Butyrylcholinesterase Genotype and Activity in Relation to Gulf War Illness:

Preliminary Evidence and Ongoing Studies of Gene-Exposure Interaction in 1991 Gulf War Veterans

Lea Steele, Ph.D.
Veterans Health Research Program,
Division of Neuropsychiatry
Baylor College of Medicine

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Background:

Large number of Gulf War-related experiences/exposures have been suggested as possible causes or contributors to Gulf War Illness

- *Psychological stress, trauma*
- *Chemical weapons*
- *Oil well fires*
- *Munitions containing depleted uranium*
- *Heavy use of insecticides/repellants*
- *PB pills (pyridostigmine bromide)*
- *Vaccines*
- *Infectious diseases*
- *Tent heaters*
- *Particulates*
- *Fuel exposures*
- *Solvents, CARC paint*

Synthesis of Evidence from Epidemiologic Studies of Gulf War Veterans

- Pyridostigmine bromide (PB) - Pesticide exposures	Evidence consistently indicates a significant association
- Low-level nerve agents - Sustained oil well smoke - Large number of vaccines - Combinations of exposures	Less clear; evidence is inconsistent or limited in important ways
- Depleted uranium - Anthrax vaccine - Fuels, solvents - Sand, particulates - Other	Little evidence of association; unlikely to have been primary contributing factor for the majority of affected veterans

Adapted from : Research Adv. Cmte Gulf War Illnesses: Gulf War Illness and the Health of Gulf War Veterans, 2008, 2014

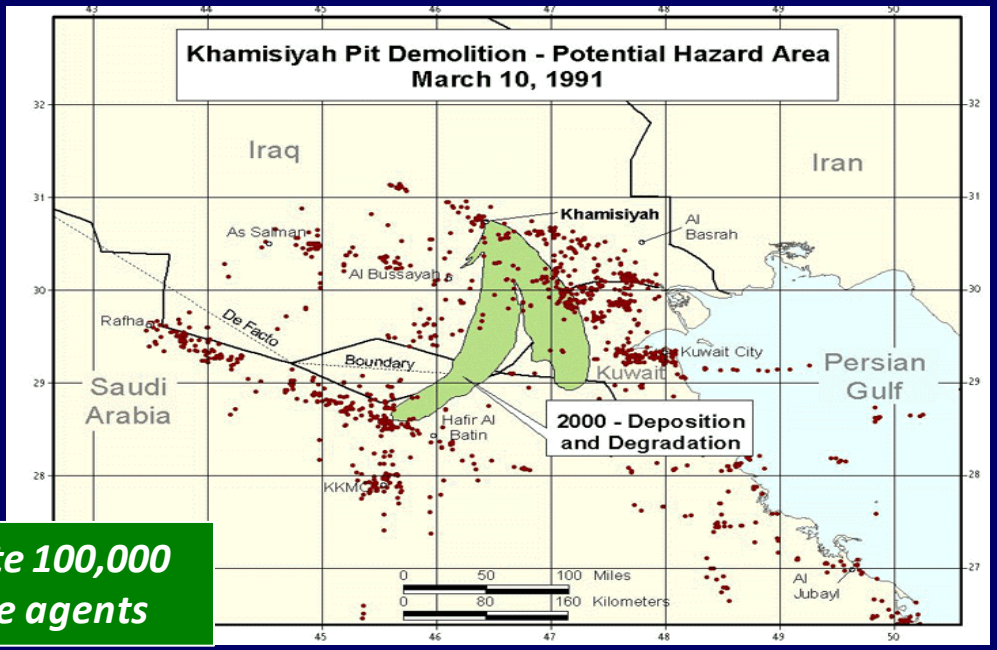
Extensive use of pesticides, insect repellants (~ 64 products; 37 active ingredients)

Table 2. Pesticides and Insect Repellants Identified as Pesticides of Potential Concern by the Deployment Health Support Directorate

