

High Prevalence of Mycoplasmal Infections in Symptomatic (Chronic Fatigue Syndrome) Family Members of Mycoplasma-Positive Gulf War Illness Patients

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SUMMARY. Immediate family members of veterans diagnosed with Gulf War Illnesses often complain of fatiguing illnesses, and upon analysis they report similar signs and symptoms as their veteran family members. Since a relatively common finding in Gulf War Illness patients is a bacterial infection due to *Mycoplasma* species, we examined military families (149 patients: 42 veterans, 40 spouses, 32 other relatives and 35 children with at least one family complaint of illness) selected from a group of 110 veterans with Gulf War Illness who tested positive (~41%) for at least one of four *Mycoplasma* species: *M. fermentans*, *M. hominis*, *M. pneumoniae* or *M. genitalium*. Consistent with previous results, over 80% of Gulf War Illness patients who were positive for blood mycoplasmal infections had only one *Mycoplasma* species, in particular *M. fermentans* (Odds ratio = 17.9, 95% CL 4.1-78.1, $P < 0.001$). In healthy control subjects the incidence of mycoplasmal infection was ~8.5% and none were found to have multiple mycoplasmal species (Multiple species Odds ratio > 25 , $\text{Chi}^2 = 8.1$, $P < 0.001$). In 107 family members of mycoplasma-positive Gulf War Illness patients there were 57 patients (53%) that had essentially the same signs and symptoms as the veterans and were diagnosed with Chronic Fatigue Syndrome (CFS) and/or Fibromyalgia Syndrome. Most of these CFS patients also had mycoplasmal infections compared to the few non-symptomatic family members (Odds ratio = 16.9, 95% CL 6.0-47.6, $P < 0.001$), and the most common species found was *M. fermentans* (Odds ratio = 40.3, 95% CL 8.7-186.4, $P < 0.001$). In contrast, in the few non-symptomatic family members that tested mycoplasma-positive, the *Mycoplasma* spp. were often different from the species found in the Gulf War Illness patients. The results suggest that a subset of Gulf War Illness patients have mycoplasmal infections, possibly obtained as contaminants from multiple vaccines given during deployment, and these infections can be transmitted to immediate family members who subsequently display similar signs and symptoms and are diagnosed with CFS and/or Fibromyalgia Syndrome. © 2003 by The Haworth Press, Inc.

KEYWORDS. Gulf War Syndrome, bacterial infections, Chronic Fatigue Syndrome, Myalgic Encephalomyelitis, Fibromyalgia Syndrome, infectious disease

INTRODUCTION

A sizable fraction (estimated at over 16%) of the veterans of the 1991 Persian Gulf War from the United States, Great Britain, Canada, Australia and other nations slowly developed, usually with a lag of 6-18 months or more after the conflict, a variety of complex chronic signs and symptoms. These were characterized by disabling fatigue, intermittent low-grade fevers, night sweats, arthralgias, myalgias, short-term memory loss, confusion, irritability, depression, headaches, skin rashes, intermittent diarrhea, respiratory complaints, photophobia and other signs and symptoms (1-4). These illnesses have been called Gulf War Syndrome or Gulf War Illnesses (Gulf War Illness) (5). Since routine laboratory test results on Gulf War Illness patients are usually not consistent with a single, specific diagnosis, veterans often did not receive a diagnosis for their condition (illness of unknown origin), or they received a diagnosis of somatoform disorder (1). The signs and symptoms of Gulf War Illness do not easily fit into ICD-10 diagnostic categories, but they loosely fall into

the category of fatiguing illnesses (2-4), and the signs and symptoms of Gulf War Illness resemble Chronic Fatigue Syndrome or Myalgic Encephalomyelitis (CSF/ME) (2, 6-9).

We (6-9) found that most of the signs and symptoms in a large subset of Gulf War Illness patients could be explained by chronic pathogenic bacterial infections. Evidence for infectious agents has been found in Gulf War Illness patients' urine (11) and blood (6-10). In studies on hundreds of U.S. and British veterans with Gulf War Illness, approximately 40-50% of Gulf War Illness patients show evidence of mycoplasmal infections compared to 6-9% in non-deployed, healthy subjects (6-10). This has also been confirmed in a large study of 1,600 veterans at over 30 veterans' and defense medical centers (VA Cooperative Clinical Study Program #475) in the United States (12).

