Acetylcholinesterase Activity in Veterans of the First Gulf War

John Concato, Mihaela Aslan, Margaret M. Palmisano, Caroline Carney Doebbeling, Peter Peduzzi, Keren Ofek, Hermona Soreq, and Bradley Doebbeling

Background: Factors affecting acetylcholine-mediated neurotransmission have been proposed as possible explanations for physical and mental health symptoms among veterans of the 1990–1991 Gulf War. This study was designed to examine relationships of deployment to the Gulf, as well as symptoms after military service, with postdeployment activity of acetylcholinesterase (AChE) and related enzymes.
Methods: The patient population included 488 veterans, originally from Iowa at enlistment, who served in the US military during August 1990 to July 1991. Demographic, military, and clinical characteristics were obtained from a population-based cohort study (in 1995–1996) and from a nested case-control study (in 1999–2002). Stored serum samples (from the 1999–2002 assessment) were analyzed for activity of AChE and related enzymes. These two data sources were merged, and multiple linear regression models estimated the association of deployment, stress (anxiety) or mood disorders, and symptoms compatible with Gulf War veterans' illnesses (GWVIs), with enzyme activity.
Results: Seventy-four percent ($n = 361$) of veterans had been deployed to the Gulf. At the time of evaluation, 23% ($n = 113$) of participants reported anxiety and 15% ($n = 71$) reported mood disorders; 49% ($n = 171$ of 347 eligible veterans) had symptoms of GWVIs, and the median AChE activity was 839 units. AChE activity was similar for compared groups across all categories, including an adjusted difference of −27 units ($p = .50$) for deployed versus nondeployed veterans and 87 units ($p = .13$) for veterans with versus without symptoms of GWVIs.
Conclusions: Neither deployment to the Gulf nor symptoms compatible with GWVIs are associated with long-term serum AChE activity.

Key words: acetylcholinesterase, Persian Gulf War, neural pathways, stress, symptoms, veterans

The health status of approximately 700,000 American troops deployed to Southwest Asia during 1990–1991 has been the subject of intense investigation. A substantial proportion of veterans of the Gulf War have reported symptoms such as unexplained fatigue, pain, and cognitive or mood disorders. In 1998, criteria were developed that designated Gulf War veterans' illnesses (GWVIs) as a potential specific syndrome, with clinical and research applications.[1] Much attention has been focused on GWVIs, but

From the Clinical Epidemiology Research Center (J.C., M.A., M.M.P.) and Cooperative Studies Program Coordinating Center (P.P.), VA Cooperative Studies Program, VA Connecticut Healthcare System, West Haven, CT; Yale University School of Medicine (J.C., M.A., P.P.), New Haven, CT; The Hebrew University of Jerusalem (K.O., H.S.), Jerusalem, Israel; the Health Services Research and Development Center on Implementing Evidence-Based Practice, Richard L. Roudebush VA Medical Center (B.D.), Indianapolis, IN; the Center for Health Services and Outcomes Research, Regenstrief Institute, Inc. (C.C.D., B.D.), Indianapolis, IN; and Indiana University School of Medicine (C.C.D., B.D.), Indianapolis, IN.

This work was supported by the VA Cooperative Studies Program (CSP#520), the Department of Defense (DAMD17-97-1), and a Centers for Disease Control and Prevention Cooperative Agreement (U50/CCU711513).

Data were collected and analyzed in the University of Iowa General Clinical Research Center, supported by grant M01-RR-59, National Center for Research Resources, General Clinical Research Centers Program, National Institutes of Health, and The Hebrew University of Jerusalem (Edmond G. Safra Campus), Givat-Ram, Jerusalem, Israel.

The views expressed in this article are those of the authors and do not necessarily represent the views of the Department of Veterans Affairs.

Address correspondence to: Dr. John Concato, VA Connecticut Healthcare System, Clinical Epidemiology Research Center 151B, 950 Campbell Avenue, West Haven, CT 06516; e-mail: john.concato@yale.edu.

Journal of Investigative Medicine 2007;55:360–367.

DOI 10.2310/6650.2007.00016

evidence is lacking to confirm an etiologic agent or explain a pathogenic mechanism.