Compound	*Use*	*Chemical Class*	*Purpose*	*Application*
Pesticides and Repellants Used by the General Military Population				
DEET, 33% cream, stick	Personal use repellant	Dialkylamide	Repel flies and mosquitoes	By hand to skin
DEET, 75% liquid	Personal use repellant	Dialkylamide	Repel flies and mosquitoes	By hand to skin, uniform, netting
Permethrin, 0.5% spray	Personal use repellant	Pyrethroid	Repel flies and mosquitoes	Sprayed on uniforms
d-Phenothrin, 0.2% aerosol	Area use repellant	Pyrethroid	Knock down, kill flies and mosquitoes	Sprayed in tents, other enclosed areas
Methomyl 1% crystals	Fly bait	Carbamate	Attract and kill flies	Placed in pans outside latrines, tents
Azamethiphos, 1% crystals	Fly bait	Organophosphate	Attract and kill flies	Placed in pans outside latrines, tents
Dichlorvos, 20% pest strip	Pest strip	Organophosphate	Attract and kill mosquitoes	Hung in tents, working areas, dumpsters
Pesticides Used by Pesticide Applicators				
Chlorpyrifos, 45% liquid	Sprayed liquid	Organophosphate	Kill flies, mosquitoes, flying insects	Sprayed in corners, cracks, crevices
Diazinon, 48% liquid	Sprayed liquid	Organophosphate	Kill flies, mosquitoes, flying insects	Sprayed in corners, cracks, crevices
Malathion, 57% liquid	Sprayed liquid	Organophosphate	Kill flies, mosquitoes, flying insects	Sprayed in corners, cracks, crevices
Propoxur, 14.7% liquid	Sprayed liquid	Carbamate	Kill flies, mosquitoes, flying insects	Sprayed in corners, cracks, crevices
Bendiocarb, 19% liquid	Sprayed powder	Carbamate	Kill flies, mosquitoes, flying insects	Sprayed in corners, cracks, crevices
Chlorpyrifos, 19% liquid	Fog	Organophosphate	Kill flies, mosquitoes	Large area fogging
Malathion, 91% liquid	Fog	Organophosphate	Kill flies, mosquitoes	Large area fogging
Delousing Pesticide				
Lindane, 1% powder	Delouser	Organochlorine	Kill lice, other insects	Dusted on prisoners, also for personal use

Source: DOD Environmental Exposure Report: Pesticides (2003)[1532]

PB: Pyridostigmine Bromide (anti-nerve gas pills)



Khamisiyah demolitions: DOD models indicate 100,000 Gulf war troops potentially exposed to nerve agents

GWI Etiology: Summary

- Of many putative "causes", studies most consistently identify pyridostigmine bromide (PB), pesticides as significant risk factors for GWI. evidence less consistent re: low-level exposure to nerve agents

- *Risk factors of greatest concern can all act as neurotoxicants -- prominently include acetylcholinesterase (AChE) inhibitors (organophosphates, carbamates)*

- *Animal studies also suggest variety of synergistic effects of exposure combinations*

Question: Why did some veterans develop GWI, while others (with similar exposures) remained well?

- Differing levels, combinations of exposures

- Genetic variability in biological pathways that underlie veterans' symptoms

- Individual Differences in Vulnerability to Adverse Effects of Neurotoxicants

- GWI risk may be associated with genetic variability in circulating enzymes that protect us from adverse effects of some neurotoxicants (e.g. carbamates, OPs)

  - Paraoxonase (PON1): Hydrolizes OPs; Previous studies have suggested possible association of GWI with PON1 activity levels or genotype

  - Butyrylcholinesterase (BChE): Enzyme that binds acetylcholinesterase inhibitors, protects from adverse effects

Study to Evaluate Association of Gulf War Illness with BChE

Steele et al. Environmental Health 2015, 14:4
http://www.ehjournal.net/content/14/1/4

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Butyrylcholinesterase genotype and enzyme activity in relation to Gulf War illness: preliminary evidence of gene-exposure interaction from a case–control study of 1991 Gulf War veterans

Lea Steele[1*], Oksana Lockridge[2], Mary M Gerkovich[3], Mary R Cook[4] and Antonio Sastre[5]

