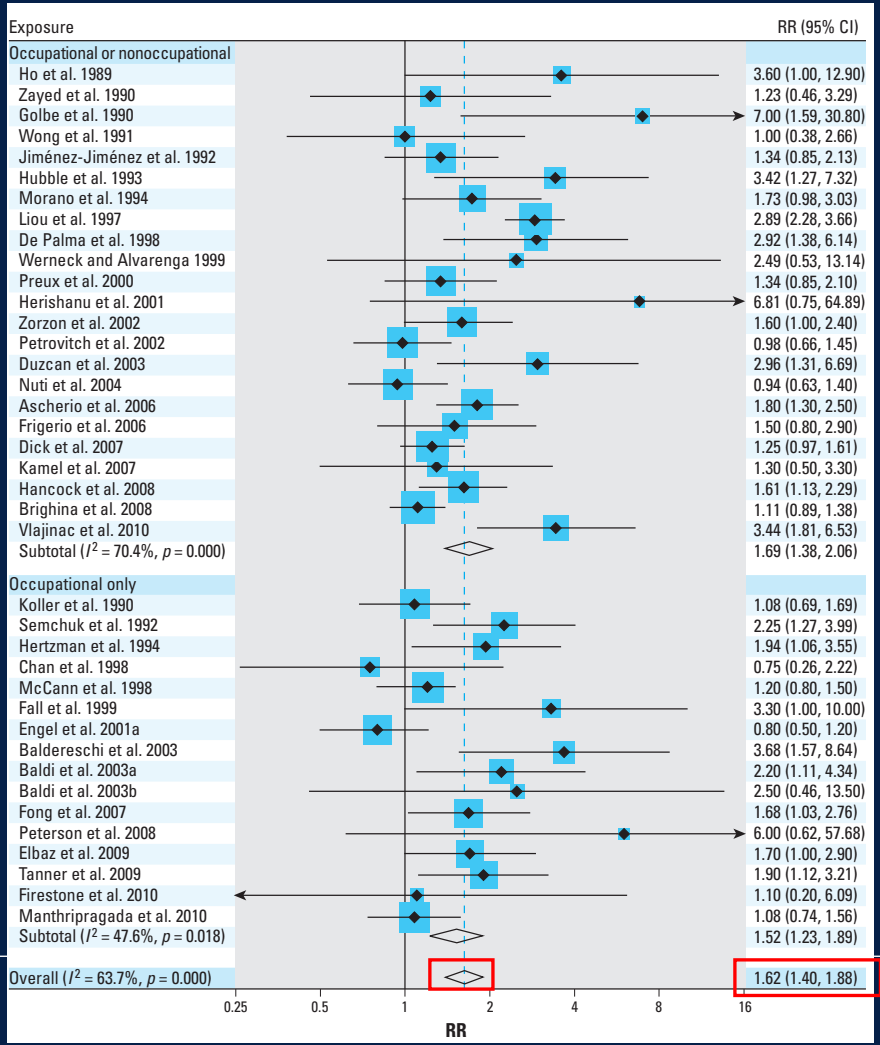
Mechanism of MPTP toxicity. Abbreviations: ATP, adenosine triphosphate; BBB, blood-brain barrier; MAO-B, monoamine oxidase B; MPP^+, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; OCT3, organic cation transporter 3; ROS, reactive oxygen species.

Goldman, Ann Rev Pharmacol Toxicol, 2014

UCSF

Pesticides & PD

- Pesticide use associated in >50 studies worldwide
- Specific compounds rarely studied



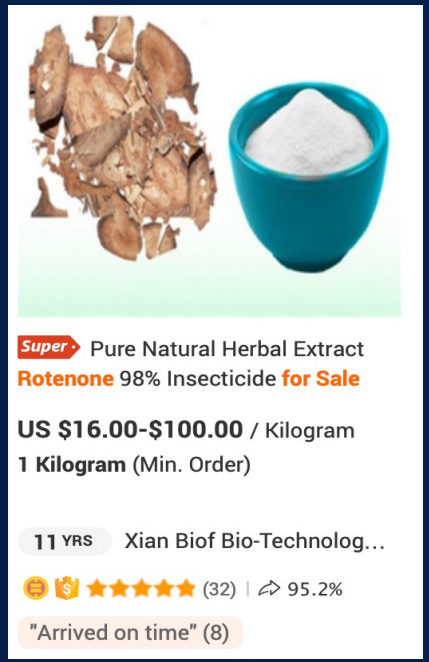
Van der Mark et al, EHP, 2012

FAME Study: PD in Agricultural Health Study

Tanner, et al, EHP, 2011

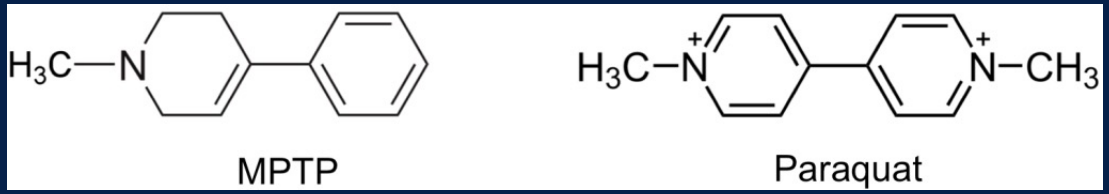
- Professional pesticide applicators (mostly farmers) & spouses
- 110 PD cases, 358 controls
- Asked about use of 31 specific pesticides
- Very good historians!
- Only 2 pesticides significantly associated

Pesticide	PD Risk	P-value
Rotenone	2.8	0.005
Paraquat	2.5	0.004



Common Toxic Mechanisms

	MPTP	Paraquat	Rotenone
Mitochondrial poison	+	+	+
Oxidative stress	+	+	+
Generates an animal model of PD	+	+	+



Solvents & Parkinson's Disease?