A report on the health of Gulf War veterans used factor analysis to identify symptom patterns (eg, impaired cognition, confusion-ataxia) in 63 patients.[2] These symptoms overlap with Alzheimer disease and chronic neuromuscular diseases, raising the question of whether neural pathways involving acetylcholine-mediated neurotransmission are affected adversely.[3] In particular, acute stress, environmental stimuli, and pharmacologic agents can increase transcription of the enzyme acetylcholinesterase (AChE), and facilitate its action to induce symptoms[4–7] In this context, deployment to the Gulf involved exposure to stressful wartime and environmental conditions, as well as to pesticides and pyridostigmine bromide (an anticholinesterase agent used as a nerve gas antidote). Although the initial report[2] did not include a comparison group to confirm the specificity of the results and the findings were not replicated,[6] questions remain about potential adverse health effects from military service in the Gulf.

The current study had three objectives, examining whether (1) deployment to the Gulf, (2) anxiety (stress) or mood disorders, or (3) symptoms of GWVIs are associated with serum activity of AChE or related enzymes at the time of evaluation (in 1999–2002).

Methods

Patient Population and Study Design

The sampling frame consists of 602 Gulf War–era veterans who had been previously enrolled in the Iowa Gulf War study, a population-based assessment of deployed and nondeployed veterans listing Iowa as their home of record at the time of enlistment.[8,9] Wave I of this study was conducted in 1995–1996 as a stratified random sample of military personnel who were either deployed or eligible but not deployed to the Gulf War in 1990–1991. Among 4,886 military personnel sampled, 3,695 veterans (76%) participated in a telephone survey that asked a variety of questions related to military and environmental factors, as well as health concerns.

A second wave of data collection was done in 1999–2002 (8–12 years postdeployment) as part of case-control studies nested within the wave I cohort. Participants were recruited for the wave II study if they had one or more of the three most prevalent conditions of interest, defined a priori, at the time of their wave I interview: symptoms of cognitive dysfunction, depression, or fibromyalgia. The goal of case selection was to enrol a representative sample chosen from strata defined by deployment, age, gender, and branch of service. A comparison group, consisting of veterans who did not have any of the three conditions of interest, was frequency-matched to case patients according to these strata. The wave II study included a full-day, in-person assessment at the University of Iowa's General Clinical Research Center, with standardized interviews, self-report assessments, examinations to assess medical and mental health problems, and phlebotomy.

The final population for the current analyses includes veterans with interview and examination data from both wave I and wave II, as well as stored serum specimens. The research design is an observational study with cross-sectional and longitudinal (retrospective) components. Interim results have been presented to the Gulf War Research Advisory Committee—an advisory group created by Congress to make recommendations to the Secretary of Veterans Affairs on government research relating to the health consequences of military service in the Southwest Asia theater of operations during the Gulf War—and the Committee provided suggestions for further analyses that are presented herein.

Questionnaire Responses

Detailed information on demographic features, military service information, and general health status were used to characterize the study population, based on wave I or wave II information, as appropriate. Various patient characteristics were identified a priori as being potentially associated with AChE activity and being possibly related independently to the exposures of interest: age, body mass index, acute physical illness symptoms, smoking status, wave I case status, antidepressant drug use, antipsychotic drug use, current drug abuse or dependence disorder, and current alcohol abuse or dependence disorder.

Veterans were classified as deployed to the Gulf War or nondeployed based on self-report. The participants underwent the Structured Clinical Interview for DSM-IV (SCID),[10] administered by trained clinicians, for anxiety or mood disorders classified as Axis I disorders by the current *Diagnostic and Statistical Manual of Mental Disorders*. Data were also collected based on a tripartite model of anxiety and depression,[11] the short-form Mood and Anxiety Symptom Questionnaire (MASQ),[12] and the Anxiety Sensitivity Index.[13]

The Centers for Disease Control and Prevention (CDC) case definition[1] for GWVI requires the presence of at least one or more chronic symptoms (present for at least 6 months) from at least two of the following three categories: fatigue, mood and cogni-

tion (eg, difficulty remembering or concentrating), and musculoskeletal (eg, symptoms of joint pain). In the Iowa study, veterans were asked about the presence of such symptoms and, if present, how much the symptom had bothered them (a little bit, moderately, quite a bit, or extremely). At wave I, if a symptom was present, a follow-up question also asked whether the symptom was present prior to August of 1990. Participants reporting symptoms—if not present prior to August of 1990, and with bother more than ''a little bit''—were coded as symptomatic in the current analysis.

Pertinent symptoms were then grouped according to the three categories of the CDC definition, and to satisfy the criterion for persistent (chronic) symptoms, GWVI was considered present in the current study if corresponding symptoms were present at the wave I and II interviews. (Patients who denied such symptoms at wave I but reported the corresponding symptoms at wave II, when phlebotomy was done, were excluded from primary analysis of GWVI. This approach focuses on the most severely afflicted veterans—those with ''earlier'' onset—and conversely avoids including nonhealthy patients in the comparison group.)

