

# Rethinking Health Outcomes Exploration in Pesticide Epidemiology

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# Pesticides

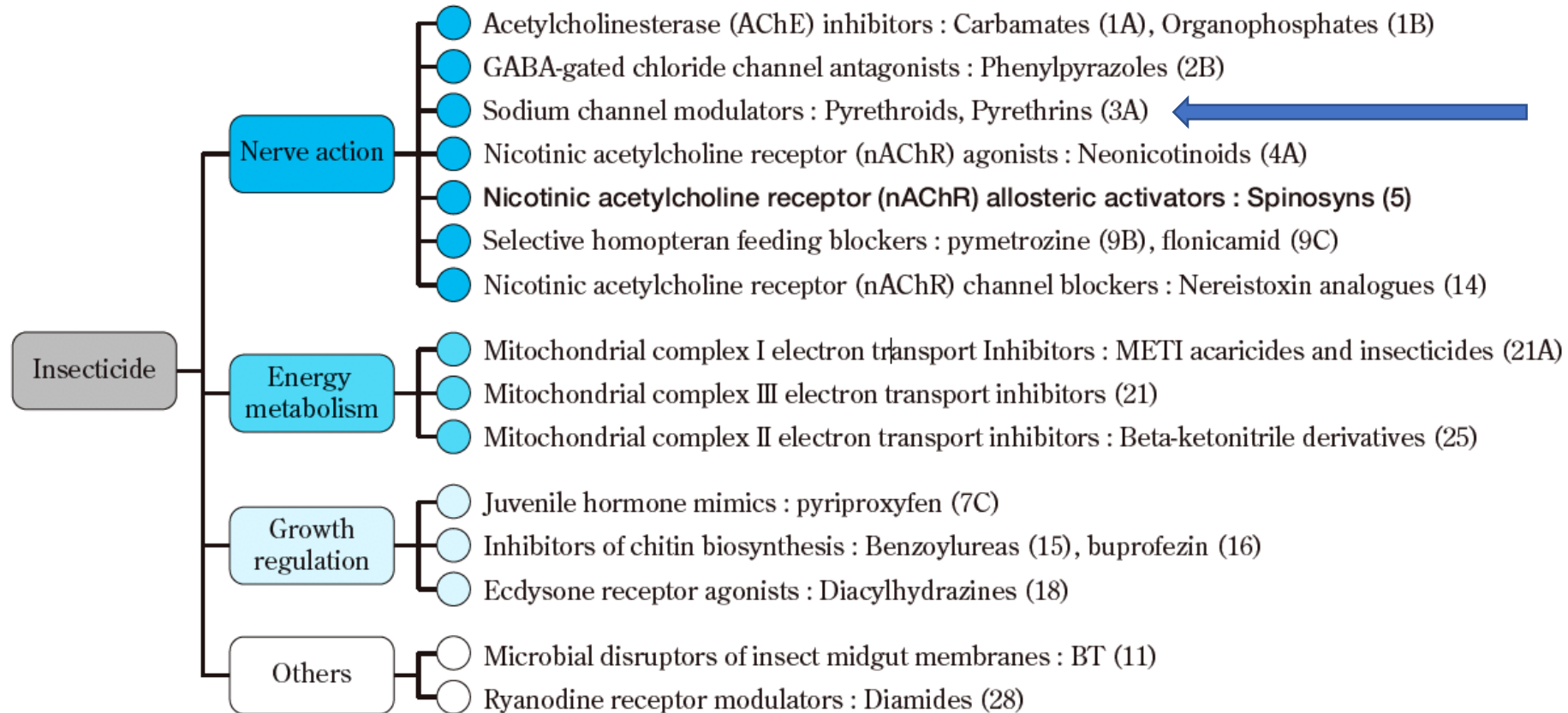


- Pesticides are a broad category
  - Insecticides, herbicides, fungicides, termiticides, bactericides, acaricides, miticides, rodenticides
  - Exposure routes: diet, agricultural drift, residential & occupational use





