Presentation 14 – Beatrice Golomb

Update on Research in Persian Gulf War Veterans Illnesses

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TOPICS

I. Epidemiology

II. Human Markers

III. Exposure Effects: Animals

IV. Human Gene or Gene-Environment
- --- ALS
- --- CFS (Dr. Kerr)
- --- MCS

V. Human Treatments

Epidemiology

- **Physical Sx Persist in GWV**
- **Mental Sx Persist but may Somewhat Abate in GWV**
- **GWV have increased pain**

Symptoms Persist in GWV

Finding: Little change sx # or severity on 5yr f/u.

Ss: 390 randomly selected from previously surveyed members of US DVA Gulf War registry who completed time-1 & time-2 survey

At time 1 divided into low vs hi sx clusters (mean 14 & 35sx).

Design: Mailed symptom survey questionnaires with 48 sx identical to time 1. Assess if "persistent or recurring" sx in the last 6 mo; and whether "mild," "moderate" or "severe"

Time 1: year 1995, 2011 GWV, 60% response

Time 2: year 2000: 60% of orig cohort "randomly" mailed 1-pg questionnaire, 2 wks B4 scheduled phone interview.

71% of these completed.

Ozakinci 2006 *Environ Health Perspect* 114:1553 Persistence of symptoms in veterans of the First Gulf War: 5-year follow-up.

Symptoms Persist in GWV

Outcomes: severity; change in # sx; cluster membership

Analysis: Effect of time on outcomes by Repeated measures Mancova

Adjusted for: age, sex, rank, race, marital, educ, branch service, duty (active vs NatGuard/Reserve), smoker. Also: cluster membership Highly symptomatic (40%) vs mod (60%) at time 1, except if this is outcome.

Ozakinci 2006 *Environ Health Perspect* 114:1553 Persistence of symptoms in veterans of the First Gulf War: 5-year follow-up.

Symptoms Persist in GWV

Result: Little change in sx number or severity with time over 5 yrs.

Sx number similar: 22.1 (12.9) → 22.7 (12.9), NS ($p = 0.14$)

Sx severity similar: 0.83 (0.62) → 0.84 (0.62), NS

Mildly symptomatic time 1: showed ↑ +2.3 sx after adjust ANCOVA

Highly symptomatic time 1: showed a ↓ -2.0, $p < 0.001$

[BUT:"regression to the mean" for vbls w measurement variability: Regression dilution bias]

Black race & older age associated with increase in sx

Similar findings for sx severity.

Interpretation: "The symptom outbreak following the 1991 Gulf War has not abated over time in registry veterans, suggesting substantial need for better understanding and care for these veterans"

Pain is increased in GWV

Finding: "A higher proportion of veterans of the PGW reported sx of pain than military comparison groups"

Design: Weighted "meta-analysis" of published studies assessing pain in GWV vs comparison veteran group.

20 studies that include prevalence of some type of pain in GWV and comparison group of nonGulf veterans

	OR	95%CI
Abd:	3.2	2.3-4.5
Muscle	3.1	2.2-4.3
Joint	2.8	2.3-3.4
Chest pain	2.5	2.2-2.9
Back pain	1.6	1.2-2.0

Thomas 2006 BMC Musculoskeletal Disorders 7:74. Pain in veterans of the Gulf War of 1991: a systematic review

Mental Health Probs in GWV

Finding: Increased mental health problems in GWV starting at deployment and present at reduced rate 10 yrs later.

Design: Assess prevalence of mental disorders in GWV vs non, beginning in deployment period, and continued prevalence 10 years later*, based on retrospective report.*

Ss: 1016 deployed veterans vs 1128 nondeployed veterans, from National Health Survey of Gulf War Veterans and their Families in 1995, a stratified random sample from 15,000 each were examined.

Evaluation period: 1998-2001

Called "Gulf Era Onset" if reported onset btn Jan 1 1991 and Jul 30 1993 (not by whether they were in the Gulf then)

Toomey,Kang, Karlinsky, Baker, Vasterling, Alpern, Reda, Henderson, Murphy, Eisen Br J Psychiatry 190: 385

Mental Health is Reduced in GWV

Outcomes:

Mental disorders diagnosed by structured clinical interviews, with onset time by self-report (not blinded to GW participation state)

- Clinician Administered PTSD Scale
- BDI Beck Depression Inventory
- BAI Beck Anxiety Inventory
- SF-36 for QOL, Mental Component Summary only
- Quality of Life Inventory: satisfaction with 16 domains of QOL.

