GENETIC STUDIES OF GULF WAR ILLNESS CSP#585 / GWECB

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Presentation to the Research Advisory Committee on GWVI

January 27, 2022

No conflicts of interest to declare.
The views expressed in here are mine and do not necessarily reflect the position or policy of the Department of Veterans Affairs or the United States government.

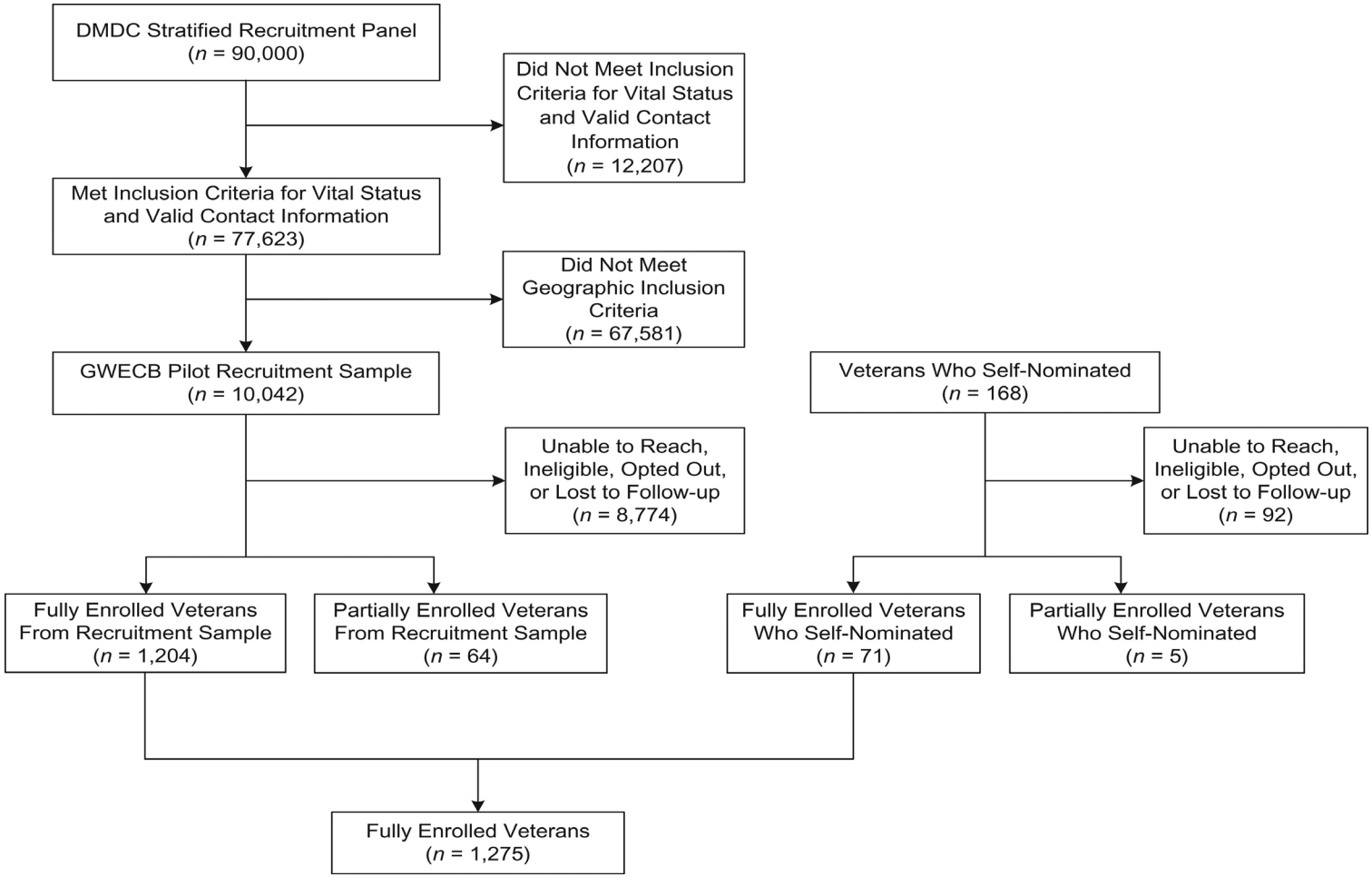
Gulf War Era Cohort and Biorepository (CSP #585) Aims

- Establish a research cohort of Gulf War Era Veterans to be used for future research studies
- Assess feasibility of and evaluate methods for recruitment, consenting, and blood collection to inform a full project and future GW/similar activities
- Perform genetic and genomic analysis on GWECB specimens
  - Hypothesis: Genetic variants are associated with differences in response to common exposures during the 1990-91 Gulf War leading to GWI
  - Discovery: Identifying the genes and pathways associated with differential response
- Provide training opportunities for new researchers

Data Collected

- GWECB paper survey
  - Developed in committee with Gulf War Era study experts and pilot tested; includes full MVP baseline survey
  - Topics: military service and geographic location; lifestyle behaviors; physical/mental health; family and family health
- Blood specimens
  - Trained phlebotomist visited Veteran's home or convenient location to draw blood (~2 tsp); sample was shipped to, processed and stored at MAVERIC
- VA and non-VA medical records
  - Accessed using electronic, administrative and other sources

Figure 1. Enrollment of participants in the Gulf War Era Cohort and Biorepository (GWECB), 2014–2016.