# Insecticides Display Wide-Ranging Toxicity



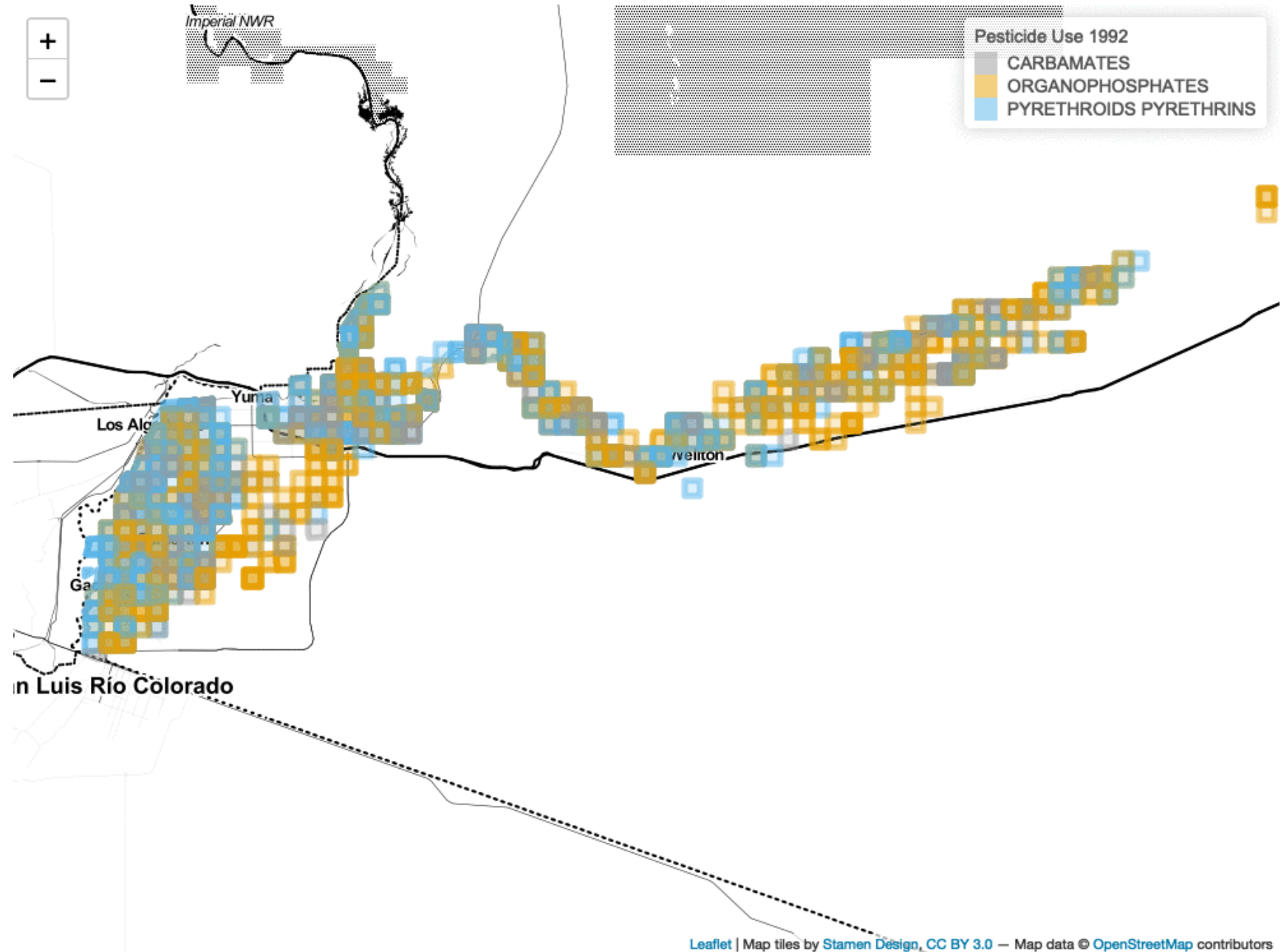
Source : IRAC MoA Classification ver.7.1 <sup>6)</sup>



## Rising Relevance of Pyrethroids: Animation of pesticide applications in Yuma county, 1992-2020

1992: mix of orange  
(OPs) and blues  
(pyrethroids)

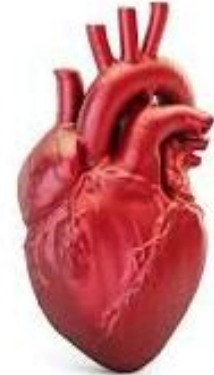
2019: mostly  
transitioned to blues  
(pyrethroids)