Toomey,Kang, Karlinsky, Baker, Vasterling, Alpern, Reda, Henderson, Murphy, Eisen Br J Psychiatry 190: 385

Mental Health is Reduced in GWV

Outcomes:

53% participation rate of those solicited to participate. Participants more likely older, white, women, reservists and National Guard, officer, and army vs other.

Deployed group 2yr younger, more AA, less educated, less likely married, less likely officers.

0.9 or higher interrater reliability on most sx; 0.77 for current PTSD

Analysis: Linear or logistic regression weighted for stratification (probability of sampling)

Adjustments: age, gender, ethnicity, education (dichotomized at 12yrs), duty type (active v reserve/guard), branch (army/marine v Navy/air force), rank (officer v not)

Toomey,Kang, Karlinsky, Baker, Vasterling, Alpern, Reda, Henderson, Murphy, Eisen Br J Psychiatry 190: 385

Mental Health in GWV: Current

	GWV	Nondeployed	p (adjusted)
SF-36 MCS mean	49	53	<0.0001
PTSD checklist score	28	23	<0.0001
BeckDI mean score	7.8	4.7	<0.0001
Mod/severe %	10.6	4.9	<0.0001 by group
BeckAI mean score	4.8	2.8	<0.0001
QOL inventory			
%V.low	15	8	0.002 by group
%"below average"	25	16	

By group p-values were were bsed on all grouPS: min,mild,mod,severe; or vlow, low, average, high

Implications: The prevalance of GEO anxiety, depression, PTSD "abates with time" – but is still increased.

Toomey,Kang, Karlinsky, Baker, Vasterling, Alpern, Reda, Henderson, Murphy, Eisen Br J Psychiatry 190: 385

Mental Health: Era Onset

Era Onset Mental Health	%GW:not	Adjusted OR	95% CI
Any mental disorder	18:9	2.1	1.4-3.1
Any mood disorder	8:4	1.9	1.1-3.3
Major Depression	7:4	1.8	1.0-3.2
Anxiety disorders	9:2	4.4	2.5-7.9
PTSD	6:1	5.8	2.6-13
Panic d/o	1.2:0.1	10.5	2.0-56
Specific phobias	1.9:0.8	2.8	1.1-7.5
Pain disorder	**0.9:0.01**	**91.7**	**10.5-798.2**
Brief psychotic rxn	**0.2:0.9**	**0.23**	**0.06-0.91**

Toomey,Kang, Karlinsky, Baker, Vasterling, Alpern, Reda, Henderson, Murphy, Eisen Br J Psychiatry 190: 385

Mental Health: Improvement over time?

War-era onset (WEO) depression 10 yrs later:

3.2%:0.8% OR 3.3 p< 0.01

Remission less likely in deployed: p = 0.048

No difference in initial severity of depression

Among depressed: ↑ co-morbid war-era onset psych d/o 46%:26%

If depression: co-morbid anxiety d/o incl PTSD: p = 0.07

PTSD- WEO 10yr later 3x ↑ (not signif: 1.8%:0.6% p = 0.12)

nonPTSD- WEO anxiety differs 2.8:1.2%, aOR=2, p = 0.01

Toomey,Kang, Karlinsky, Baker, Vasterling, Alpern, Reda, Henderson, Murphy, Eisen Br J Psychiatry 190: 385

Mental Health Problems w/ WEO abate

Medication Use 4 War-Era Onset (WEO) "mental health"

Taking Meds4This @ Time of Assessment

	%GWV	%Nondeployed	p
WEO anxiety d/o still active: N=78	12	22	NS
WEO anxiety d/o that remitted N=57	5	37	0.02
WEO anxiety d/o: Current med use	*8*	*27*	

Possibilities: Different disease; different tolerance to treatment; differential care; chance

WEO depression still active N=44 (36:8)	17	33	0.43
WEO depression that remitted N=89	13	5	0.25
WEO depression: Current med use	*15*	*11*	*NS*

In this sample: more deployed had preGW (pre Jan 1 1991) onset nonPTSD anxiety d/o13:9% p = 0.02

Toomey,Kang, Karlinsky, Baker, Vasterling, Alpern, Reda, Henderson, Murphy, Eisen Br J Psychiatry 190: 385
Vasterling, Alpern, Reda, Henderson, Murphy, Eisen Br J Psychiatry 190: 385

Mental Health is Reduced in GWV

Satisfaction with QOL reduced in GWV vs. nondeployed

QOL DOMAIN	p-value
health	0.0001
learning	0.001
play	0.01
self-esteem	0.02
love	0.03
goals and values	0.04
children	0.049

Toomey,Kang, Karlinsky, Baker, Vasterling, Alpern, Reda, Henderson, Murphy, Eisen Br J Psychiatry 190: 385