Am J Epidemiol, Volume 187, Issue 11, November 2018, Pages 2279–2291, https://doi.org/10.1093/aje/kwy147

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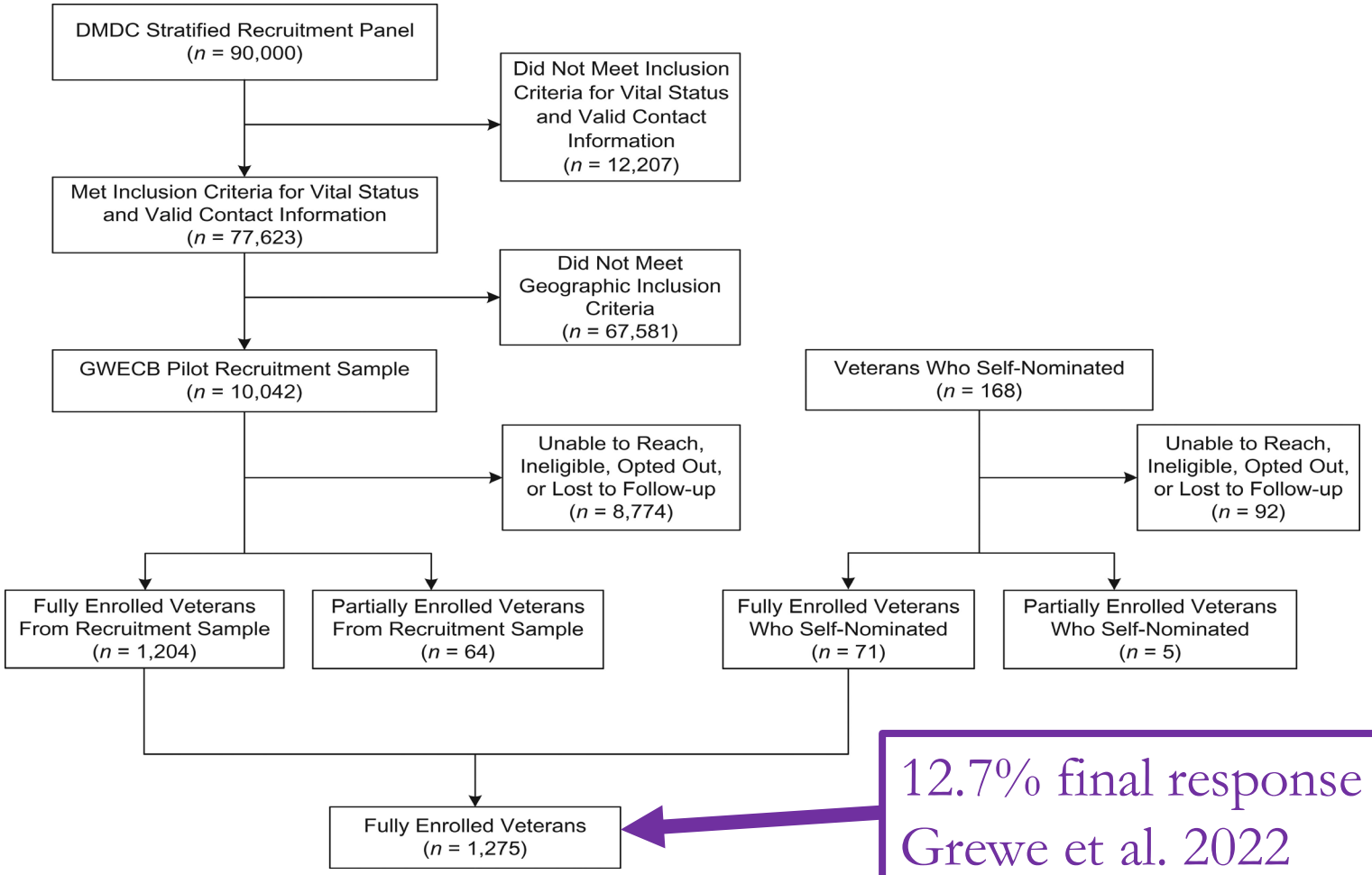


12.7% final response rate
Grewe et al. 2022

Am J Epidemiol, Volume 187, Issue 11, November 2018, Pages 2279–2291, https://doi.org/10.1093/aje/kwy147

Progress in Genetic Studies of Gulf War Illness

- GWI Case status phenotypes based on analysis published in:
  - Elizabeth J. Gifford, Jacqueline Vahey, Elizabeth R. Hauser, Kellie J. Sims, Jimmy T. Efird, Erin K. Dursa, Lea Steele, Drew A. Helmer, Dawn Provenzale. **Gulf War illness in the Gulf War Era Cohort and Biorepository: The Kansas and Centers for Disease Control definitions.** Life Sciences Volume 278, 1 August 2021, 119454.
  - Vahey J, Hauser E, Sims KJ, Helmer DA, Provenzale D, Gifford EJ. **Research tool for classifying Gulf War Illness using survey responses: Lessons for writing replicable algorithms for symptom-based conditions.** Life Sci. 2021 Jul 6:119808. doi: 10.1016/j.lfs.2021.119808. Epub ahead of print. PMID:34242657
- Completed two main analyses examining germline genetic susceptibility to GWI:
  - Candidate gene studies of Gene-by-Exposure interactions
  - Initial Genome-wide Association Study (GWAS)
- Trainee Opportunity: Jacqueline Vahey, Computational Biology and Bioinformatics Program, Duke University