# Pyrethroid Targets

- Designed as **Sodium Channel** Modulators
  - Brain and Central Nervous System
    - Central, peripheral neurons, glial cells, dorsal root ganglion cells
    - Epilepsy, seizures, pain, Autism Spectrum Disorder, brain malformations, migraine, fibromyalgia
  - Heart
    - Brugada Syndrome, atrial fibrillation
  - Skeletal muscle
    - Paralysis, myotonia, ataxia, dystonia
  - Uterus
  - Gastrointestinal cells
    - Irritable bowel syndrome
- Pyrethroids also modulate **Other Ion Channels**
  - **Calcium, Potassium**





# The Case for an Untargeted Outcomes Approach

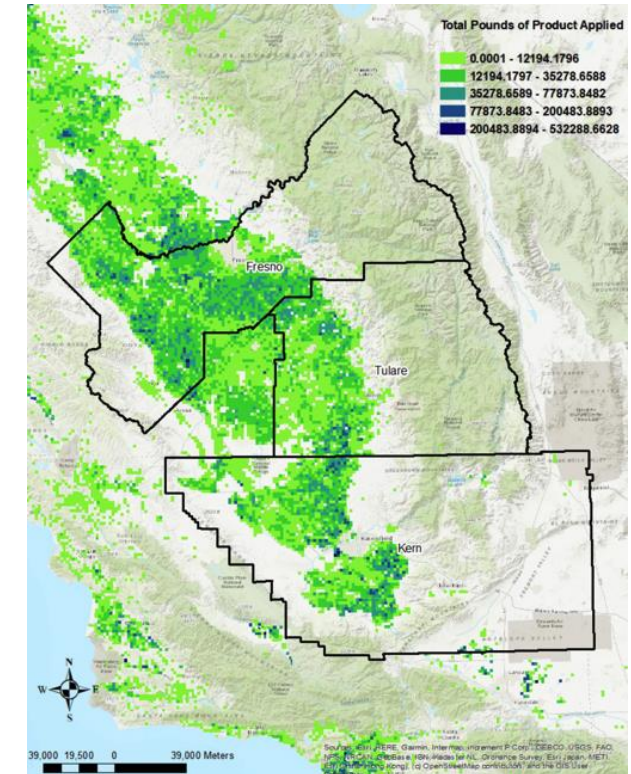
- Pyrethroid targets have broad biological relevance
- Epidemiology studies show wide range of associated health outcomes
- Untargeted studies can generate hypotheses for novel health targets





# Epigenome-Wide Association Study of Pyrethroid Exposure in California

- Controls from a case-control study of Parkinson's disease in California, PEG1 (PI Ritz)
  - Study Sample: PEG1 controls (n=237)
  - Recruited 2001-2007 from three counties in Central CA
  - Restricted to Non-Hispanic Whites and those of Hispanic ethnicity
- Exposure: Pyrethroid Pesticides
  - Pesticide Use Registry
  - Pounds of active ingredient within 500m of residence
    - 5 year averages for each pyrethroid pesticide at enrollment
    - We used binary variable to indicate exposure to any pyrethroid at >median levels for the study sample



*Figure of pesticide distribution for PEG study from Paul et al, in prep*



# Epigenome-Wide Association Study of Pyrethroid Exposure in California

## Methylation

- Untargeted study of differential methylation
  - Peripheral whole blood samples at enrollment
- Illumina 450k methylation chip
  - Methylation:
    - CpG sites are either methylated or unmethylated (0 or 1), varies by cell
    - Groups of cells are lysed and have a percent methylated (from 0 to 1)
  - Cleaning/Filtering:
    - Filtered out cross-reactive probes, Normalized for type1/type2 probe bias with BMIQ
    - Assessed for batch effects with SVA
  - Total sites assessed = 446,571 (filtered out about 40,000 sites)

## Analysis

- Beta Regression
  - Bounded values between 0 and 1
  - Control for education, age, sex, Hispanic ancestry, blood cell composition (Houseman method)
  - FDR  $q < 0.05$
- Pathway Analysis
  - Overrepresentation analysis (GO) for CpG sites at  $p < 0.05$
  - Overrepresentation analysis of diseases (Webgestalt's OMIM and GLAD4U databases) for genes annotated to CpG sites at  $p < 0.05$