Human Markers

Changes in Cortisol /ACTH

Ss: 31 GWV: 20 current PTSD, 11 never PTSD,no psych d/o
16 healthy nondeployed, no gulf theater

Marker: AUC Plasma ACTH & cortisol, q 30min for 24h

Result:

GWV w/o PTSD or psych d/o, vs health OR GWV+PTSD had

- **No difference in cortisol**
- **Signif lower 24h plasma ACTH**
- **Signif higher cortisol: ACTH**

Comment: Self reported acute effects of pesticides and PB during deployment were assoc with lower ACTH controlling for BMI and PTSD

Changes in Cortisol / ACTH

Baseline comparability table:

- Sim demographics
- Diff sx expression

Area under the curve:

	Nondeployed			
	Healthy	PTSD+	PTSD-	P
Cortisol (C):	158	154	154	NS
ACTH (A)	638	619	419 ↓	<0.05
Ratio C/A:	0.30	0.28	0.45 ↑	<0.0005

Ug/dl-hr cortisol; pg/ml-hr ACTH

Golier 2007 *Biol Psychiatry* early e-pub

Changes in Cortisol / ACTH

Previously reported increased cortisol and ACTH suppression to dexamethasone in GWV assoc with chronic musculoskel sx

– enhanced responsivity to cortisol feedback effect may be partly responsible.

Golier 2007 *Biol Psychiatry* early e-pub

Markers in CFS: RBC oxidative stress

Finding: Increased RBC oxidative stress, morphol changes

Background: RBC metabolism and shape relate to deformability/morphology; altered morphology seen in CFS; and morphology correlates with RBC oxidative damage.

Goal: confirm these correlations.

Ss: 31 CFS; 41 "age and gender matched" "healthy controls"

10/33 male and 18/41 male; age 41+/-28 CFS, 40+/-25 control.

Outcomes:

- RBC levels of reduced glutathione (GSH); malondialdehyde (MDA); methemoglobin (metHb)
- 2,3-diphosphoglyceric acid (2,3 DPG): an RBC mb regulator, ↓ deformabil, ↓ O2 affinity
- Scanning EM of RBC

Richards 2007 Arch Med Res 38: 94

Markers in CFS: RBC oxidative stress

Results:

Signif ↑ oxidative damage (prooxidant>antioxidant protection)

↑ 2,3 DPB $p < 0.05$: ↓ RBC deformability, ↓ O2 affinity (release more

↑ MetHb, $p < 0.005$. Oxid damage Hb, ↓ *RBC deformability,* ↑*rigidity*

↑ MDA, $p < 0.01$: Marker of lipid peroxidation

Significant RBC morphological abnormality

↑ Stomatocytes in blood vs nl ($p < 0.005$)

Interpretation:

RBC changes could be a marker of oxidative stress that may be a common cause for symptoms

RBC changes could also causally affect energetics if they affect oxygen transport to tissue – but may ↓ or ↑

Richards 2007 Arch Med Res 38: 94

Markers in FM: Oxidative Stress

Ss:

- 30 fem w/ Primary FM by ACR criteria (Am Coll Rheumatol)
- 16 "age-matched" healthy controls

Markers: TBARS/MDA, SOD, adenosine deaminase (ADA), xanthine oxidase, nitrite as index of NO production

Clinical assessments: TMS (aggregates 21 pain pressure thresholds), HAM-D, HAM-A, FM-impact questionnaire (FIQ)

Finding:

- FM have Higher TBARS (lipid peroxidation marker), Lower nitrite
- No correlation to clinical measures

-- TMS scale corr. negatively with xanthine oxidase, an antioxidant

Ozgocmen 2006 Rheumatol Int 26: 598

Markers in FM: Oxidative Stress

Test		FM	Ctrl	P
TBARS	μM/L	1.4 ± 0.7	1.0± 0.5	<0.05
Nitrite	μM/L	51.9 ±24.5	69.5 ±10.9	<0.05
ADA	U/L	219 ± 73	181± 67	NS
SOD	U/ml	6.2 ± 2.1	6.4 ± 2.4	NS
XO	U/L	2.4 ± 0.9	2.6 ± 1.0	NS

Ozgocmen 2006 Rheumatol Int 26: 598

ANIMAL STUDIES OF EXPOSURES

- Aluminum adjuvants cause health problems
- Jet fuel promotes noise-induced hearing loss – associated with oxidative stress
- Uranium causes oxidative stress in brain
- Chlorpyrifos affects microtubules (which transport mitochondria to different parts of the cell)

Anthrax vaccine adjuvants

Goal: To examine whether adjuvant compounds might contribute to neuronal deficits associated with GWI, an animal model for examining the potential neurological impact of aluminum hydroxide, squalene, or aluminum hydroxide combined with squalene was developed.