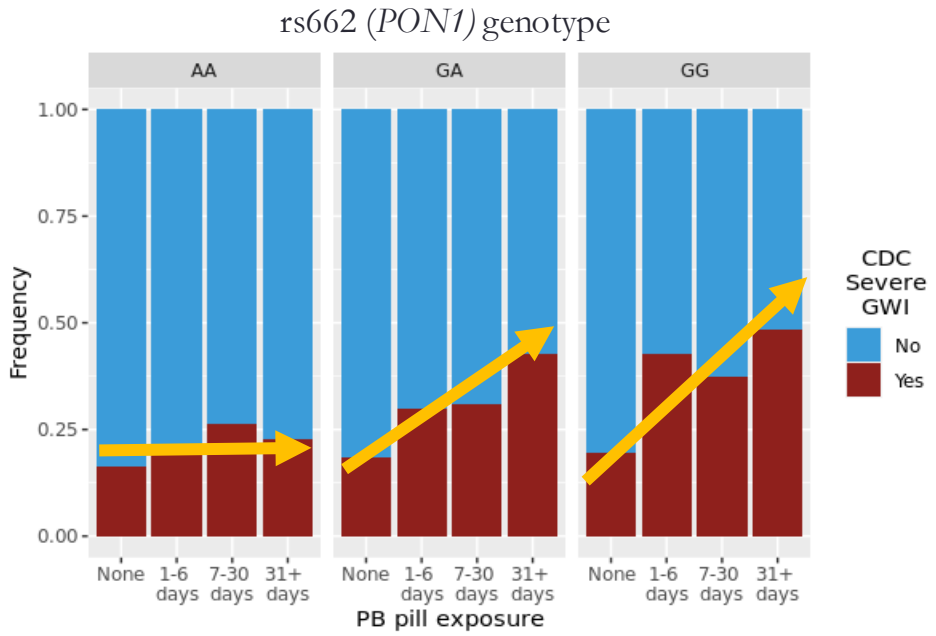
Gene–toxicant interactions in Gulf War illness: Differential effects of the PON1 genotype

Ms. Jacqueline Vahey (PhD Candidate)
Computational Biology & Bioinformatics,
Duke University Graduate School

Dr. Elizabeth Hauser, PhD
Dept of Biostatistics and Bioinformatics,
Duke Molecular Physiology Institute
Durham VA Cooperative Studies Program Epidemiology Center

Overview: GWI and gene-environment interactions

- BLUF: Exposure and risk gene together are associated with greater risk of GWI
- We replicated a previously-identified gene-environment interaction that is significantly associated with Gulf War illness.
  - Replication: get the same results as a study done by someone else with different data
  - Significantly associated: GWI and the gene-environment interaction occur together more often than they should by chance, suggesting that they are connected in some way
- This interaction could help identify a biological basis for GWI
- Exposure alone increases risk; exposure and genetic risk factor together increases risk significantly.



Gene-environment interactions

- Some genes can cause people to be higher risk for environmentally-caused disease.
  - Skin cancer: people who are in the sun without sunscreen are higher risk for skin cancer, but some families seem to have higher risk. They might have a gene that makes them more susceptible that then allows the sun damage to cause skin cancer at a higher rate.

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- We think that Gulf War illness might have a genetic risk factor that causes a more severe response to one or more of the toxic exposures experienced during deployment
- **Targeted genetic search**: we chose specific candidate genes that we think are most likely to be involved in the development of Gulf War illness, then tested these specific candidate genes.
  - These targets were chosen based on published work from other groups
  - More on broad, unbiased searches later

High prevalence of GWI

- 26.9% CDC Severe GWI among deployed veterans and 13.5% among non-deployed veterans
- High prevalence requires common genetic variants and widespread exposures
- Whatever happened had to affect enough people to explain this prevalence

	Deployed	Not deployed	All
Number of Veterans	849	267	1116
CDC Severe GWI	26.9%	13.5%	23.7%

Published in E. J. Gifford *et al.*, "Gulf War illness in the Gulf War Era Cohort and Biorepository: The Kansas and Centers for Disease Control definitions," *Life Sci.*, vol. 278, p. 119454, Aug. 2021, doi: 10.1016/j.lfs.2021.119454.

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  - Pesticides: 55% of deployed Veterans
  - PB pills: 59% of deployed Veterans

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Number of Veterans	849	267	1116
CDC Severe GWI	26.9%	13.5%	23.7%
PB pill use	59%	NA	NA
Pesticide use	55%	NA	NA

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Nerve gas and pesticides can cause long term neurological symptoms

- Acetylcholine is necessary for nerve signaling
- Degradation of acetylcholine is necessary for cessation of nerve signaling
- Organophosphates (OPs), including some pesticides and sarin gas, competitively inhibit acetylcholinesterase
- **PB pills and pesticides are inhibitors of acetylcholinesterase**
- **PB pills and quantity of pesticides are unique exposures to service in the Gulf**