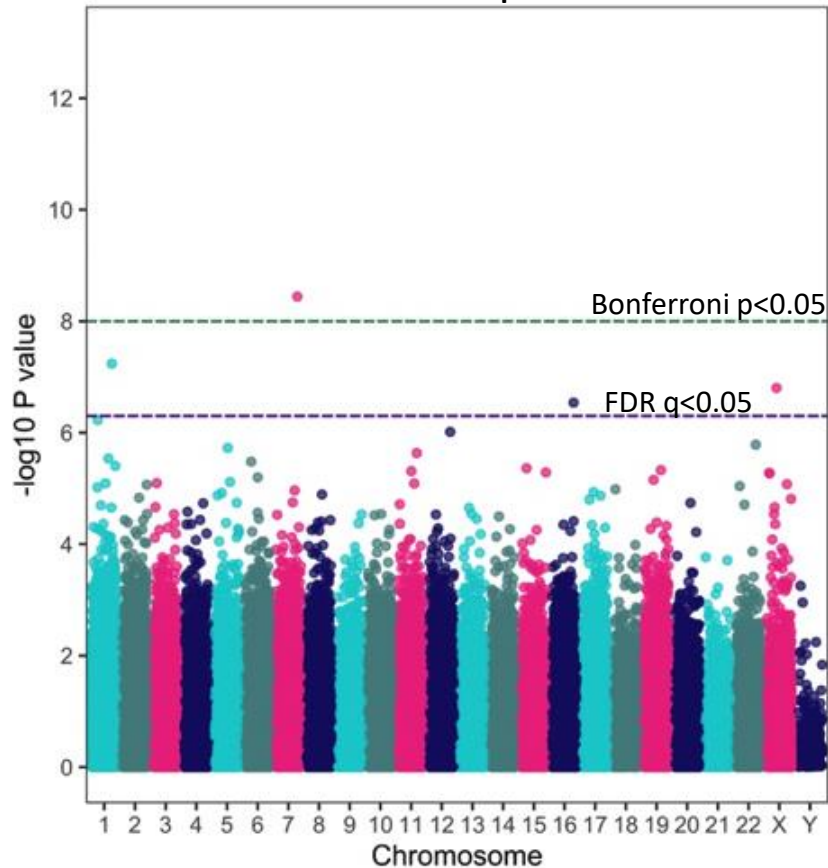
Demographic characteristics of the study participants (N = 237).

Characterstics	Study Participants
Age (mean, sd)	67.4 (12.8)
Sex (n, %)	
Male	126 (53.2)
Female	111 (46.8)
Ethnicity	
Non-Hispanic White	218 (87.3)
Hispanic White	19 (12.6)
Hispanic Ancestry Markers (mean, sd)	0.07 (0.17)
Pyrethroid Exposure	
< Median for All Pyrethroids in Past 5 Years	192 (81.0)
> Median for Any Pyrethroid in Past 5 Years	45 (19.0)
Organophosphate Exposure	
< Median for All OPs in Past 5 Years	164 (69.2)
> Median for Any OP in Past 5 Years	73 (30.8)