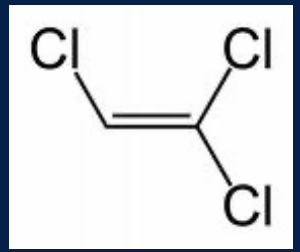
- Acute solvent intoxications can cause parkinson*ism*
  - Methanol, n-hexane, hydrocarbon mixtures….
  - Carbon disulfide in rubber and rayon manufacturing (Delpech, 1850s; Hamilton, 1930s)
- Associations with ***Parkinson's Disease*** *not* consistent
- Specific solvents rarely studied

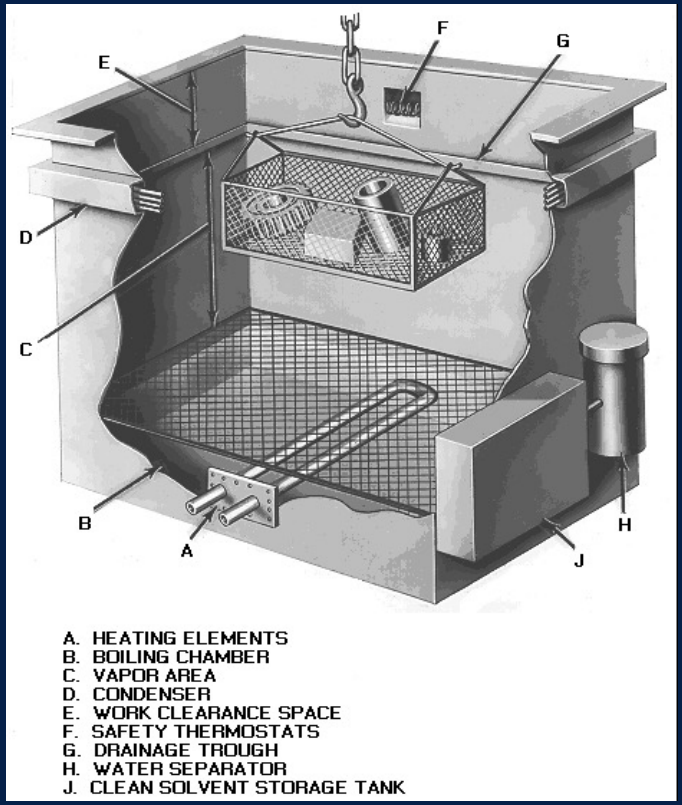
A PD Cluster

Gash et al, Ann Neurol, 2008

- Small Kentucky industrial plant with 30 employees
- PD in 3 co-workers
- mild parkinsonian signs/symptoms in 14 others

- Open vapor-degreasing vat containing trichloroethylene (TCE)





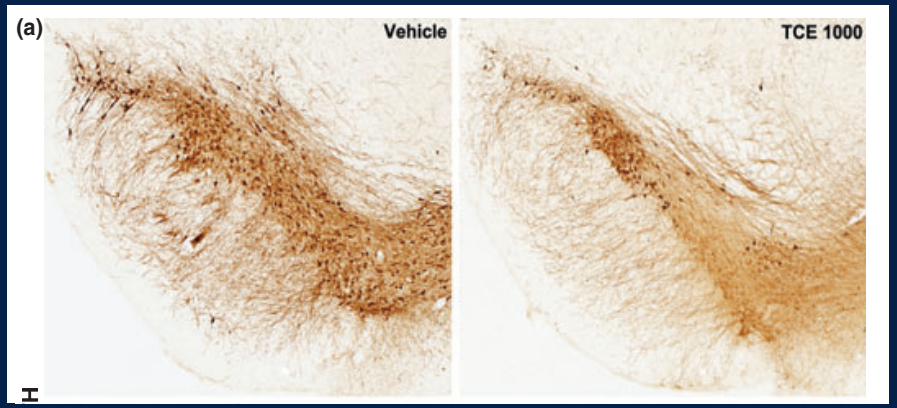
Occupational Solvent Exposures in PD-Discordant Twins

Goldman, Tanner, et al, Ann Neurol, 2012

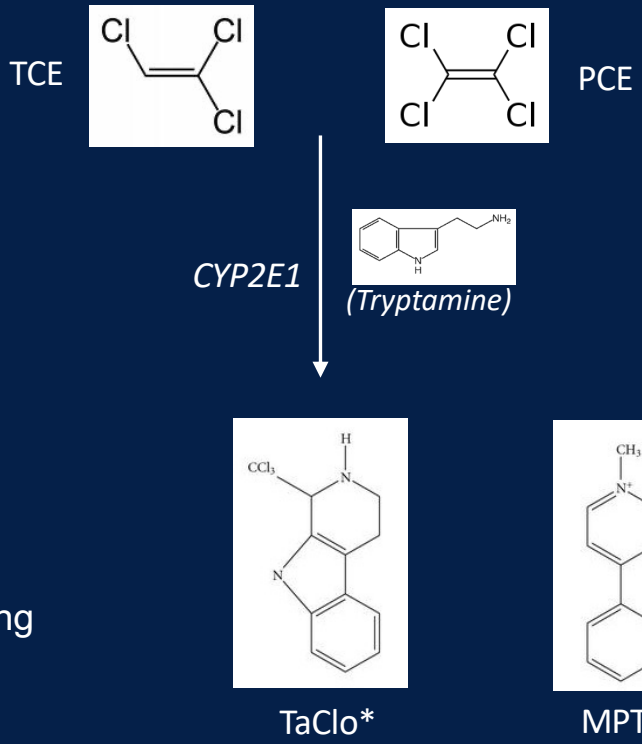
- 99 male twin pairs discordant for PD
- Lifelong job-task-specific occupational history
- Exposure assigned by industrial hygienist unaware of case status

Compound	Odds ratio ever/never	95% Confidence Interval	p-value
N-hexane	1.3	0.40-4.1	0.69
Toluene	1.3	0.49-3.3	0.61
Xylene	2.2	0.43-12	0.34
CCl_4	2.3	0.88-6.1	0.088
TCE	**6.1**	**1.2-33**	**0.034**
PCE	**10.5**	**0.97-113**	**0.053**

Oral TCE causes selective dose-related degeneration of dopaminergic neurons in rat substantia nigra