Ss: Young, male colony CD-1 mice

Exposure: injected with "the adjuvants" at doses equivalent to those given to US military service personnel.

Outcomes: battery of motor and cognitive-behavioral tests over a 6-mo period postinjections.

Postmortem: Following sacrifice, central nervous system tissues were examined using immunohistochemistry for evidence of inflammation and cell death.

Anthrax vaccine adjuvants

Behavioral Results:

Aluminum: Motor deficits in form of progressive ↓ strength by the wire-mesh hang test (final deficit at 24 wk; about 50%).

Combined aluminum-squalene: Cognitive deficits (signif) in water-maze learning (4.3 error/trial) vs controls (0.2 error/ trial) @ 20 wk.

Postmortem Results:

Aluminum: Apoptotic neurons with signif ↑ activated caspase-3 labeling in lumbar spinal cord (255%) & primary motor cortex (192%) vs controls.

Signif motor neuron loss (35%)

↑ # of astrocytes (350%) in the lumbar spinal cord.

"The findings suggest a possible role for the aluminum adjuvant in some neurological features associated with GWI and possibly an additional role for the combination of adjuvants."

Petrik 2007 Neuromolecular Med 9:83-100: FROM ABSTRACT ONLY (couldn't access in time)

Low dose sarin + stress affects rats

Finding: "low dose" repeat sarin + shaker stress led to delayed behavioral changes in rats and delayed endocrine effects.

Subjects: 65 Male mice

Exposures:

- Shaker stress: horizontal excursion of 2.9cm at 150 cycles/min x 90min/d x 7 days, as 45 2min shaking periods separated by randomized still periods of mean duration 30min (13-44min)
- Sarin: 64ug/kg sq x 3 days or saline control, given on shaker day 4-6 in the shaker animals

Mach 2007 J Applied Toxicol e-pub in advance of publication

Low dose sarin + stress affects rats

Test Times: 1h after 1st dose; 24h after last dose; weekly to 21d p last.

Behavioral outcomes:

1. Acoustic startle response
2. Prepulse inhibition: % inhibition of startle to noise, after give a pre-noise.

White burst noise stimulus with or without prepulse"

- Background 60dB
- "Prepulse" 70dB
- "Pulse" 100 or 120dB
- Prepulse + pulse

Each trial type 10x in 10 blocks in random order; with interval between 9-16sec

Tested in a commercial "Startle Monitor System" Version 4.0

Movements of the body (jerks) transformed into analog signal

Mach 2007 J Applied Toxicol e-pub in advance of publication

Low dose sarin + stress affects rats

Test Times: 1h after 1st dose; 24h after last dose; weekly to 21d p last.

Outcomes, continued:

3. Open field locomotion by infrared photobeams
- Locomotor activity
- Fine movement
- Rearing

4. Catecholamine determinations in homogenized adrenal glands: animals killed 7wk p last exposure. NE, Epi, DA.

Other outcomes

- Blood Cholinesterase inhibition 24h & 3wk after last sarin inj

Mach 2007 J Applied Toxicol e-pub in advance of publication

Low dose sarin + stress affects rats

Test Times: 1h after 1st dose; 24h after last dose; weekly to 21d p last.

Results:

Acoustic startle & PPI: no change (delayed, vs during AChEi w/ PB)

Activity: Sarin ↓ activity, all 3 types, $p < 0.05$. (No interaxn w stress.)

Enzymes: Sarin ↓ enz activity: ChE, AChE, BChE: all $p < 0.001$

~40% ↓ ChE, 40% ↓ BChE; 60% ↓ AChE (by graph)

Stress ↑ enzyme activity

Combination: ↓ enz activity, but less than sarin alone

Catechol content & adrenal weight: 7 wks later, homogenized adrenal

Sarin ↓ catechols: $p<.01$ NE and Epi; $p<.05$ DA

Sarin + stress increase adrenal weight vs other groups

Sarin + stress: Catechols normal

IMach 2007 J Applied Toxicol e-pub in advance of publication

Low dose sarin + stress affects rats

Test Times: 1h after 1st dose; 24h after last dose; weekly to 21d p last.