# Results- Associated CpG Sites & GO pathways

Manhattan Plot for CpG Sites



Associated CpG Sites

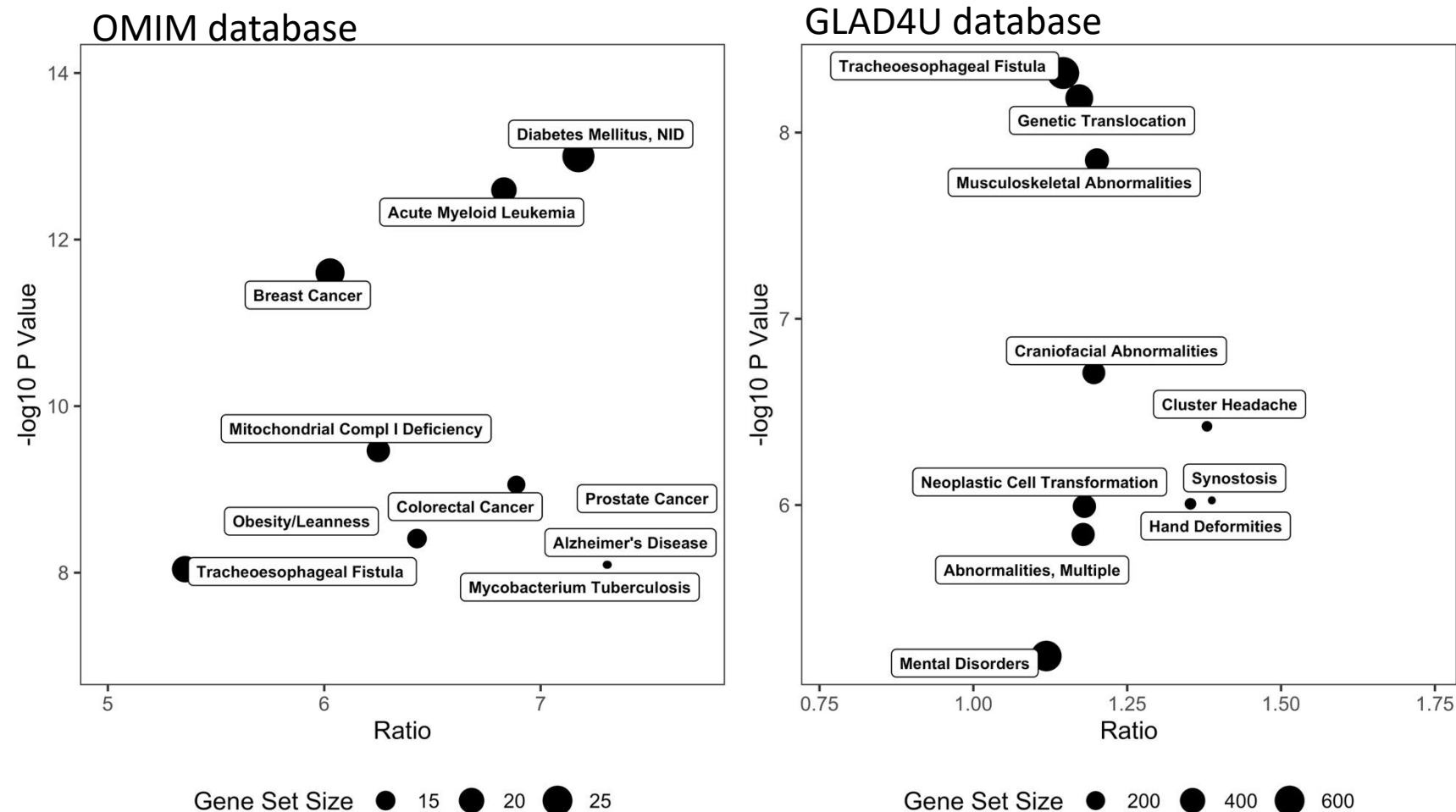
CpG Site	Exp ( $\beta$ )	$\Delta P$	P	FDR q	Gene	Gene Description
cg18234533	1.08	0.06	3.6e-9	<0.01	FAM20C	Calcium Ion Binding
cg12459932	1.04	0.03	5.8e-8	0.01	RUNX3	Tumor suppressor, transcription, Notch signaling, dysplasia
cg26578373	0.76	-0.05	1.6e-7	0.02	PIN4	Cell cycle, mitochondria
cg10065825	0.70	-0.04	2.9e-7	0.03	CDH11	Calcium ion binding

Associated GO pathways

Ontology	Term	N	DE	P.DE	FDR
CC	membrane	1853	1313	8.0E-08	7.0E-04
CC	nucleoplasm	3123	2164	1.4E-06	7.9E-03
CC	cytosol	4871	3288	1.3E-05	3.9E-02
MF	protein binding	9664	6483	2.0E-12	3.6E-08
MF	ATP binding	1451	1041	4.0E-06	1.8E-02
MF	transcription factor binding	264	213	5.3E-06	1.9E-02



# Diseases



These plots show the Enrichment ratios and  $-\log_{10} p$  values of disease gene sets from the OMIM database (left) and GLAD4U database (right). These sets are overrepresented at an FDR  $q < 0.05$  level, in the list of genes annotated to CpG sites that are associated with pyrethroid exposure at a raw  $p < 0.05$  ( $n = 32,695$  CpG sites, annotating to 5,782 unique gene names), using BMIQ normalization. The size of the markers indicates the relative size of the gene sets.