Liu, et al, J Neurochem, 2010



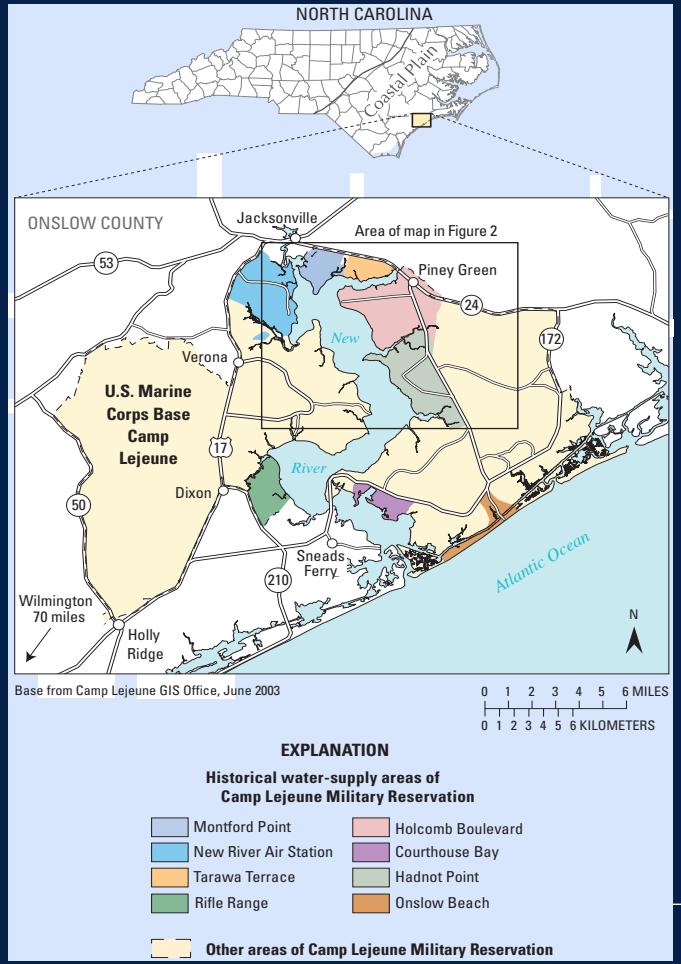
TaClo forms spontaneously in rat brain after TCE dosing

Liu, et al, Mol Neurobiol, 2017

*1-trichloromethyl-1,2,3,4-tetrahydro-β-carboline

UCSF

Camp Lejeune, NC



History

- 1953 onward: wells contaminated with TCE (on-base dumping), PCE (off-base dry-cleaner)
- 1987: Contaminants discovered, wells closed
- 1989: EPA Superfund site

Median water levels 1975-85 *(EPA MCLs 5ug/l)*:
TCE 366 ug/l; PCE 85 ug/l

Sources of exposure: Drinking water (oral), cooking (oral, inhalation), bathing (inhalation, dermal), swimming/recreation (inhalation, dermal)

UCSF

JAMA Neurology | Original Investigation

JAMA Neurol. doi:10.1001/jamaneurol.2023.1168
Published online May 15, 2023.

Risk of Parkinson Disease Among Service Members at Marine Corps Base Camp Lejeune

Samuel M. Goldman, MD, MPH; Frances M. Weaver, PhD; Kevin T. Stroupe, PhD; Lishan Cao, MS; Beverly Gonzalez, PhD; Kalea Colletta, DO; Ethan G. Brown, MD; Caroline M. Tanner, MD, PhD

- Cohort established by Agency for Toxic Substances and Disease Registry (ATSDR)
- All servicemembers stationed during 1975-1985 at:
  - Camp Lejeune, NC (water contaminated), n=172,128
  - Camp Pendleton, CA (water uncontaminated), n=168,361
- VHA & Medicare (1999-2021) ICD code search: inpatient, outpatient, pharmacy
- Diagnoses validated by review of all medical records (n > 1500)

Results: PD risk

- 430 PD cases ascertained: 398 diagnosed in VHA EMR; 32 Medicare data only
- 57 other neurodegenerative parkinsonism (no differences between camps)

	n	PD n (%)	Adjusted OR (95%CI)
Lejeune	84,824	279 (0.33)	**1.70 (1.39-2.08)**
Pendleton	73,298	151 (0.21)	Ref

- Prodromal diagnoses also more common in Lejeune (tremor, olfaction, ED….)

TCE (trichloroethylene)

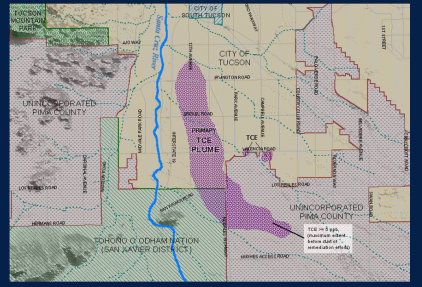
Common exposures since the 1920s

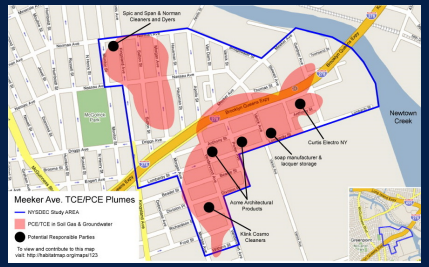
- Degreasing metal parts (85%), computer circuits
- Dry-cleaning
- Surgical anesthetic (until 1977)
- Decaffeinated coffee (until 1977)
- Typewriter correction fluids, adhesives, paints, carpet cleaners, spot removers…..