Recap:

Low dose, repeat sarin causes delayed effects, reduces rat activity; & reduces adrenal catechols

Stress modestly increases enzyme activity

Stress + sarin causes adrenal hyperplasia -- almost doubles adrenal weight, and is associated with reduced catechol activity

Elsewhere: adrenal size and catechol activity not change immediately after sarin exposure (cite their own unpublished data)

"We hypothesize that coexposure could disrupt biogenic amine balance" causing longlasting changes in autonomic fxn. (Don't seem to need coexposure, though)

IMach 2007 J Applied Toxicol e-pub in advance of publication

AChEi Effects on Microtubules

Finding: OP decreases neuron viability – that may be associated with degradation of microtubules

"Subjects": slice cultures of rat hippocampus and bovine tubulin

Exposure: chlorpyrifos oxon 0.1-10 µM x 1-7d

- This exposure reduces AChEi activity by 15-50%

Outcomes: assessed 1, 3, and 7 days after start of exposure

Cytotoxicity: somatic uptake of the marker propidium iodide, a "nonvital" marker; and cell damage by fluorescence microscopy

Alpha tubulin (immunoreactivity – IR) by fluorescence microscopy

Microtubulin associated protein-2 (MAP-2 IR)

Bovine microtubule outcome: Tubulin polymerization

Prendergast 2007. Microtubule associated targets in chlorpyrifos oxon hippocampal toxicity. Neuroscience 146: 330-9

AChEi Effects on microtubules

Result:

MAP: 35-45% ↓ MAP-2 (microtubule associated protein) as early as 1st measurement (1day – 18% reduction, $p < 0.05$) and with lowest concentration 0.1μM, dramatic after 7d, max in area CA1 & CA3 subregions of slice cultures.

Cytotoxicity: Concentration-dependent neuron injury in pyramidal cell layer of area CA1 of hippocampus and to lesser degree area CA3 and dentate cells after 3 days, at all doses, greatest after longest exposure (35% and 21% increase in PI uptake, $p < 0.005$)

Marked inhibition of polymerization of purified tubulin and MAP rich tubulin, especially the latter

No change in alpha tubulin at any time

Prendergast 2007. Microtubule associated targets in chlorpyrifos oxon hippocampal toxicity. Neuroscience 146: 330-9

AChEi Effects on brain microtubules

Conclusion:

μtubule damage was greatest in CA1-area w/ densest neurons, high energy demand

Cytotoxicity followed evidence of μ tubule injury, and *might* be related to it.

Cell death also greatest in CA1, tho ∃ higher cholinergic input to the dentate

The authors express uncertainty as to why this may be

They sugg hypotheses about AChE shown nec 4 neurite outgrowth– and speculate about apoptosis cascades sensitive to cytoskeletal integrity -- but note poor temporal assn of AChE inhibition to and increased PI uptake (24h exposure 0.1uM not cell damage till 7 days)

They note AChEi/ CPO inhibit axon and dendrite growth at conc far below those that inhibit AChE

Comment:

CA1 especially vulnerable to cell death with energy deficits –Robert Sapolsky

Massive die-off of CA1 HC cells in mild hypoxemia coupled with stress

Possibly relevant: μ tubules transport mitochondria within the cell.

Prendergast 2007. Microtubule associated targets in chlorpyrifos oxon hippocampal toxicity. Neuroscience 146: 330-9

AChEi Effects on Learning (and cytotox)

Old Finding: "low dose" (no acute toxicity) repeated chlorpyrifos exposure (x14d) in rats impaired spatial learning; inhibited axonal transport longterm after exposure; and in cell culture led to cell toxicity and death

(Exposure: CFO in peanut oil vehicle, or peanut oil injection)

Terry 2003 J Pharmacology and Exp Therapeutics 305:375. Repeated exposures to subthreshold doses of chlorpyrifos in rats: HC damage, impaired axonal transport and deficits in spatial leaning.

Uranium and Oxidative Stress

Finding: Prooxidant effects of uranium; stress doesn't add much
Ss: Adult male rats
Exposure:
-- Uranyl acetate dihydrate in drinking water, 0,10,20,40mg/kg/d x 34mo
-- Restraint stress 2h/d through study in 4 of 8 groups.
Outcome: Endogenous antioxidant capacity; oxidative damage in several areas of the brain:
Reduced glutathione (GS): oxidized glutathione (GSSG); glutathione reductase (GR); glutathione peroxidase (GPs); superoxide dismutase (SOD); catalase (CAT); thiobarbituric acid reactive substances (TBARS); uranium concentration

Linares 2007. Toxicology 236:82. Pro-oxidant effects in the brain of rats concurrently exposed to uranium and stress.