The original Iowa Gulf War Study also collected data on self-reported exposures during deployment. For example, participants were asked: ''Did you have direct contact with/were you exposed to [smoke from oil well fires]?'' and a ''yes'' response prompted further questions. Input from the Gulf War Research Advisory Committee led to post hoc analyses examining associations of these self-reported exposures with long-term levels of enzyme activity.

Enzyme Activity Measurements

Stored sera were shipped to The Hebrew University of Jerusalem for performance of kinetic assays, ''blind'' to deployment and symptom status, using a Molecular Dynamics (San Diego, CA) multi-well plate reader for the selected enzymes. The protocol specified that spectrophotometric analyses of AChE[14] would be conducted first, with analyses of butyrylcholinesterase,[14] paraoxonase (PON),[15] and arylesterase[16] serving potential explanatory roles. Immunochemical analyses of the R variant of acetylcholinesterase (AChE-R)[17] were also done, based in part on input from the Gulf War Research Advisory Committee.

Statistical Analysis

Descriptive statistics were used to report the distributions of baseline and adjustment factors, diagnoses, symptoms, enzyme activity, and self-reported exposures. Continuous and categorical variables, as appropriate, were used for anxiety and mood measures, deployment, and GWVI; and unadjusted mean enzyme activities for subjects with and without each exposure were first compared. Analyses were then adjusted for the effects of potential confounding factors, using linear regression models to estimate mean enzyme activity differences between groups. Given the multiple (> 10) associations examined, the probability of one or more p values being less than .05 owing to chance alone was elevated; we therefore identified a two-sided p value of .005 as statistically significant. Statistical power was not calculated because the sample size was fixed, and data are limited regarding clinically relevant levels of enzyme activity. Analyses were done using *SAS* version 9.1 (SAS Institute, Cary, NC).

Results

Baseline Characteristics

Among 602 veterans who completed both waves I and II of the Iowa Gulf War Study, 11 had no serum sample available and 14 samples were discarded after being damaged in shipment, leaving 577 (96%) study participants. Among this group, 488 veterans (85%) were white and male, 71 (12%) were white and female veterans, and 18 (3%) were nonwhite veterans (including 2 women). To preserve validity, albeit at the expense of generalizability, only white male participants were included in subsequent analyses. The mean elapsed time between the wave I and wave II interviews was 4.4 years, with a range from 3.1 to 7.1 years.

The mean age of the final analytic sample of 488 veterans was 39 (SD 9) years; 71% ($n = 345$) had served in the Army, 15% ($n = 73$) reported a history of high blood pressure, and 2% ($n = 11$) reported a history of coronary artery disease (Table 1). The mental and physical component summary scores of the MOS 36-Item Short-Form Health Survey Health Survey (SF-36)[18] were lower than population norms, as expected based on the sampling strategy.

Among the 361 veterans (74%) who were deployed to the Gulf during the war, the mean time spent in the theater was 156 days (SD = 77). Deployed subjects reported receiving a median of 8 vaccinations (range 0–213). A majority (58%) of deployed veterans reported taking pyridostigmine bromide tablets while in the military; the median number of pills taken among subjects who reported any use was 16 (range 1–720).

Table 1 Demographic, Military, and Clinical Characteristics ($N = 488$)*

Characteristic	*Mean ± SD*	*% (n)*
Age (yr)	39 ± 9	
Education (missing = 5)		
Less than high school		1 (3)
High school graduate		37 (177)
Some college		46 (220)
College graduate		17 (83)
Marital status (missing = 5)		
Married or living with partner		75 (363)
Divorced/annulled/separated		17 (82)
Never married		7 (35)
Widowed		1 (3)
Service branch		
Army		71 (345)
Marines		14 (69)
Navy/Coast Guard		10 (49)
Air Force		5 (25)
Health conditions		
Hypertension		15 (73)
Coronary artery disease (missing = 1)		2 (11)
Acute illness† (missing = 8)		39 (185)
Body mass index (missing = 3)	29 ± 5	
SF-36 scales in prior 4 wk		
PCS (missing = 7)	46 ± 10	
MCS (missing = 7)	49 ± 10	
Current tobacco use		35 (171)
Drug use (see text)		
Drug abuse or dependence disorder (missing = 1)		2 (9)
Alcohol abuse or dependence disorder (missing = 1)		6 (27)
Antidepressant medication use		9 (42)
Antipsychotic medication use		< 1 (1)

MCS = mental component summary; PCS = physical component summary; SF-36 = MOS 36-Item Short-Form Health Survey.
*$N = 488$ unless number missing noted.
†Acute illness symptoms from wave II data: sore throat, diarrhea, nausea, or cough.