Most frequently reported organic contaminant in groundwater: detected in up to 33% of US water supplies

Subsurface "plumes" common, with vapor intrusion into homes through foundations

Tucson



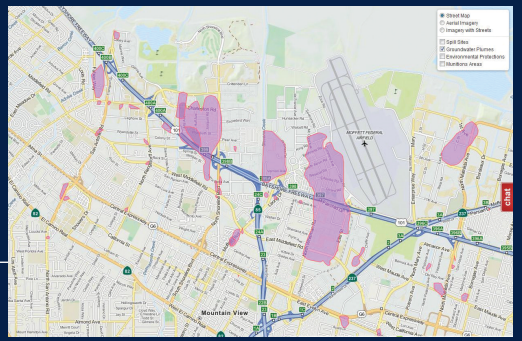


Brooklyn

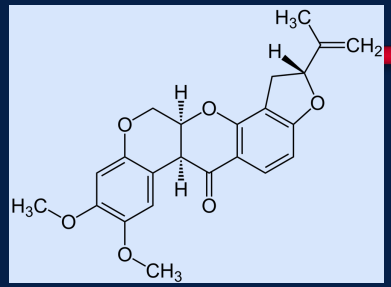
Dayton

Minneapolis

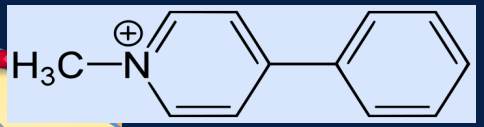
Mountain View



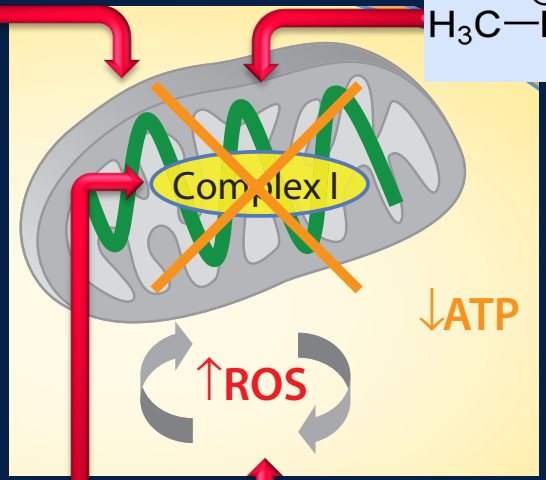
Toxicants Associated with PD: Mechanistic & Structural Similarity



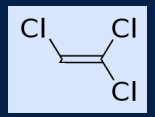
Rotenone

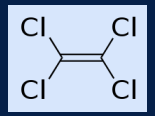


MPP+



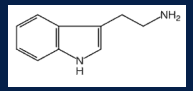
TCE



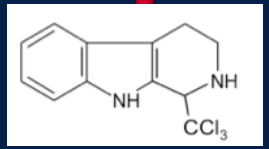


PCE

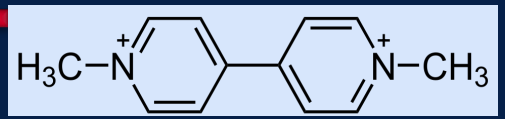
CYP2E1



Tryptamine



TaClo*



Paraquat

*1-trichloromethyl-1,2,3,4-tetrahydro-β-carboline

PD Environmental Associations

Increased Risk

Head trauma
Diet: dairy products, animal fats
Environmental exposures
- MPTP
- **Pesticides (paraquat, rotenone, others)**
- Industrial agents (PCBs)
- **Solvents (TCE, PERC)**
- Metals (lead, iron)
- **Rural residence**
- Drinking well water

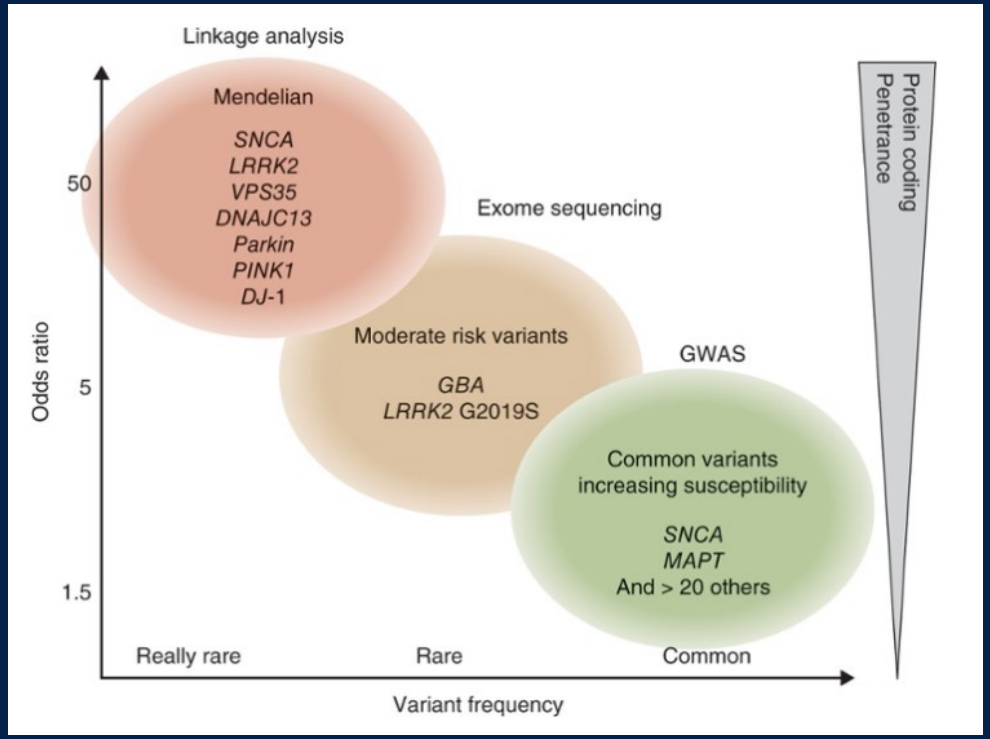
Occupations: health care, teaching, carpentry, lawyer, clergy, **farming**

Reduced Risk

Cigarette smoking
Coffee and tea drinking
Diet
- "Mediterranean" diet
- polyunsaturated fats
- uricogenic diet

Physical activity
Medications
- Calcium-channel blockers
- **NSAIDs**
- Statins

Mendelian variants are rare causes of PD. Individually, most common variants impart very modest increases in risk.