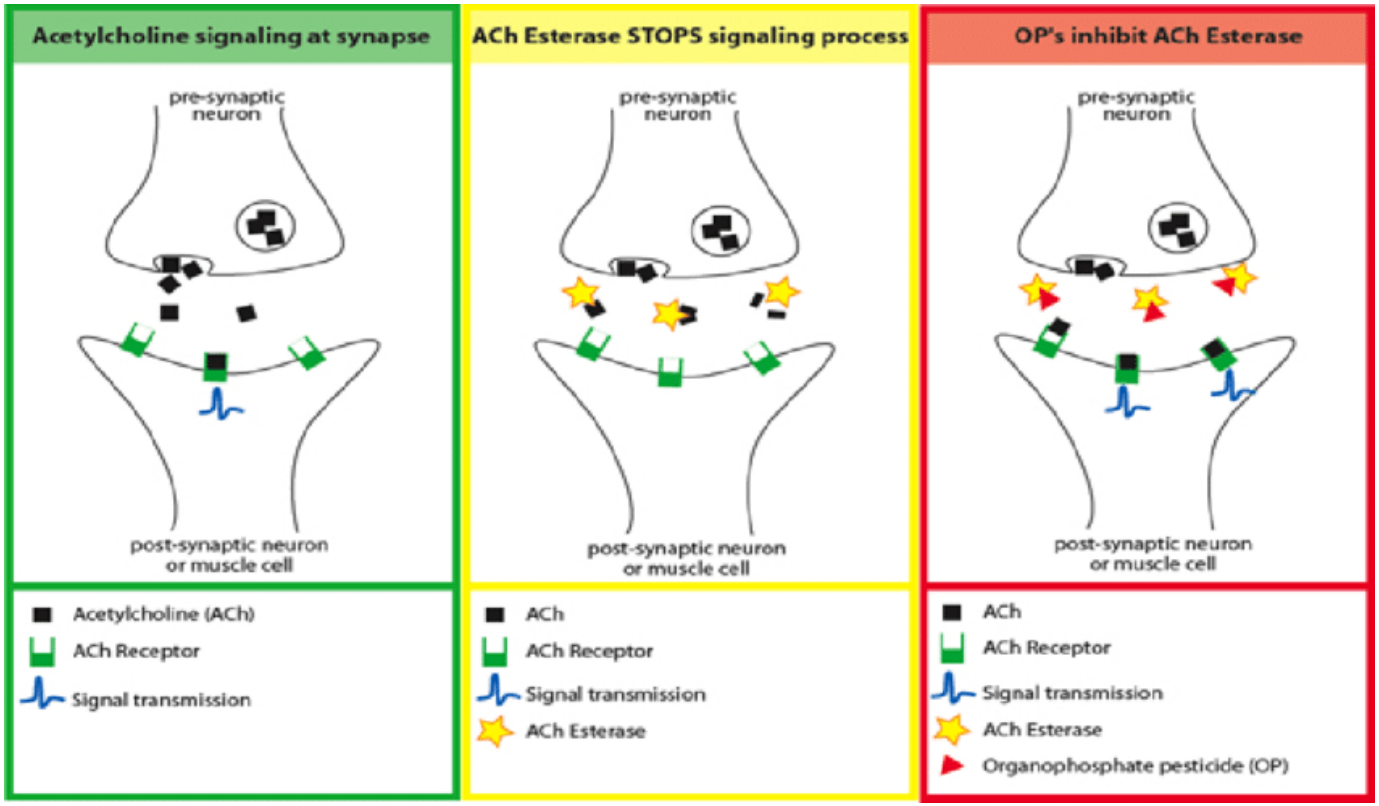


Figure: Karki, Parag & Manandhar, Kiran & Shah, Ram. (2014). Study of cardiac abnormalities in acute organophosphate poisoning. 10.13140/rg.2.2.36515.68642.

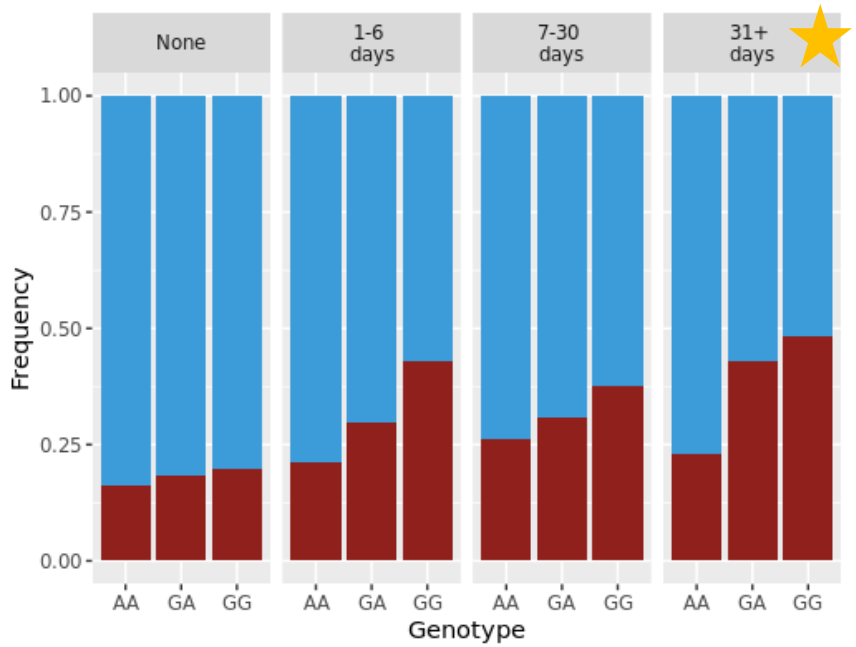
Candidate SNPs have biological meaning and prior evidence