Current anxiety disorders, as determined by the SCID, were present at wave II in 23% of subjects (Table 2). The most common diagnoses were posttraumatic stress disorder (7%), generalized anxiety disorder (7%), and social phobia disorder (6%). Current mood disorders by SCID were present at wave II in 15% of subjects, including major depressive (8%) and dysthymic (4%) disorders.

The primary analysis of GWVIs excluded 141 veterans, either with missing data ($n = 7$) or those who reported corresponding symptoms at wave II (when enzyme activity was determined) but not at wave I ($n = 134$). Among the remaining 347 participants, 49% ($n = 171$) met the CDC criteria[1] for GWVIs, including 53% ($n = 142$ of 266) in the deployed group and 36% ($n = 29$ of 81) in the nondeployed group (see Table 2).

AChE activity (nmol substrate hydrolyzed/min^{-mL}) ranged from 130 to 2,908 in the study population, with a median of 839 and a mean of 891 (SD = 377).

Associations with AChE activity

Deployment to Southwest Asia during the Gulf War was not associated with postdeployment AChE activity (Table 3); the adjusted mean difference was −27 units for deployed versus nondeployed subjects ($p = .50$). The adjusted difference in mean AChE for subjects with (vs without) a current anxiety disorder was −9 units ($p = .84$); see also Figure 1. A similar lack of association was observed for the relationship between mood disorders and measured AChE activity.

Comparing veterans with ($n = 171$) and without ($n = 176$) GWVI symptoms, the adjusted mean difference in AChE activity was 87 units ($p = .13$). In an analysis restricted to veterans who were deployed to the Gulf, the adjusted difference in AChE activity for GWVI symptoms was also statistically nonsignificant (91 units; $p = .21$). In a sensitivity analysis that included the 134 veterans with late-onset symptoms of unknown duration in the coding of GWVIs, the adjusted mean difference in AChE activity was 9 units ($p = .83$).

In secondary analyses, no significant associations were found for measures of deployment, anxiety or mood, or GWVIs with activity of butyrylcholinesterase, PON, or arylesterase (data not shown). Sufficient (remaining) serum was not available to conduct follow-up immunochemistry assays for the AChE-R variant among all participants, but significant differences in baseline characteristics were not found for the groups of veterans with ($n = 218$) versus without ($n = 270$) values. Among the 218 patients with available assays, no significant associations regarding deployment, anxiety or mood, or GWVIs were found for AChE monomers (data not shown).

Among associations of self-reported exposures (for $n = 361$ deployed) and postdeployment AChE activity, none were statistically significant (Table 4). Among other enzymes, including AChE monomers, the only statistically significant association—and the

Table 2 Deployment, Anxiety and Mood Measures, and Gulf War Veterans Illnesses ($N = 488$)

Measurement	*Mean ± SD*	*% (n)*
Deployment to Gulf		74 (361/488)
Anxiety		
Any current anxiety disorder (SCID; missing = 1)		23 (113/487)
Any lifetime anxiety disorder (SCID; missing = 1)		29 (139/487)
Anxiety arousal (MASQ; range 17–61; missing = 24)	24 ± 7	
Anxiety sensitivity index (range 16–66; missing = 20)	32 ± 10	
Mood		
Any current mood disorder (missing = 1)		15 (71/487)
Any lifetime mood disorder (missing = 1)		31 (153/487)
Anhedonic depression (MASQ; range 27–103; missing = 25)	59 ± 14	
Gulf War veterans' illnesses (missing = 7)		
Total		49 (171/347)
Deployed		53 (142/266)
Nondeployed		36 (29/81)

MASQ = Mood and Anxiety Symptom Questionnaire; SCID = Structured Clinical Interview for DSM-IV.

strongest by an order of magnitude—was for self-reported exposure to solvents or petrochemicals and PON (difference = −16; $p = .002$). When compared to the overall mean of 42 units, however, the "abnormal" value was found in the nonexposed group (58 units) rather than the exposed group (42 units), and only 6% ($n = 22$) of veterans reported their status as nonexposed.