From Brockmann & Gasser, 2015, in Parkinson's Disease & Movement Disorders, Jankovic & Tolosa eds

UCSF

Meanings of the Term "Interaction" in Epidemiology

Biological interaction:

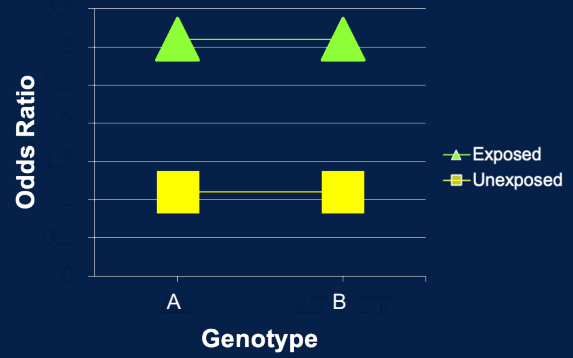
- act on the same or intersecting pathophysiologic pathways
- may involve multiple environmental, genetic, epigenetic factors
- this is what we really care about and hope to measure

Statistical interaction:

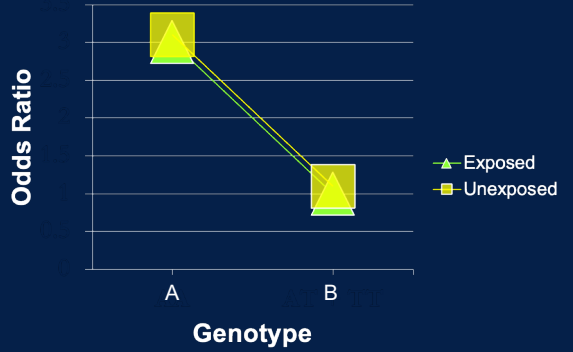
- deviation of a model from the expected joint effects of two or more factors
- this is what we actually measure in epi studies

Gene-Environment Interaction in Epidemiologic Studies

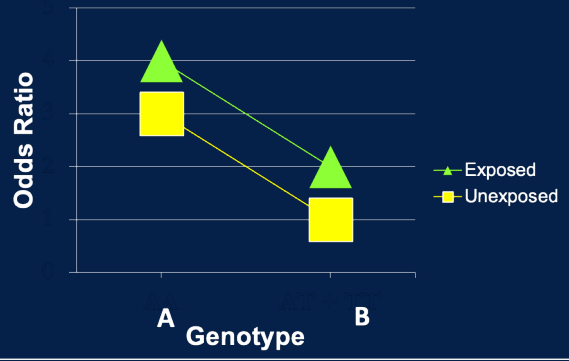
Environment association, no genotype association



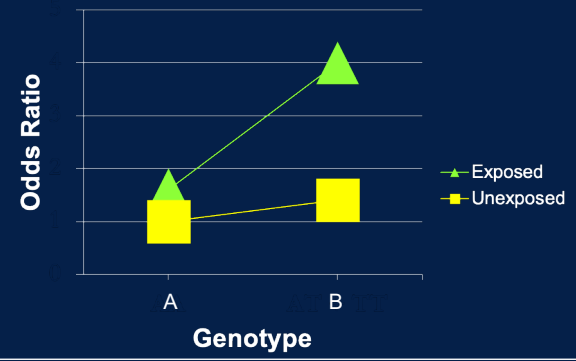
Genotype association, no environment association



Environment association, genotype association: no interaction



Environment association, genotype association: Synergistic statistical interaction



UCSF

Challenges in Studying Gene-Environment Interactions

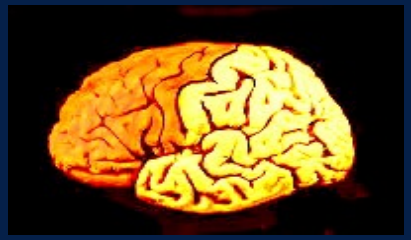
- Biological:
  - Mechanistic effects of genes & exposures often poorly understood
  - “Pathways” likely more relevant than single genes
  - Environmental exposures don’t occur in isolation
- Epidemiological:
  - PD is difficult to diagnose
  - Exposure measurements imprecise, highly variable across studies
  - Population heterogeneity may limit replication
- Statistical: sample size!
  - Allele prevalence = 0.1, exposure prevalence = 0.1
  - Odds ratio of interaction = 4 (this is high!)
  - ~ 700 cases & 700 controls needed for 80% power

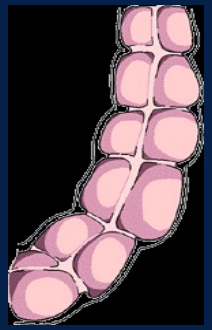
Practical Examples of G*E: Pharmacogenetics

- Pythagoras noted that some persons became ill after eating fava beans (hemolytic anemia in G6PD deficiency)

- Numerous modern examples:
  - Isoniazid * *NAT2*: peripheral neuropathy, hepatitis
  - Anti-depressant * *CYP2D6*: efficacy/toxicity
  - Anti-neoplastics * *ABCB1*: efficacy/toxicity
- Due to variation in "ADME" genes: absorption, distribution, metabolism, excretion

Some Potential Biological Mechanisms of Gene * Environment Interaction

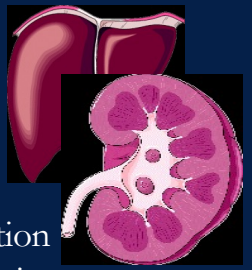




Toxicant

- Transporters (*OCT3*)
- Metabolism (*CYP2D6*)
- Injury Protection (*SOD1*)
- Injury Response (*COX2*)
- Repair/plasticity (*BDNF*)
- Protein expression (*SNCA*)