# Summary of Pyrethroids EWAS

- Findings Support & Extend Literature
- Some findings highly consistent with prior literature
  - Ion binding (calcium vs sodium)
  - Membrane action
- Some findings consistent with targets, but understudied in epidemiology
  - Musculoskeletal abnormalities, Headaches, Mental Disorders, obesity/leanness, Mitochondrial Complex I Deficiency
- Some are understudied/novel across fields
  - Prostate Cancer, Alzheimer's disease, Diabetes
- Differential methylation can paint a picture of sub-acute, non-clinical phenotypes

Pyrethroid Targets

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# Assumptions

- Differential methylation is biologically significant
  - Differential methylation is meaningful for the annotated gene
  - P-values are a relevant indicator
  - Specific pyrethroids have equivalent mechanisms of action & toxicity
  - Time window of exposure is relevant (5 years before blood draw)
- Subclinical disease indications at the molecular level translate to chronic disease implications at population levels



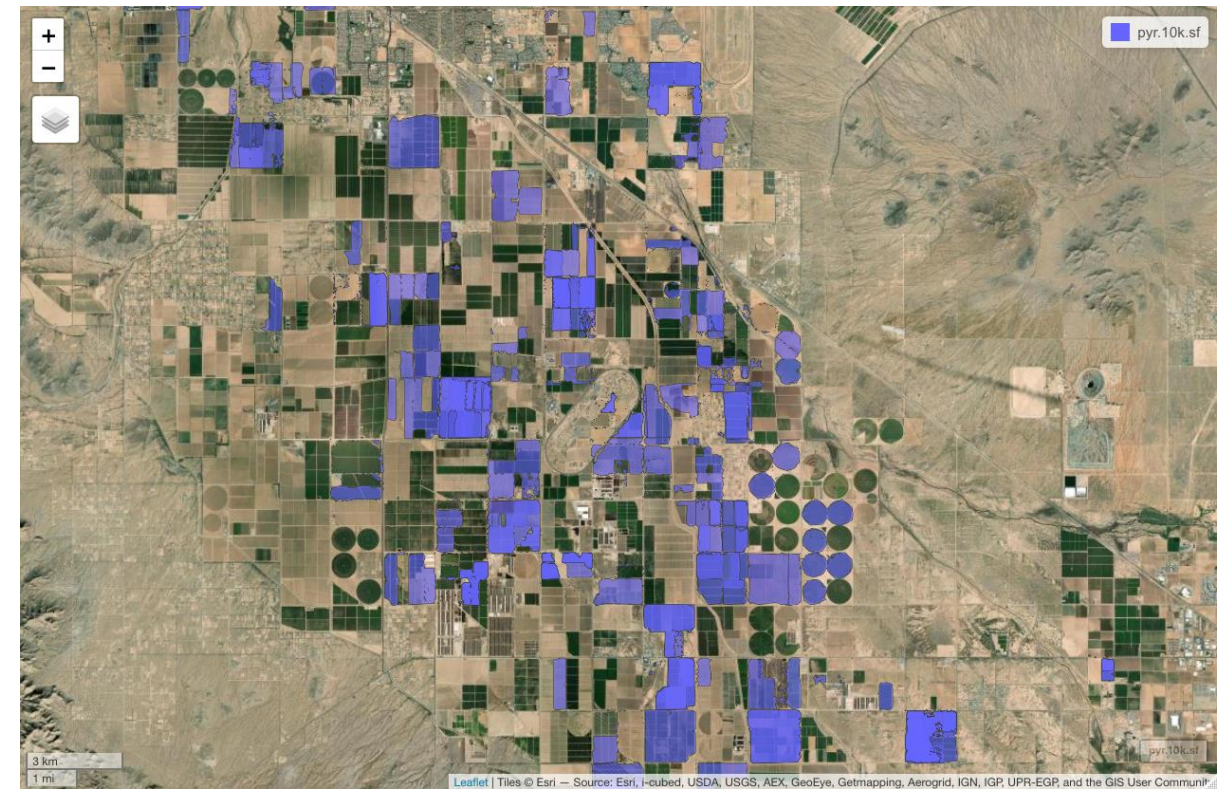
# Rich Arizona-Specific Resources for Hypothesis Testing

- From hypothesis generation to hypothesis testing: Challenges
  - Rare outcomes require big data or is \$\$\$
  - Pesticide biomarker measurement is \$\$\$
    - Retrospective assessment using biomarkers is usually impossible
- Arizona's resources:
  - Statewide pesticide use registry (PUR) 1992-present
  - University and State-wide support for population research
    - Medicaid, birth certificates, cancer registries, birth defects registries, EMRs, hospital admissions data
- Goals:
  - Use extant resources to link >2million pesticide applications from 1992-present with available health records in AZ (birth certificates, Medicaid, registries)
  - This will allow:
    - Deep dives into specific pesticides ←
    - Research into sensitive windows ←
    - Untargeted outcomes research
    - Mixtures research
    - Others
- Sneak peek: birth certificates linkage & power to evaluate rare outcomes