Discussion

No significant association was found linking military service in the Gulf, or subsequent symptoms, with postdeployment (long-term) serum AChE activity. This null finding was robust across domains related to deployment status, anxiety or mood symptoms, and criteria for GWVIs. A similar lack of significant

Table 3 Association of Deployment and Symptoms with Acetylcholinesterase Activity ($N = 488$)

	Mean AChE when Factor:					
Factor (% of participants)	*Present*	*Absent*	*Unadjusted Difference*	p	*Adjusted Difference**	p
Deployment (74)	885	906	−21	.59	−27	.50
Anxiety or mood disorders						
Current anxiety (23)	880	894	−14	.72	−9	.84
Lifetime anxiety (29)	882	895	−13	.74	−14	.74
Anxiety arousal†	—	—	13	.46	23	.24
Anxiety symptom index†	—	—	1	.97	−1	.98
Current mood (15)	897	890	7	.89	2	.97
Lifetime mood (31)	920	877	43	.25	46	.27
Anhedonic depression†	—	—	−6	.72	−12	.55
Gulf War veterans' illnesses						
Overall (49)	937	889	47	.26	87	.13
Deployed (53)	929	884	45	.37	91	.21
Nondeployed (36)	976	903	73	.37	−4	.97

AChE = acetylcholinesterase.

*Adjusted for age, body mass index, physical illness symptoms, smoking status, wave I case status, antidepressant drug use, antipsychotic drug use, current drug abuse or dependence disorder, and current alcohol abuse or dependence disorder ($n = 12$ missing). For adjusted models, $n = 476$, except $n = 457$ for anxiety arousal, 461 for anxiety sensitivity, 456 for anhedonic depression, and 344 for overall Gulf War veterans' illness (265 for deployed and 79 for nondeployed).

†Differences are for increase of 1 SD (anxiety arousal 7.4; anxiety symptom index 9.7; anhedonic depression 14.4).

Unadjusted differences may not equal the difference of the mean values of AChE owing to rounding.

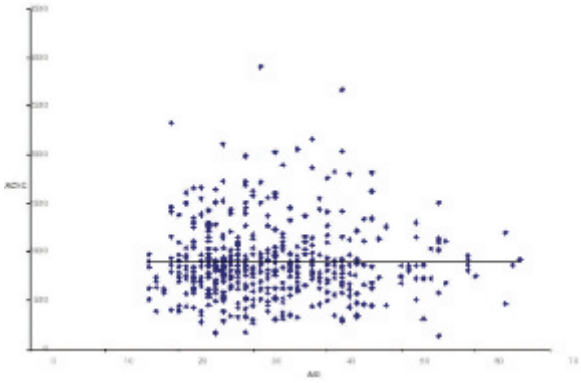

Figure 1 Relationship between Anxiety Symptom Index and acetylcholinesterase activity.

associations was observed for butyrylcholinesterase, PON, arylesterase, and AChE monomers as outcome variables.

Explanatory models involving AChE and related enzymes involved in neural pathways have been proposed to help explain illness among Gulf veterans. For example, transcriptional activation of AChE is associated with a shift in its splicing pattern, leading to accumulation of the (otherwise) rare AChE-R variant, which may help mitigate the impact of transient stress on progressive neurologic disease.[3,19,20] Our results do not support a persistent mode of these explanatory models; for example, symptomatic and asymptomatic participants had similar enzyme activity levels. With regard to PON enzyme activity, our results are consistent with a study of veterans from the United Kingdom Armed Forces, which found that symptomatic and nonsymptomatic Gulf War veterans "did not differ in PON1 activity."[21] Also of note, a substantial proportion of nondeployed Gulf War–era veterans reported symptoms consistent with GWVIs, as found

Table 4 Association of Self-Reported Exposures during Deployment with Acetylcholinesterase Activity (n = 361)

	Mean AChE when Factor:					
Factor (n)	*Present*	*Absent*	*Unadjusted Difference*	p	*Adjusted Difference**	p
Days in Gulf War theater†	—	—	19	.35	17	.42
Vaccinations†	—	—	3	.87	5	.81
Pyridostigmine bromide tablets†	—	—	−23	.27	−25	.26
Chemical attack alert (284)	891	878	13	.80	14	.79
Chemical warfare agents (32)	773	896	−123	.08	−126	.09
Pesticides (230)	862	926	−64	.13	−55	.20
Solvents/petrochemicals (339)	887	861	25	.77	6	.95
Smoke/combustion products (340)	884	898	−14	.87	−4	.96
Sources of infectious agents (327)	880	937	−57	.41	−64	.37
Sources of lead from fuels (314)	873	970	−98	.11	−98	.11
Ionizing/nonionizing radiation (82)	873	889	−15	.75	−6	.91
Physical trauma (18)	746	893	−146	.12	−126	.21
Heat stress (86)	949	865	83	.08	74	.13
Psychological stressors (341)	884	905	−21	.82	11	.90
Other exposures (124)	890	883	7	.87	4	.94

AChE = acetylcholinesterase.