} Absorption
Distribution
Metabolism
Excretion

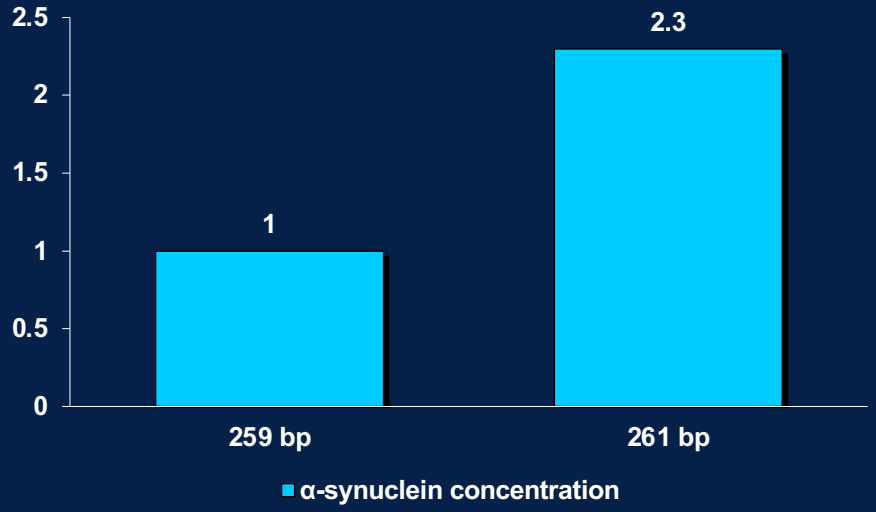


Example 1: Gene*Environment Interaction in PD

Gene: *SNCA* (alpha synuclein)

Environment: Head injury

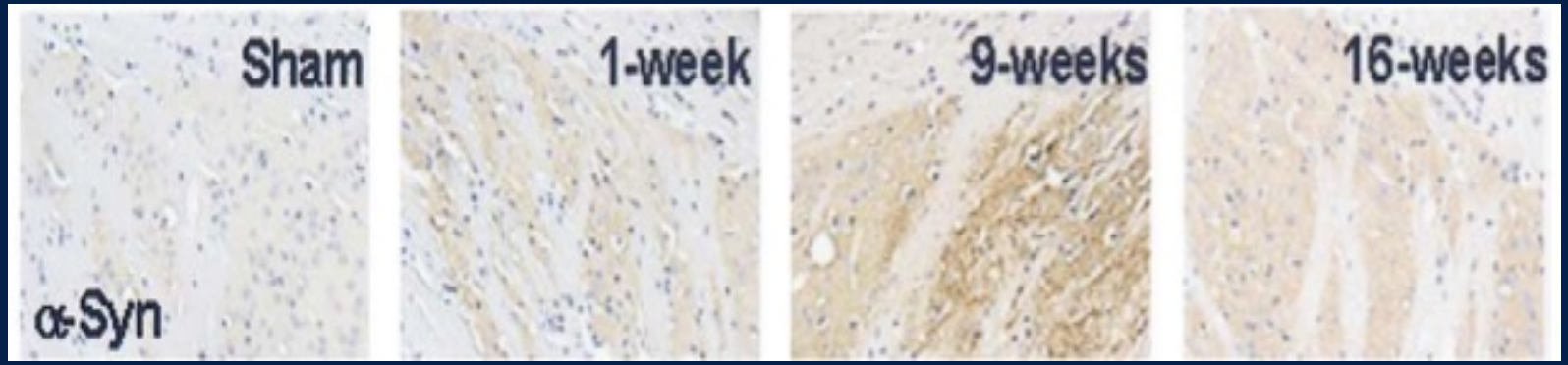
In rodent, *SNCA* Rep1 expansion increases α-synuclein expression in brain



Cronin, et al, Human Molecular Genetics, 2009

Rodent Models of Moderate Head Injury

Percussive TBI increases α-synuclein immunoreactivity and kills dopaminergic neurons in mouse striatum



Uryu, et al, Experimental Neurology, 2003

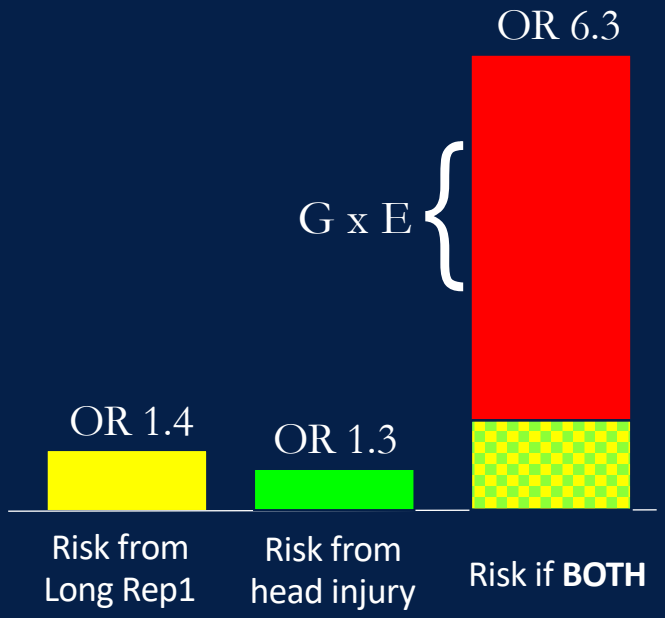
Research Question

Does SNCA Rep1 length modify the effect of head injury on PD risk?

Head Injury & SNCA Rep1 length

Goldman, Tanner et al, Ann Neurol, 2012

- Study population: 2 large case-control studies (n=964)