Uranium and Oxidative Stress

Result: U significantly accumulated in HC, c'bellum, cortex p 3 mo
UAD promoted oxidative stress in these cerebral tissues
TBARS correlated with U content in CX and Cbellum.
In Cbellum GSSG and GSH were pos and neg correlated with U respectively.
Stress "scarcely showed additional adverse effects"

Conclusion: UAD can cause progressive perturbations on physiological brain levels of oxidative stress markers.

Linares 2007. Toxicology 236:82. Pro-oxidant effects in the brain of rats concurrently exposed to uranium and stress.

JP-8 Jet Fuel

Finding: JP-8 associated with GSH depletion some tissues; and promotion of noise induced hearing loss
Background: JP-8 jet fuel = std jet fuel for US and NATO military.
Includes many compounds: toluene, xylene, nonane, undecane, octane, ethylbenzene among many others.
Ss: 98 rats (pigmented male Long Evans)
Exposure 1: JP-8 fuel 1000mg/m3, nose only inhalation x 4hr
Current permissible exposure is 350mg/m3
Exposure 2: noise: half rats immed subjected to an octave band of noise btn 97 dB 4h x 1 day; 97dB x 5 d and 105dB 4h x 5d
Noise alone produces a small auditory impairment
Exposure Groups:

- Neither
- Jet fuel alone
- Noise alone
- Jet fuel f/b noise

Fechter 2007 Toxicological Sciences 98: 510

JP-8 Jet Fuel

Outcomes:
Hair Cell Loss: Histology of cochlea to assess % hair cell loss (cochlea is organized tonotopically)
Oxidative stress: glutathione tissue levels
Hair cell function assessment by drop in amplitude of DPOAE = "distortion product otoacoustic emissions" (distorted tone sound energy generated in cochlea after stim with two simultaneous "primary" tones)
Hearing: CAP = compound action potentials to pure tones at ½ octave steps

Result: Jet fuel increased cochlea susceptibility to noise
GSH depleted esp in liver (antioxidant depletion)
DPOAE test especially sensitive (hair cell fxn)
Greater outer hair cell death than with noise alone.

Context: previously shown, but with longer exposure times.
Humans: toluene exposed workers had reduced auditory brainstem response vs nonexposed, Abbatae 1993; Vrea 1996 (2 yrs exposure); Morata 1997.
Rats: benzene exposed outer cell damage/ ototoxicity.
Fechter 2007 Toxicological Sciences 98: 510

Jet Fuel Context: mito mechanisms

Mitochondrial mechanisms are shown to play a role in noise induced and toxin induced hearing loss. And mitochondrial mechs in protection…

Mitochondrial dysfunction in hearing loss.
Fischel-Ghodsian 2004. Mitochondrion;4:675.

Mechanisms of noise-induced hearing loss potentiation by hypoxia.
Chen 2005. Hear Res 200:1.

Mitochondrial DNA deletion is a predisposing cause for sensorineural hearing loss. Ueda 1998. Laryngoscope108:580.

A BAD link to mitochondrial cell death in the cochlea of mice with noise-induced hearing loss. Vicente-Torres 2006. J Neurosci Res 831:564.

Audiovestibular findings in patients with mitochondrial A1555G mutation.
Noguchi 2004. Laryngoscope 114:344.

Mitochondrial role in hair cell survival after injury.
Hyde 1995. Otolaryngol Head Neck Surg113:530.

Audiologic findings in a family with mitochondrial disorder.
Elverland 1991. Am J Otol 12:459.

Pharmacological rescue of noise induced hearing loss using N-acetylcysteine and acetyl-L-carnitine. Coleman 2007. Hear Res;226:104.

Gene-Environment

ALS vs glutathione genes

Genetic factors associated with environmental susceptibility to ALS

Ss: 186 sporadic ALS cases, 186 controls

Glutathione synthetase haplotype interacted with both metals and solvents/ chemicals to increase risk of ALS (glutathione involved in detox of both)

(Glutathione is important for protection against free radical injury/ oxidative stress; and elsewhere shown e.g. to biotransform methyl parathion, …)

Morahan 2007 Genetic susceptibility to environmental toxicants in ALS
Am J Med Genetics Part B: Neuropsychiatric Genetics only 14May 2007

Recall: ALS increased in GWV

1. Armon . Excess incidence of ALS in young Gulf War veterans. Neurology 2004;63(10):1986-7; author reply -7.
2. Coffman. Estimating the occurrence of amyotrophic lateral sclerosis among Gulf War (1990-1991) veterans using capture-recapture methods. Neuroepidemiology 2005;24(3):141-50.
3. Haley. Excess incidence of ALS in young Gulf War veterans. Neurology 2003;61(6):750-6.
4. Horner. Prospective study of military service and mortality from ALS. Neurology 2005;65(1):180-1; author reply -1.
5. Horner. Occurrence of amyotrophic lateral sclerosis among Gulf War veterans. Neurology 2003;61(6):742-9.
6. Weisskopf. Prospective study of military service and mortality from ALS. Neurology 2005;64(1):32-7.