*See Table 3 for list of adjustment variables; n = 356 with nonmissing data, except n = 355 for days in Gulf War theater, 332 for vaccinations, 345 for pyridostigmine tablets, and 352 for chemical alert attack.

†Differences are for increase of 1 SD (days in Gulf theater 77.3; vaccinations 18.9; pyridostigmine tablets 69.2).

Unadjusted differences may not equal the difference of the mean values of AChE owing to rounding.

initially[1] and subsequently[6,22] when such comparison groups are examined.

Our study minimized common problems[23] of research on the health of Gulf War veterans by having a high response rate, ''thorough'' ascertainment, and a suitable comparison group. Limitations of the current analysis include a lack of prewar data on participants' clinical status or enzyme activity. Our protocol was also not designed to examine enzyme activity shortly after the time of exposure or in the central nervous system (especially pertinent for AChE-R[19]), and serum enzyme activity may not fully reflect in vivo mechanisms. In addition, our analyses include cross-sectional components that provide limited inference regarding cause-effect associations (eg, neurotoxins may affect enzyme activity and cause symptoms, or having symptoms, regardless of etiology, may affect enzyme activity). Yet our study included longitudinal analyses (eg, deployment and subsequent enzyme activity) that also yielded null results.

The sampling scheme used in the original Iowa Veterans Cohort Study added complexity to our project, and the final study population (of white males) was enriched regarding symptomatic veterans. We tailored our analyses to match each corresponding design feature in our study, however, and the results were similar (data not shown) when analyses did not adjust for wave I case-control status. Also, mean values of AChE activity in our population were notably higher than values measured in other studies, including both healthy volunteers and patients with Parkinson disease,[24–26] but the determinants of levels (including ''normal'' values) in individual patients are not established. Accordingly, the implications of across-study comparisons are unknown. Finally, we did not have data collected directly according to formal criteria for GWVIs, mainly because the initial Iowa project predated their promulgation, but secondary analyses (data not shown) that varied the case definition also yielded null results.

Given various viewpoints and areas of emphasis among different groups,[6,27] questions will likely arise regarding the design, analyses, and interpretation of the results of our study, for example, What priority should be given to anxiety or stress when assessing the health of Gulf War veterans? The current project was designed to assess these important health concerns, and available evidence[28,29] supports such a focus as part of a comprehensive and scientific approach. Another potential question is: What inference should be made from the isolated finding of a paradoxical association between nonexposure to solvents or petrochemicals and PON activity? This finding could be considered a ''positive'' result or an illustration of ''data dredging'' given no previous hypothesis and a lack of a similar association for AChE. Future scientific investigations related to these topics should be evaluated by the scientific community.

In conclusion, veterans of the Persian Gulf War manifest a variety of symptoms related to physical and mental health. Research on this topic, and on affected veterans, is important in terms of explicatory science and is crucial regarding diagnostic and therapeutic implications.[30–32] Our study serves an important purpose by providing information to guide future efforts and resources. Our findings do not ''rule out'' a role of AChE and related enzymes in the etiology or pathogenesis of symptoms among veterans of the First Gulf War. Postdeployment (long-term) serum AChE activity does not, however, appear to be strongly associated with having been deployed to the Persian Gulf or established symptoms of GWVIs.

Acknowledgments

We are grateful to investigators at the VA Clinical Epidemiology Research Center and Cooperative Studies Program Coordinating Center at West Haven, Yale University, the University of Iowa, Iowa State University Stat Lab, Iowa Department of Public Health, CDC, and The Hebrew University of Jerusalem for their input and assistance. In particular, we thank M. Antonelli, D. Black, W. Coryell, P. Peloso, R. Roth, D. Schwartz, J. Torner, C. Viscoli, M. Voelker, D. Watson, and C.K. Wells for their contributions. We also thank the Gulf War Research Advisory Committee for their input. We greatly appreciate the participation of veterans and other military personnel who volunteered their time to participate in this study.

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