Example 2: Gene*Environment Interaction in PD

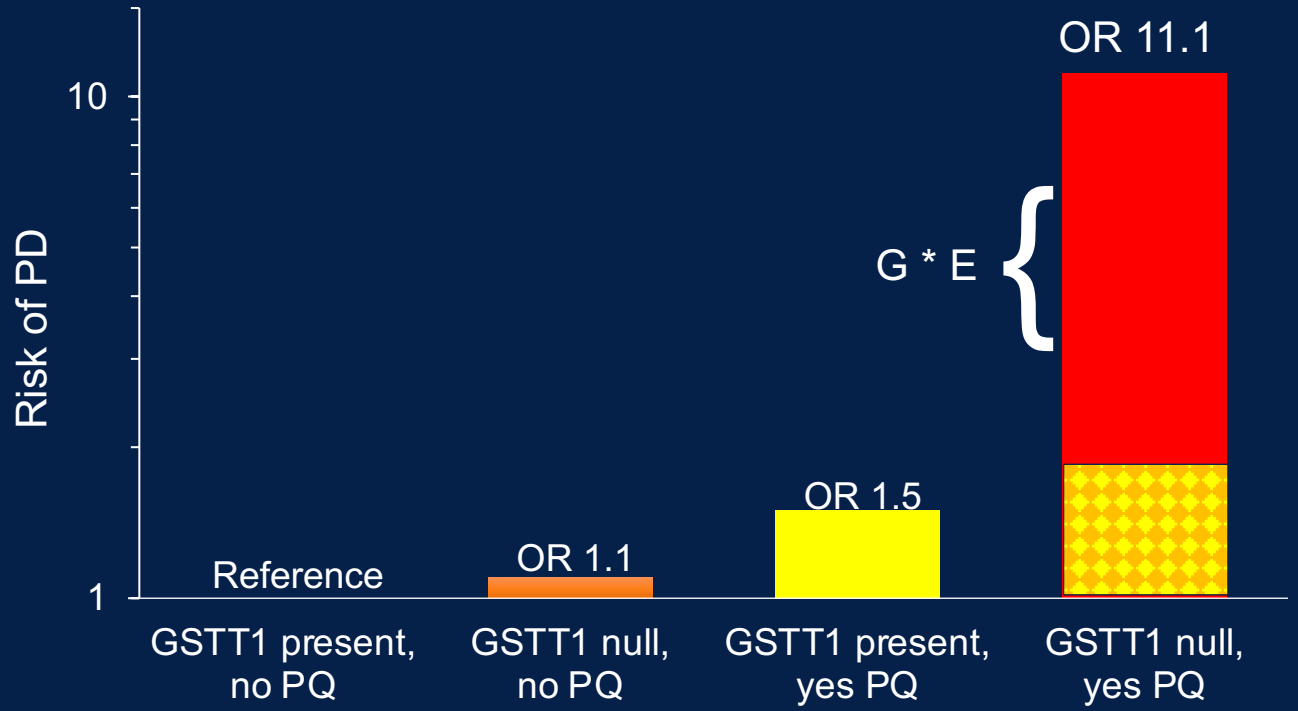
Gene: *GSTT1* (glutathione-S-transferase T1)

Environment: Paraquat

Paraquat and *GSTT1* Gene

Goldman, Tanner, et al, Movement Disorders, 2012

- Paraquat: animal model, redox-cycling, oxidative stress, associated in several human studies
- Glutathione-S-transferase T1: anti-oxidant, glutathione reduced in PD, 20% homozygous null



Example 3: Environment in a genetically at-risk cohort

Gene: *LRRK2*

Environment: NSAIDs

NSAIDs & LRRK2-PD

San Luciano et al, Movement Disorders, 2020

- All participants (n=577) carry *LRRK2* mutations (30% penetrant)
- Regular NSAID use (at least twice weekly > 6 months) prior to index date
- Is NSAID use associated with penetrance?

Regular Use of NSAIDs			
NSAID	**OR**	**95%CI**	**p**
Any NSAID	0.38	0.21-0.67	< 0.001
Ibuprofen	0.19	0.07-0.50	< 0.001
Aspirin	0.79	0.38-1.67	0.5

G*E Associations in PD

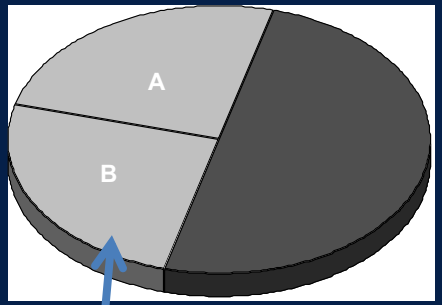
Exposure	Gene	Risk variant[&]	Design for interaction	PD	CT	Interaction (p)	Joint effect OR [95% CI] or (SE)
Pesticides	*ABCB1*	rs1045642 C>T, p.Ile1145Ile	Case-only	415	—	—	4.74 [1.01–22.31]
Organochlorines	*ABCB1*	rs2032582 G>[A,T], p.Ser893Ala/Thr	Case-only	207	—	—	5.4 [1.1–27.5][*]
Organophosphorus	*ABCB1*	rs1045642 C>T, p.Ile1145Ile rs2032582 G>[A,T], p.Ser893Ala/Thr	Case-control	350	724	NA	1 allele 2.1 [1.3–3.2][*] Both alleles 3.7 [2.0–7.0][*]
Diazinon Chlorpyrifos	*PON1*	rs854560 T>A, p.Leu55Met (SM)	Case-control	351	363	NA	2.2 [1.1–4.5] 2.6 [1.3–5.4]
Organophosphates	*PON1*	rs854560 T>A, p.Leu55Met (SM)	Case-control	357	807	NA	2.62 [1.4–4.8]
Pesticides	*CYP2D6*	rs3892097 G>A, null allele (PM)	Case-control	190	419	0.02	4.74 [1.29–17.45]
Pesticides	*CYP2D6*	rs3892097 G>A, null allele (PM)	Case-control	393	389	0.05	8.41 [1.01–69.76]
Caffeine	*CYP1A2*	rs762551 C>A rs2470890 C>T, p.Asn516Asn	Case-control	925	1249	0.05 0.04	0.33 [0.16–0.68][$] 0.43 [0.27–0.69][$]
Paraquat	*GSTT1*	Null allele	Case-control	87	343	0.027	11.1 [3.0–44.6]
Solvents	*GSTM1*	Null allele	Case-only	959	—	—	2.34 [1.08–4.62]
Smoking	*GSTP1*	GSTP1*C haplotype	Case-only	400	—	—	2 [1.11–3.60]
Pesticides	*SLC6A3*	5′ A clade and 3′ VNTR 9-repeats	Case-control	178 men	239 men	0.02	5.66 [1.73–18.53][*]
Paraquat, maneb	*SLC6A3*	5′ A clade and 3′ VNTR 9-repeats	Case-control	324	334	<0.001	4.53 [1.70–12.09]
Smoking	*MAO-B*	rs1799836 A>G	Case-control	82	118	NA	0.24 [0.10–0.55][$]
Pesticides	*MnSOD* *NQO1*	rs4880 T>C p.Val16Ala rs1800566 C>T p.Pro153Ser	Case-control	153	155	<0.001	2.49 [1.18–5.26] 2.42 [1.16–4.76]
Pesticides	*NOS1*	rs12829185 T>C rs10774910 T>C rs2682826 A>G rs2314810 G>C	Case-control	156	174	0.034 0.026 0.028 0.024	3.12 [1.71–5.71][$] 4.15 [1.85–9.34][$] 3.52 [1.78–6.95][$] 0.56 [0.34–0.92][$]
Smoking	*NOS2A*	rs2248814 A>G rs1060826 T>C	Case-control	179	204	0.021 0.013	0.23 [0.09–0.59][$] 0.17 [0.06–0.49][$]
Caffeine	*GRIN2A*	rs4998386 C>T	GWAIS + replications	2472	2848	3×10^{-5}	0.41 (0.05)
Caffeine	*GRIN2A*	rs4998386 C>T	Case-control	193	377	<0.001	0.38 [0.20–0.70]