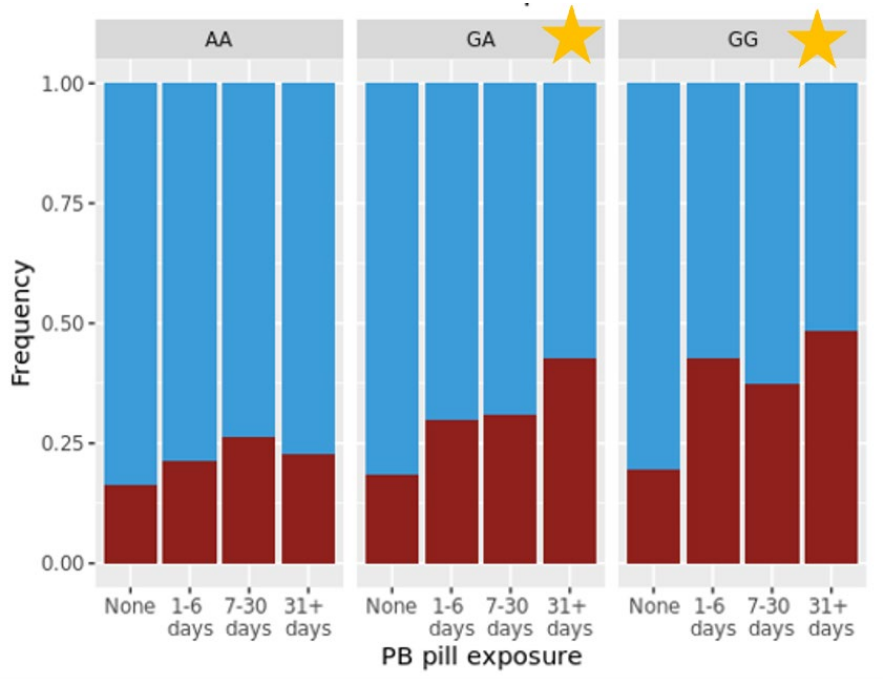
- Environment: PB pill exposure
- Gene: rs662, a functional variant in PON1
  - PON1 helps process and break down toxic chemicals
  - Changes catalytic efficiency
  - The higher risk variant from prior functional studies maps to the G allele
    - Slower processing of most toxicants
  - Identified by early GWI studies
  - Identified by occupational hazards studies on organophosphate exposure

Tier 1 SNPs: BCHE, PON1, and ACHE from prior studies				
SNP	Gene	Variant	MAF	Citations
rs1799807	BCHE	Atypical (A); lower catalytic rate; succinylcholine susceptibility	0.020	Zhu et al. 2020, Goodall 2004, Steele et al. 2015
rs662	PON1	192Q/R; functional variant of Pon1 Modifier for risk of sporadic ALS	0.396	Dardiotis et al. 2018, Davies et al. 1996, Haley et al. 1999; Verde et al. 2019
rs1799805	ACHE	H322N; Yt blood group;	0.037	Shapira et al. 2000

Tier 2 SNPs: remaining SNPs in BCHE, PON1, ACHE, and SOD1			
	Gene	**Location**	**Number of SNPs**
All SNPs in GWECB that are within 50kb of the gene	BCHE	Chromosome 3	84
	PON1	Chromosome 7	178
	ACHE	Chromosome 7	59
	SOD1	Chromosome 21	53