ALS linked to metal detoxifying genes

Genetic factors associated with environmental susceptibility to ALS (186 sporadic ALS cases, 186 controls)

Metalothionein gene and metal transcription factor gene differed significantly.

Morahan 2007 Genetic susceptibility to environmental toxicants in ALS. Am J Med Genetics Part B: Neuropsychiatric Genetics only 14May 2007

ALS linked to PON genes

Case control: 143sALS patients and 143 matched controls

PON1 promoter allele reduces PON expression

- **Strongly associated with sALS**

Haplotypes associated with increased expression

- **Associated with controls.**

Morahan 2007 Gene environment study of PON1 gene and pesticides in ALS. NeuroToxicology 28: 532-40.

Prior reports link other PON polymorphisms to ALS:

1. **Saeed M, Siddique N, Hung WY, et al. Paraoxonase cluster polymorphisms are associated with sporadic ALS. Neurology 2006;67(5):771-6.**
2. **Slowik A, Tomik B, Wolkow PP, et al. Paraoxonase gene polymorphisms and sporadic ALS. Neurology 2006;67(5):766-70.**

And: ALS Linked to Chem Exposures

Ss: 179 sALS cases and 179 age-, ethnicity- and sex-matched controls in Australia

sALS linked to

- Solvent/chemical exposure: OR 1.9 (1.3-2.9)
- Overall herbicide/pesticide exposure: OR 1.57 (1.03-2.4)
- Industrial herbicide/pesticide exposure: OR 5.58 (2.1-15)

Exposure to herbicides/pesticides showed a dose-response effect

Morahan 2006 Amyotrophic lateral sclerosis and exposure to environmental toxins: an Australian case-control study. Neuroepidemiology 27: 130

MCS and Detoxifying/ Antioxidant Genes

Ss: 521 unrelated Ss agreed to participate, of 800 Ss who answered a Q-aire of 10 items re: severity of chemical sensitivity

Genetics: Analyze variants of genes involved in detoxification of ubiquitous chemical substances for which variants have been shown to influence metabolism of ubiquitous chemicals.

N-acetyltransferases: NAT2 gene

Glutathione S-transferase: GSTM1, GSTT1, GSTP1 genes

Result: With vs w/o self-reported chemical sensitivity differ signif in distribution of gene variants for NAT2, GSTM1, GSTT1:

- NAT2 slow acetylator: OR 1.8 (1.3-2.6)
- Homozygous deletion of GSTM1: OR 2.1, 1.5-3.0, p = 0.0001
- Homozygous deletion of GSTT1: OR 2.8, 1.7-4.8, p = 0.0001

Schnakenberg 2007. *Environmental Health* 6:6. A cross-sectional study of self-reported chemical-related sensitivity is associated with gene variants of drug-metabolizing enzymes. (Germany)

MCS and Genes

GST enzymes (GSTM1, GSTT1, GSTP1) protect cells and organs from oxidative stress by conjugation of glutathione: detoxify a large range of compounds generated by reactive oxygen species induced damage to intracellular molecules.

Deletion of GSTM1 or GSTT1 lead to loss of protection against oxidative stress.

N-acetylation is an important mechanisms for inactivating arylamines.

Schnakenberg 2007. *Environmental Health* 6:6. A cross-sectional study of self-reported chemical-related sensitivity is associated with gene variants of drug-metabolizing enzymes. (Germany)

MCS-Gene - Context

Complements prior finding of altered chemical detoxification in MCS[1]

Complements prior study presented here

Relevance to GWV:

GWV – esp exposed to pesticides – have high rates of MCS [2]

1997-8 cross-sectional survey of 3 UK military cohorts

MCS in Gulf, Bosnia, Era cohorts was 1.3%, 0.3%, and 0.2%

GWV (n=3,531); Bosnia (n=2,050); Gulf Era nondeployed (n=2,614)

GWV: MCS associated pesticide exposure

adjusted OR: 12.3 95% CI: 5.1- 30.0

[1] McKeown-Eyssen 2004, Case-control study of genotypes in multiple chemical sensitivity: CYP2D6, NAT1, NAT2, PON1, PON2 and MTHFR. Int J Epidemiol 33(5):971-8.

[2] Reid 2001. Multiple chemical sensitivity and chronic fatigue syndrome in British Gulf War veterans. Am J Epidemiol;153(6):604-9.