Polito, et al, Parkinson's Disease, 2016

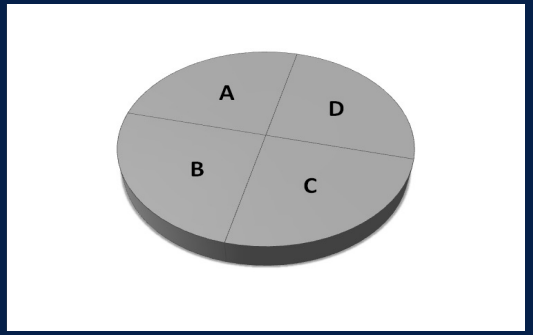
UCSF

Causation: What determines "penetrance" in the individual?

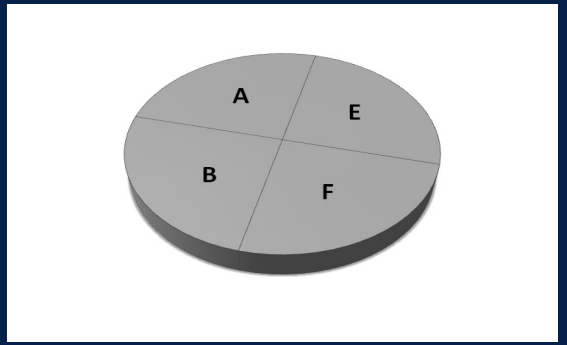
Disease: A Broken Wrist



Component cause



Sufficient cause 1:
A = stairs
B = ice
C = osteoporosis
D = porch light burned out



Sufficient cause 2:
A = stairs
B = ice
E = high heels
F = alcohol

Predicting Individual Risk for PD

- DoD funded ongoing work
- FAME study of professional pesticide applicators
  - Whole-genome sequencing
  - Gene burden tests of deleterious rare variants
  - Exposed-only analyses identify genes interacting with paraquat, rotenone, permethrin, dieldrin
  - Machine learning to derive pesticide-specific cumulative interaction risk scores
- CRISPR to knock down suspect genes in iPS-derived dopamine neurons
  - Test neuron sensitivity to the specific pesticides

ML-based gene-burden tests with Rotenone

- Highest importance gene*E

CYP1B1	0.150696
GLRX	0.053897
CTNS	0.049122
ABCB1	0.04593
NQO1	0.044611
CEP68	0.030758
CYP2S1	0.03039
NXN	0.029461
TXNDC11	0.028275
ABCB11	0.025

- ROC AUC predictive accuracy: 0.83

Current Directions for G*E Studies of PD

- Old-school hypothesis-driven “candidate” mechanistic approaches
- Genome-wide gene-environment studies (GEWIS)
  - Power is a major limitation
  - Exposure data rarely collected or inconsistent quality
- GWAS in exposed populations (e-GEWIS): ongoing work in our group
  - Can validate in vitro
- EWAS in populations defined by G
  - How E affects penetrance of Mendelian forms (e.g., NSAIDS in LRRK2-PD)
  - Can extend to animal models
- Individual susceptibility
  - Burden tests, ML

Our Collaborators

UCSF & SFVAMC
- **Carlie Tanner**
- Ethan Brown
- Marta San Luciano
- Patricia Quinlan
- Kathleen Comyns
- Cheryl Meng
- Monica Korell
- Farah Kausar
- Raymond Swanson

VA Pacific Islands Healthcare
- G. Webster Ross
- Helen Petrovitch
- Robert Abbott

German Center for Neurodegenerative Diseases (DZNE)
- Sarah Jewell
- Dino DiMonte

Toronto Western Hospital
- Connie Marras
- Anthony Lang

Univ of Lübeck
- Meike Kasten

Gladstone Institute
- Julia Kaye
- Steve Finkbeiner
- Leandro Lima

Kaiser Permanente DOR
- Stephen Van Den Eeden
- Kathleen Albers

NIEHS
- Freya Kamel
- Jane Hoppin
- David Umbach
- Marie Richards
- Dale Sandler

Stanford University
- Lorene Nelson
- J. William Langston
- Birgitt Schüle
- James Tetrud

Columbia University
- Ruth Ottman
- Richard Mayeux
- Karen Marder

Capital Univ Med Sci
- Piu Chan
- Kitty Gu

Favaloro University
- Anabel Chade

Buddhist Tzu Chi Hospital
- Raymond Lo

University of Pennsylvania
- Jonas Ellenberg

IND
- Kenneth Marek

University of Rochester
- David Oakes
- Ray Dorsey

Hines VA
- Frances Weaver
- Kevin Stroupe
- Lishan Cao
- Beverly Gonzalez
- Kalea Colletta

NCI
- Aaron Blair

NIA
- Andrew Singleton