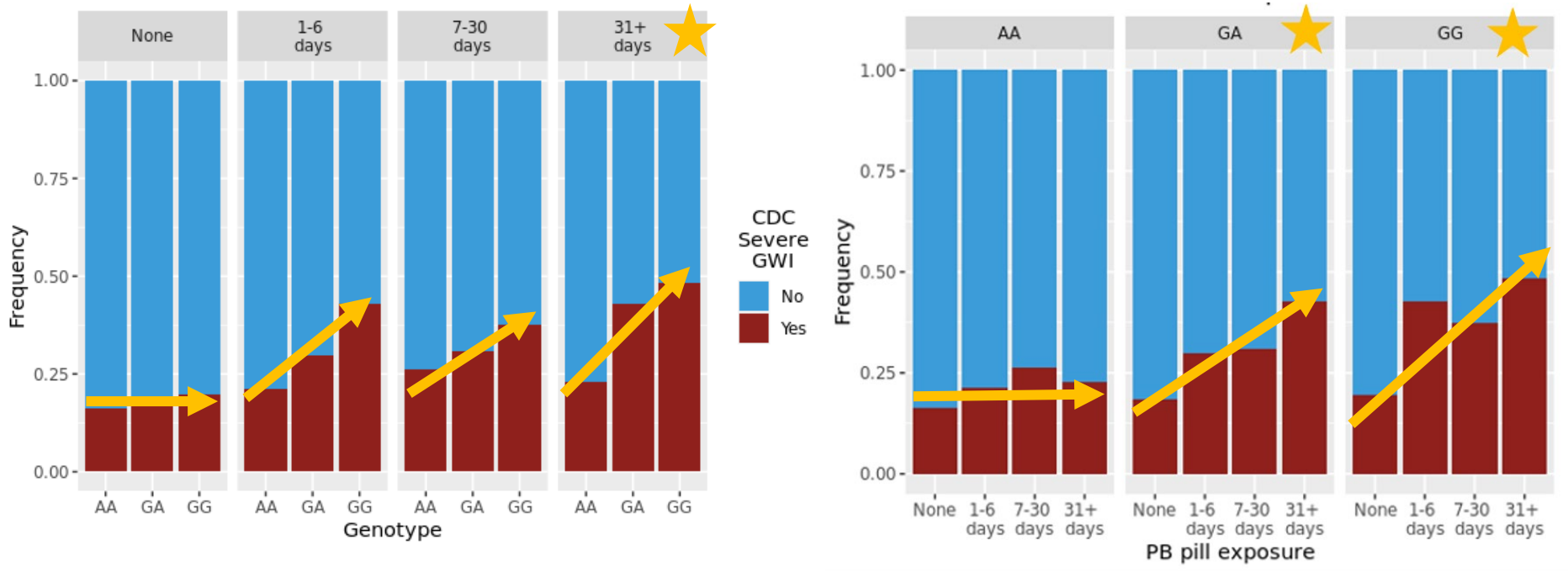
Pon1 Candidate SNP, rs662





★ Statistically significant

Pon1 Candidate SNP, rs662



★ Statistically significant slope

Main takeaways

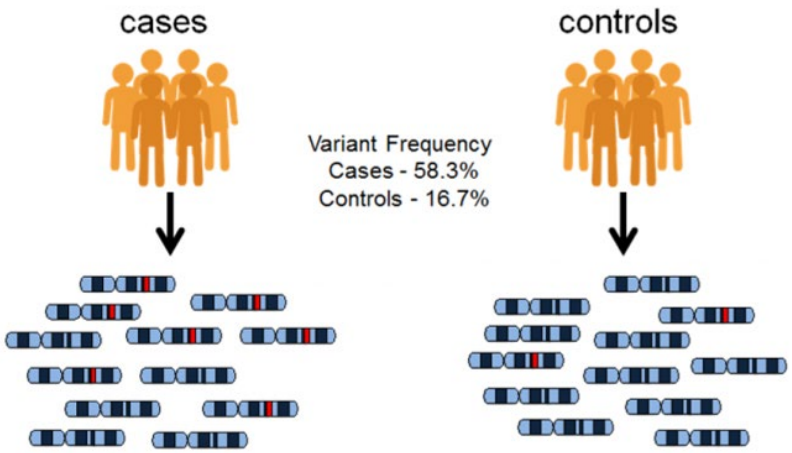
- Suggestive evidence supporting association of CDC Severe GWI with *PON1* (rs662) interaction with PB pills
  - Replicates prior work from Haley et al., 1999
  - Manuscript published in 2021
- Other candidate SNPs or genes and interactions were not associated in our dataset
- Gene-environment interaction study in a larger dataset could confirm or refute these results
  - MVP dataset is large enough to do this work
- None of this work would be possible without the self-reported exposure data or the matched genetic data.
  - All of this work is made possible by the Veterans who participated in CSP585

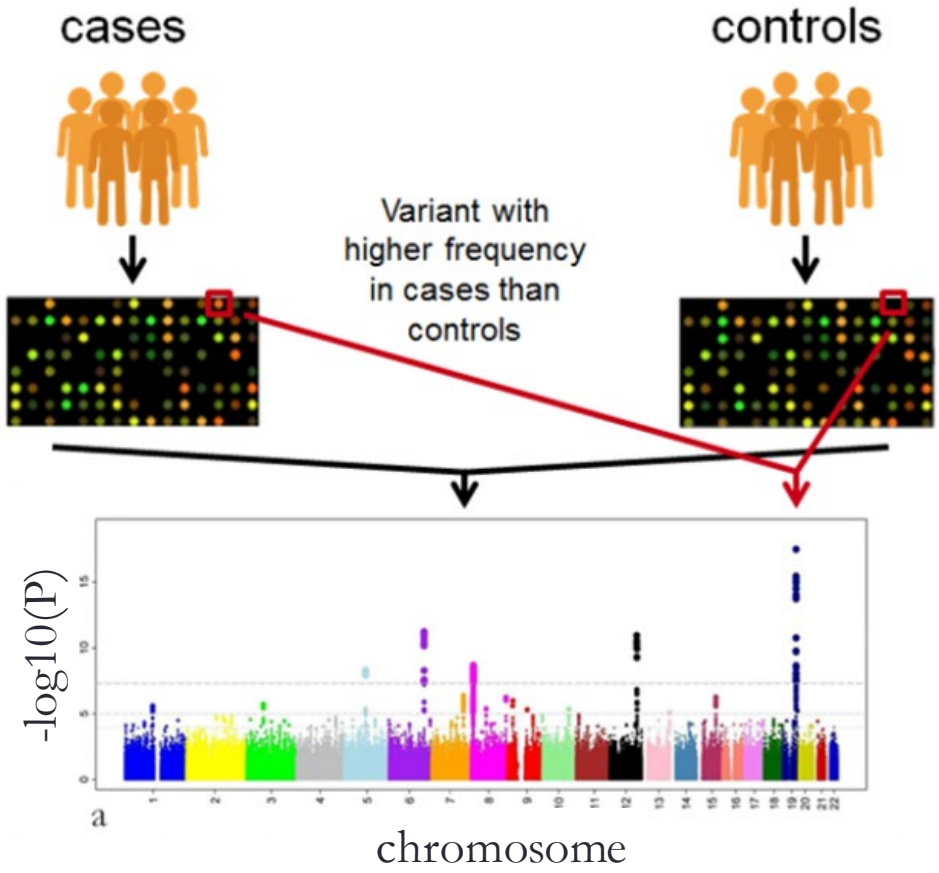
Genome-wide association study: Unbiased search

- Looking for any genetic signal across the whole genome, without a prior hypothesis
- Allows for an unbiased search!
- Helpful for hypothesis generation and pathway analysis.
- Not published yet but results are encouraging!
  - Gene set results appear to support prior work