Abstract

Background: Epidemiologic studies have implicated wartime exposures to acetylcholinesterase (AChE)-inhibiting chemicals as etiologic factors in Gulf War illness (GWI), the multisymptom condition linked to military service in the 1991 Gulf War. It is unclear, however, why some veterans developed GWI while others with similar exposures did not. Genetic variants of the enzyme butyrylcholinesterase (BChE) differ in their capacity for metabolizing AChE-inhibiting chemicals, and may confer differences in biological responses to these compounds. The current

Collaborators:

Midwest Research Institute (now MRI Global)
Antonio Sastre, PhD
Mary Cook, PhD
Mary Gerkovich, PhD

Eppley Institute, University of Nebraska Medical School
Oksana Lockridge, PhD

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Butyrylcholinesterase (BChE): Overview

- BChE: present at > 10X level of AChE in blood; physio functions not fully understood. Acts as scavenger, binds organophosphates, carbamates, other compounds
- Early pharmacogenetics: Patients with abnormal response to succinylcholine (e.g. given for surgery) found to have inherited BChE ("pseudocholinesterase") deficiency. Produces prolonged paralysis/inability to breathe after dose that normally acts for few minutes
- One BChE gene (chromosome 3q26), multiple variations: Wild type "U" allele most common; then K, A, F
- Activity level variable; e.g. BChE activity testing routinely used in monitoring exposures among pesticide workers
- Other conditions: BChE variants associated with higher rates of symptoms and PD in pesticide-exposed populations; differential response to AD medications

Is BChE Associated with GWI?

Early Studies re: BChE Enzyme Activity and GWI

- No BChE activity differences identified between symptomatic and healthy Gulf War veterans (Haley 1999, Concato 2007)

Early studies re: BChE Genotype

- Lowenstein-Lichtenstein (1995) [Soreq lab]: Case report of Israeli soldier—severe symptoms after taking PB pills during the Gulf War; found to be BChE AA homozygote, poor BChE affinity for carbamates

- Lockridge: 1999 DOD project: Nebraska GW veterans: most (73%) carriers of A, F alleles had reported they had Gulf War syndrome (vs < 30% overall)

- Early animal studies: delayed/persistent CNS effects following low-dose PB exposure only in rat strain (WKY) w/ low BChE activity (Servatius 1998, Beck 2001)

Current Study: BChE Genotype and Enzyme Activity in Relation to GWI
(part of multipart DOD-funded project, Midwest Research Institute)

Design and Methods

- Primary Objective: Determine if GWI case status and/or GWI risk associated with "cholinergic exposures" differs with veterans' BChE status
- Case-Control study: Population-based sample of n=304: 144 GWI cases (KS case def), 160 GW veteran controls
- Assessed both BChE enzyme activity and BChE genotype

Risk Factor Analyses:

Evaluated Assoc. of Cholinergic Exposures with GWI in 2 BChE genetic subgroups:
- 1. "Common"/normal BChE variants (UU and UK): enzyme effectively neutralizes AChE inhibitors

- 2. "Less common" variants (LCV): enzyme acts more slowly, less effective at neutralizing some chemicals (K/K, U/AK, U/A, A/F, AK/F)

Results-1: Butyrylcholinesterase Enzyme Activity Levels Vary by Genotype

	n	BChE Mean (SD) Activity*
All veterans in study	304	1.10 (0.26)
BChE genotype		
U/U	189	1.19 (0.24)
U/K	87	1.01 (0.21)
K/K	13	0.80 (0.15)
U/AK	10	0.76 (0.18)
U/A	3	1.03 (0.12)
A/F	1	0.92
AK/F	1	0.69
Common variants combined (U/U and U/K)	276	1.13 (0.24)
Less common variants combined (K/K, U/AK, U/A, A/F, AK/F)	28	0.81 (0.17)↑

*Enzyme activity expressed in umoles benzoylcholine hydrolyzed per minute per ml. serum;
↑Enzyme activity differs in common variants vs. less common variants, p< 0.001

Results-2: Butyrylcholinesterase Enzyme Activity Levels in GWI Cases vs. Controls