# Arizona's Pesticide Use Registry & Birth Certificates

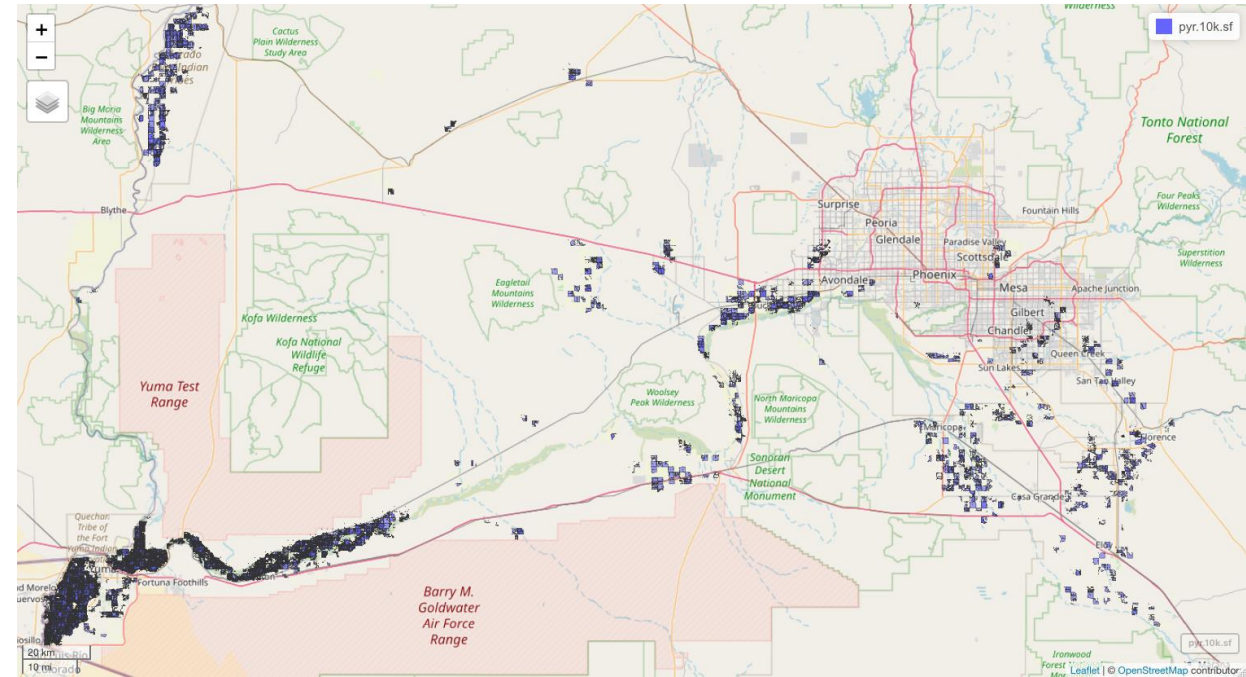
- Comparative analysis of pesticides during pregnancy with extremely preterm birth (<28 weeks)
- Linked AZ pesticide applications to births from 2011-2016
- Binary exposures for living within 500m of a pesticide application, by trimester & pre-conception (90 days prior to LMP)
- Restricted to births in agricultural zones
- Logistic regressions of pesticides by trimester with extremely preterm birth
  - Controlled for maternal race/ethnicity, child sex, maternal education, birth year
- FDR adjustment





# Key Characteristics

- ~11% births from 2011-2016 lived near an actively used agricultural field
- In agricultural areas, <1% of births were extremely preterm (<28 weeks)
- >150 unique active ingredients were applied >250 times over this time period



Random Snapshot of 10,000 Pyrethroid Pesticide Applications, Arizona



Unpublished data – not for reproduction



# Summary

- Implicated new/understudied and familiar pesticides
- New windows of sensitivity
  - Pre-conception appears important
- Able to study a rare birth outcome (<1% of births) & rare exposures
  - Caveat that cell counts for exposed cases tended to be small



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Thank you!