GWAS: Genome-wide sweep for association

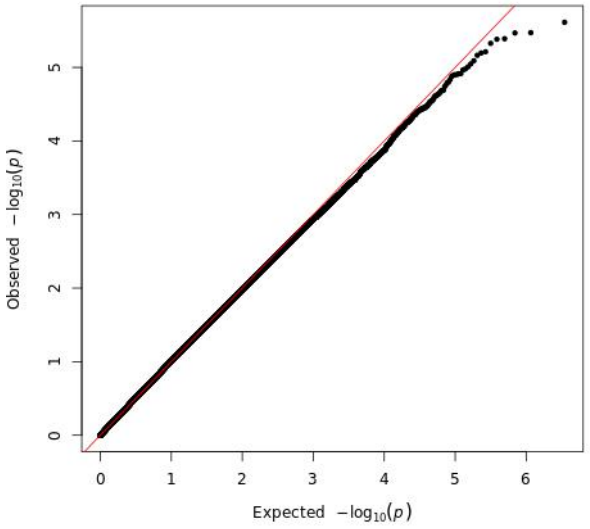
- Is there a locus in the genome that separates the people with GWI from the people who do not have GWI?
- Unbiased!

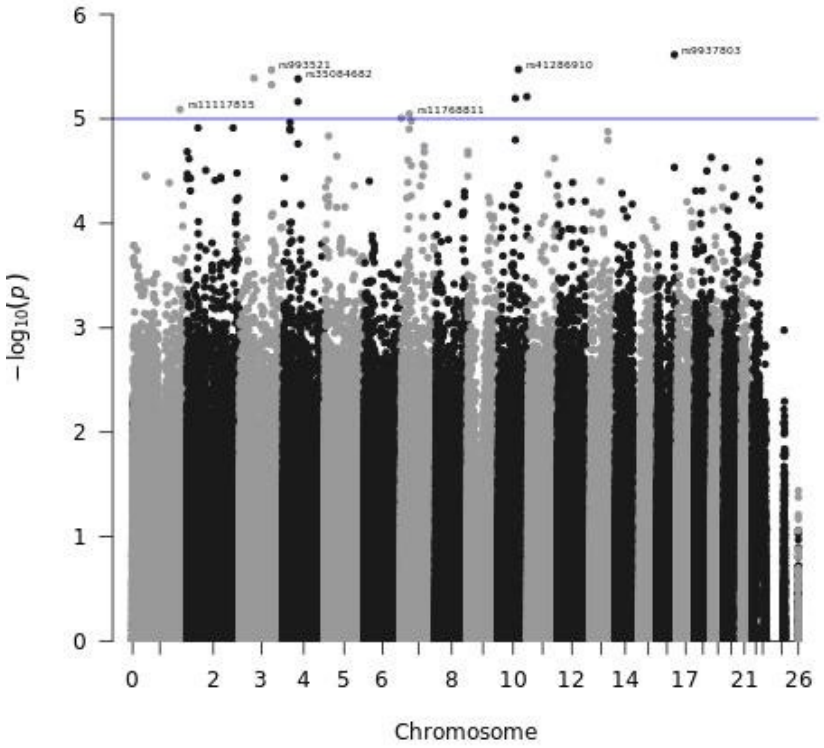




GWAS Results: Overview (CDC Severe, All)

- Nothing statistically significant at a genome level





Next Steps for GWECB / CSP#585

- Continue the genetic analysis
- Functional annotation of identified SNPs and genes
- Pathway analysis
  - Do we identify any of the pathways highlighted in previous GWI research?
- Analysis of epigenetic data
  - Genome-wide methylation screen array in those with genetic data
  - More informative for exposures and gene regulation differences among cases and controls?
- Replication of genetic findings/ Collaboration with CSP#2006/MVP029

Veterans Health Administration R&D www.research.va.gov VA Cooperative CSP Studies Program

GWECB Gulf War Era Cohort and Biorepository

DUKE MOLECULAR PHYSIOLOGY INSTITUTE

Acknowledgements

Thank you to all the Veterans who participated, **the CSP#585 Team**, the CSP #585 Executive Committee and our collaborators on CSP#2006/MVP029

Jason Aguilar
Sarah Ahmed
Carlos Amezcua
Mihaela Aslan
Karen Block
Steve Boyle
Alyssa Bullard
David Burnaska
Brenda Cabrera-Mendoza
Brian Charest
Kei-Hoi Cheung
Kelly Cho
John Concato
Patricia Crutchfield
Teresa Day
Flavio De Angelis
Antonella De Lillo
Linh Duong

Erin Dursa
Jimmy Efird
Margaret Freeman
Amanda Garcia
Michael Gaziano
Elizabeth Gifford
Kelly Harrington
Elizabeth Hauser
Drew Helmer
Grant Huang
Vales Jeanpaul
Joel Gelernter
Regina Joseph
Nancy Klimas
John Ko
Dora Koller
Rene LaFleur

Daniel Levey
Yuli Li
Alysia Maffucci
Rebecca McNeil
Sumitra Muralidhar
Shree Nadkarni
Rajeevan Nallakkandi
Theresa Nguyen
Alice Nono-Djotsa
Meghan O'Leary
Gita Pathak
Renato Polimanti
Dawn Provenzale
Rachel Quaden
Krishnan Radhakrishnan
Charles Ramos
Mazhgan Rowneki