Gulf War illness case status	n	BChE Mean (SD) Activity
Gulf War illness cases	144	1.10 (0.24)
Gulf War veteran controls	160	1.10 (0.27)

Enzyme activity expressed in umoles benzoylcholine hydrolyzed per minute per ml. serum

Results-3: Comparison of BChE Genotype in GWI Cases vs Controls

	Total Sample (n = 304)		GWI Cases (n = 144)		Controls (n = 160)	
BChE Genotype	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**
U/U	189	(62%)	89	(62%)	100	(62%)
U/K	87	(29%)	41	(28%)	46	(29%)
K/K	13	(4%)	7	(5%)	6	(4%)
U/AK	10	(3%)	5	(3%)	5	(3%)
U/A	3	(1%)	1	(<1%)	2	(1%)
A/F	1	(<1%)	0	(0%)	1	(<1%)
AK/F	1	(<1%)	1	(<1%)	0	(0%)
Less common variants combined (K/K, U/AK, U/A. A/F, AK/F)	28	(9%)	14	(10%)	14	(9%)

Results-3: Association of Gulf War Illness (GWI) with Cholinergic Exposures, by BChE Genetic Subgroup

Experience/Exposure	All Gulf War Veterans
Took PB pills	OR = 3.21*
Wore pesticide-treated uniforms	OR = 3.72*
Used pesticides on skin	OR = 2.89*
Living area sprayed with pesticides	OR = 1.33
Heard chemical alarms sounded	OR = 1.31

*p< 0.001

Ref: Steele et al (2015) Environ Health 14:4

Association of Gulf War Illness Case Status with Cholinergic Exposures, by BChE Genetic Subgroup

Experience/Exposure	All Gulf War Veterans	BChE Most Common Variants (normal activity) UU and U/K (n=276)
Took PB pills	OR = 3.21*	OR = 2.68*
Wore pesticide-treated uniforms	OR = 3.72*	OR = 3.63*
Used pesticides on skin	OR = 2.89*	OR = 3.14*
Living area sprayed with pesticides	OR = 1.33	OR = 1.30
Heard chemical alarms sounded	OR = 1.31	OR=1.26

*p< 0.001

Ref: Steele et al (2015) Environ Health 14:4

Association of Gulf War Illness with Cholinergic Exposures, by BChE Genetic Subgroup

Experience/Exposure	All Gulf War Veterans	BChE Common Variants (normal activity) UU and U/K (n=276)	BChE Less Common Variants (slower-acting) KK,UAK,UA,AF,AKF (n=28)
Took PB pills	OR = 3.21*	OR = 2.68*	OR = 40.00*
Wore pesticide-treated uniforms	OR = 3.72*	OR = 3.63*	OR = 4.80
Used pesticides on skin	OR = 2.89*	OR = 3.14*	OR = 1.33
Living area sprayed with pesticides	OR = 1.33	OR = 1.30	OR = 1.64
Heard chemical alarms sounded	OR = 1.31	OR=1.26	OR = 1.80

*p< 0.001

Ref: Steele et al (2015) Environ Health 14:4

Can Apparent BChE x PB Interaction be Explained by Other Study Factors?

- **Did BChE-Variant subgroup differ from other Gulf War veterans in other ways?**
  - Demographically (sex, age, education) almost identical
  - Deployment/exposure characteristics: nearly identical (e.g. 58% vs. 57% reported PB use)

- **Was association with BChE an anomaly of KS GWI case definition?**
  - Test by reassigning case/control status using CDC criteria
  - Yields 187 CMI cases, 117 controls

Association of PB with Gulf War Illness in BChE Genetic Subgroups

Evaluated Using Two Case Definitions

Case Definition	All Gulf War Veterans	BChE Common Variants (normal activity) UU and U/K (n=276)	BChE Less Common Variants (slow-acting) KK,UAK,UA,AF,AKF (n=28)
GWI—Kansas Case Definition	OR = 3.21*	OR = 2.68*	OR = 40.00*
CMI—Fukuda/CDC Case Def	OR = 1.99*	OR = 1.73*	OR = 11.37*