Chemical Sensitivity and Genes

Cases: Hamburg, Germany 8800 randomly selected volunteers from a general practice completed the questionnaire.

Rates severity of sensitivity to each of 10 common items from 1=not at all, 2 = moderate, 3 = disabling.

Possible score: 10-30.

Score >20 defined as sensitive. Score <= 20 defined as controls.

Items: Diesel or gas engine. Tobacco smoke. Insecticide. Gasoline. Paint or paint thinner. Cleaning products such as disinfectants, bleach, bathroom cleaners or floor cleaners. Certain perfumes, air fresheners or other fragrances. Fresh tar or asphalt. Nail polish, nail polish remover, or hair spray. New furnishing, carpeting, plastic shower curtain or interior of new car.

Schnakenberg 2007. *Environmental Health* 6:6. A cross-sectional study of self-reported chemical-related sensitivity is associated with gene variants of drug-metabolizing enzymes. (Germany)

Chemical Sensitivity and Genes

Genotyping:

DNA extraction: DNA isolated from EDTA blood (kit).

NAT2 gene amplified: sgl nt pmorphisms (SNPs) nt481, nt590, nt857 analyzed by RFLP/PCR or real-time PCR.

Use NAT2 nomenclature of the Arylamine N-Acetyltransferase Nomenclature Committee.

Genetic variants analyzed lead to a 4-allel model of the NAT2 gene which can predict the acetylator phenotype with accuracy >95% for slow and rapid acetylation.

Multiplex PCR to detect homozygous deletions of GSTM1 and GSTT1.

Schnakenberg 2007. *Environmental Health* 6:6. A cross-sectional study of self-reported chemical-related sensitivity is associated with gene variants of drug-metabolizing enzymes. (Germany)

Treatment

D-ribose treatment for CFS & FM

Finding: D-ribose reportedly tolerated and helpful in 5 tested domains – but in uncontrolled open label study

Rationale: D-ribose has been shown to increase cell energy synthesis in heart and skeletal muscle

Design: Uncontrolled open-label pilot study: intervention and survey mailed to subjects once enrolled.

Ss: 41 pts with physician dx of FM (by ACR criteria) and/or CFS (by CDC criteria)

Exclusion: known "severe" med or nutrient sensitivity; prior D-ribose

Intervention: D-ribose at 5gm tid till 280g used up (19 days); then send survey

Outcome: Visual analog scales of energy, sleep, mental clarity, pain intensity and well being. Patient global assessment.

Result: 66% of subjects experienced "significant improvement" on D-ribose with average increase in energy on VAS of 45%; and average improvement in well-being

Teitelbaum 2006. J Alternative & Complementary Med 12: 857-62

D-ribose treatment for CFS & FM

Scales from 1-10

Outcome	N	Pre	Post	Difference	p-value
Energy	36	3.8 (1.1)	5.5 (1.5)	1.7 (1.1,2,2)	<0.0001
Sleep	36	4.8	6.0	1.2	0.0001
Mental clarity	36	4.9	5.7	0.8	0.003
Pain	36	4.9	5.6	0.7	0.026
Well-being	36	4.3	5.6	1.3	<0.0001

Global subjective

Much better:	14%
Somewhat better:	49%
Somewhat better/no change	2.9%
No change:	26%
No change/somwhat worse	3%
Somewhat worse	6%
Much worse	0%

Teitelbaum 2006. J Alternative & Complementary Med 12: 857-62

Effect of Treatment in FM

Ss:

30 females with primary FM by ACR criteria (Am College Rheumatology), age 37

16 "age-matched" healthy controls mean age 33

Design: uncontrolled, unblinded pre-post

Intervention: 8 wk

- amitripyline 20/d or
- sertraline 100/d

Outcomes: HAM-A; HAM-D; FIQ =FM impact Q-aire; TMS = summed 21 point pain pressure thresholds

Ozgocmen 2006 Rheumatol Int 26: 598

Effects of Treatment in FM

	Amitriptyline N=12			Sertraline N=18		
Test	Pre	Post	P	Pre	Post	p
FIQ-pain	70	38	.008	57	35	.02
FIQ-fatigue	65	46	.12	55	27	.006
TMS	87	95	.24	88	91	.08
Ham-D	17	9	.005	17	9	.001
HAM-A	18	11	.005	21	10	.001

SOD, XO, TBAES, NO, ADA: no effect

TMS is sum of pain pressure thresholds at 21 sites

FIQ = fibromyalgia impact questionnaire

Ozgocmen 2006 Rheumatol Int 26: 598