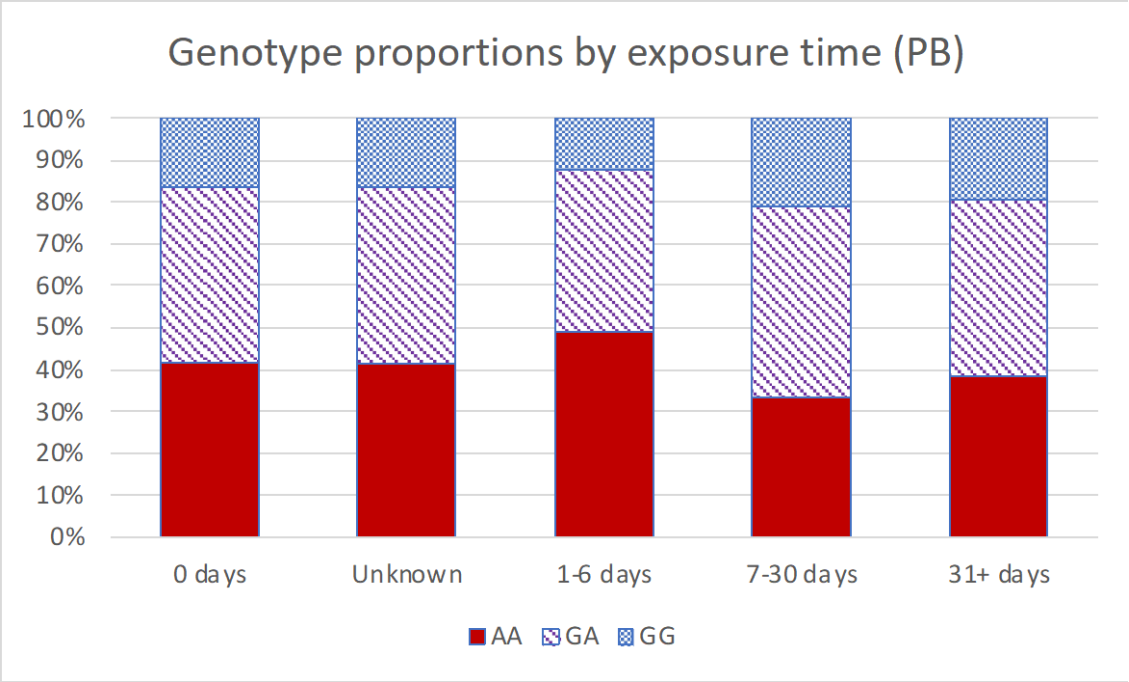
Frederick Sayward
Kellie Sims
Crystal Stafford
Lea Steele
Kim Sullivan
Coveannda Sumpter
Marsha Turner
Daniel Tylee
Jacqueline Vahey
Frank Wendt
Stacey Whitbourne
Donna White
Christina Williams
Rebekah Ryanne Wu
Xuejun Qin
Hongyu Zhao

Extra slides

- Independence of genotype and exposure
- LocusZoom among CDC Severe candidate genes

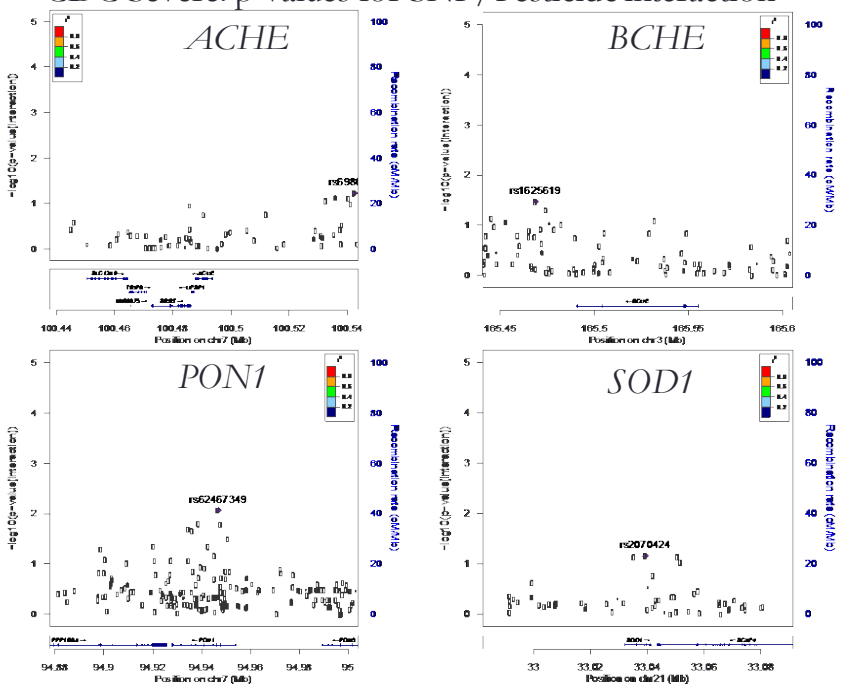
Pon1 Candidate SNP, rs662

- Three genotypes:
  - AA (41%)
  - GA (42%)
  - GG (17%)
- P(G) is overall 38% in CSP585
- European ancestry P(G): 27%
- African ancestry P(G): 65%
- Genotype proportions are constant across exposure time
  - Genotype is not associated with exposure time



No significant results among CDC Severe candidate genes

CDC Severe: p-values for SNP/Pesticide interaction



CDC Severe: p-values for SNP/PB interaction