*p< 0.01

Ref: Steele et al (2015) Environ Health 14:4

Conclusions

Study provides preliminary evidence that BChE-LCV subgroup (KK,UAK,UA, AF,AKF) who used PB could be at substantially increased risk for GWI

Caveats:

- **Small sample (n=304) included only 28 veterans with "at risk" variant genotypes**
- **Number of GW veterans potentially affected by this interaction unknown; based on our study, this interaction might explain ~ 10 % of GWI cases**

This study also illustrates a key methodological issue for research that evaluates potential genetic associations with GWI

- As in other areas of Gulf War research, essential to evaluate subgroups (as appropriate to the study question)
- Key point: If a genetic factor confers ↑ or ↓ vulnerability to certain chemical(s), those exposures must be considered in the analyses. (i.e., no effect of variant in absence of exposure).
- Most studies have not evaluated GWI genetic associations in relation to specific exposures. Genotype/enzyme activity evaluated in:
  - *GW deployed vs. nondeployed*
  - *GWI cases vs controls*

In Brief: 2nd Exploratory/Pilot Project (in multipart MRI Study) Evaluated Paraxonase (PON1)

Background

- PON1: Hydrolyzes (inactivates) organophosphate (OP) compounds (AChE inhibitors), including insecticides and nerve agents
- PON1-192 variants differ in their capacity for neutralizing specific OP compounds:

  $PON1_{192}$ - Q variant most effective at hydrolyzing nerve agents

  $PON1_{192}$ - R variant most effective at hydrolyzing some pesticides (e.g. chlorpyrifos, parathion)

In Brief: Exploratory GWI - PON1 Evaluation Study

- **2nd population-based sample (n=72); "high risk" 1991 Gulf War veterans**
  - 40 GW veterans with GWI (GWI cases)
  - 18 GW veteran controls
  - 14 nondeployed/era veteran controls

- **GW sample: GW veterans at highest risk for GWI**
  - All served in Army, enlisted ranks, forward deployed
  - In units identified as potentially exposed to nerve agents

- **Compared Assoc. of GWI with exposures in $PON1_{192}$ QQ vs. QR+RR**

Exploratory: Association of GWI with GW Exposures, by $PON1_{192}$ Genotype

ORs for GW Cases (n=40) vs. GW controls (n=18)

Experience/Exposure	All Gulf War Veterans	PON1 QQ Genotype (n=31)	PON1 QR/RR Genotypes (n=27)	sign. intrxn?
Chemical alarms sounded	OR = 1.90 (ns)	OR = 0.75 (ns)	OR = 7.56*	~
Wore pesticide-treated uniforms	OR = 3.10 (ns)	OR = 21.0* [logit est]	OR = 0.72 (ns)	0.02*
Took PB: ever	OR = 1.40 (ns)	OR = 2.28 (ns)	OR = 0.43 (ns) [logit est]	no

*statistically significant, $p<0.05$

In Brief: Preliminary/Exploratory Results raise hypotheses re:

Association of Specific Exposures w/ GWI May Differ w/ PON1 Status

- ✓ **Identified associations were in the "expected" directions in relation to PON1 genotype**
- ✓ ***Veterans with R allele were at sign. greater risk for GWI if they were in areas where chemical alarms sounded (no association for QQ homozygotes)***
- ✓ **Personal pesticide use sign. associated with GWI only in PON1 QQ homozygotes (not in veterans with PON1 R allele)**

Follow-up:

Two Current Multisite Collaborative Projects to Evaluate Gene-Exposure Interaction in Relation to GWI

1. *Examination of Plasma PON1 Paraoxonase Activity and Genotype in Gulf War Veterans*[1]

- PI: Linda Chao (UCSF, SF VAMC)
- Evaluating PON1 Assoc with GWI in 4 independent Cohorts
- Preliminary analyses: significant gene x exposure interactions

2. *Investigating Gene-Environment Interactions in Multiple Cohorts of 1990-1991 Gulf War Veterans*[2]

- PI: Tricia Janulewicz Lloyd (Boston Univ SPH)
- Evaluating BChE Assoc with GWI in 4 independent Cohorts
- Lab analyses completed, analyses underway

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Thank you