Mycoplasmas are small intracellular bacteria lacking cell walls and extensive intracellular organelles. Historically, mycoplasmal infections were thought to produce relatively mild diseases limited to particular tissues or organs, such as urinary tract or respiratory infections. However, the *Mycoplasma* species detected in Gulf War Illness patients with molecular techniques are highly virulent, colonize a wide variety of organs and tissues, and are difficult to treat (13-15). The mycoplasma most commonly detected in Gulf War Illness, *Mycoplasma fermentans*, was found in the overwhelming majority of those Gulf War Illness patients positive for any mycoplasma (7-10). *M. fermentans* is an intracellular mycoplasma, and it is unlikely that this type of infection will result in a strong antibody response. This may explain the lack of a high prevalence of serologic evidence for these types of intracellular infections in Gulf War Illness patients compared to nondeployed controls (15).

When examining Gulf War Illness patients for illness, we noticed that immediate family members often had similar complaints (9, 16). Previously a U.S. Congressional committee had questioned approximately 1,200 families where one member was a veteran with Gulf War Illness and found that 77% of spouses and 65% of children born after the Gulf War had similar health complaints as the veteran (17). This suggested that in these families Gulf War Illness was being transmitted from veterans with Gulf War Illness to immediate family members. The most likely type of transmitted agent would be the chronic infections found in Gulf War veterans, such as mycoplasmal infections (6-10). Therefore, we examined the family members of Gulf War veterans who were positive for mycoplasmal infections to see if their signs and symptoms and chronic infections were similar. We found that most family members of Gulf War Illness patients that were symptomatic with signs and symptoms of CFS/ME also had mycoplasmal infections.

MATERIALS AND METHODS

Patients

Gulf War veterans with Gulf War Illness and a positive test for mycoplasmal infection and their immediate family members (149 patients: 42 veterans, 40 spouses, 32 other relatives and 35 children) were enrolled (Table 1). Seventy age-matched healthy volunteers were recruited and used as control subjects.

All subjects underwent a medical history and routine laboratory tests. If necessary, medical records were also reviewed to determine if patients suffered from organic or psychiatric illnesses that could explain their symptoms (3). When positive results were found in any of the evaluations of civilians that met the Fukuda et al. (18) exclusionary criteria for CFS, the patients were not included in the study. All subjects completed an illness survey questionnaire (Illness Survey Form, www.immed.org/signsympt.htm), which included demographic information, known environmental exposures, dates of illness onset, health status before and immediately after the Gulf War and current health status. Additionally, all subjects were questioned about medication use during the three months prior to the study, and they had to be free of antibiotic treatment for three months prior to blood collection. Controls had to be free of disease for at least three months prior to data collection.

Blood Collection

Blood was collected in EDTA-containing tubes and immediately brought to ice bath temperature as described previously (19, 20). Samples were shipped with wet ice by air courier to the Institute for Molecular Medicine and International Molecular Diagnostics, Inc. for analysis. All blood samples were blinded. Whole blood (50 μ l) was used for preparation of DNA using Chelex (Biorad, Hercules, USA) as follows. Blood cells were lysed with nano-pure water (1.3 ml) at room temperature for 30 min. After centrifugation at 13 000 \times g for 2 min, the supernatants were discarded. Chelex solution (200 μ l) was added, and the samples were incubated at 56°C and at 100°C for 15 minutes each. Aliquots from the centrifuged samples were used immediately for PCR or stored at -70°C until use. Multiple Mycoplasma tests were performed on all patients (19, 20).

Amplification of Gene Sequences

Amplification of the target gene sequences (Table in Nasralla et al., ref. 19) containing 0.1% Triton X-100, 200 μ m each of dATP, dTTP, dGTP, dCTP, 100 pmol of each primer, and 0.5 – 1 μ g of chromosomal DNA. Purified mycoplasmal DNA (0.1 – 1 ng of DNA) was used as a positive control for amplification. The amplification was carried out for 40 cycles with denaturing at 94°C and annealing at 60°C (genus-specific primers and *M. penetrans*) or 55°C (*M.*

pneumoniae, *M. hominis*, *M. fermentans*, *M. genitalium*). Extension temperature was 72°C in all cases. Finally, product extension was performed at 72°C for 10 min. Negative and positive controls were present in each experimental run (19, 20).

Southern Blot Confirmation

The amplified samples were run on a 1% agarose gel containing 5 µl/100 ml of ethidium bromide in TAE buffer (0.04 M Tris-Acetate, 0.001 M EDTA, pH 8.0). After denaturing and neutralization, Southern blotting was performed as follows. The PCR product was transferred to a Nytran membrane. After transfer, UV cross-linking was performed. Membranes were prehybridized with hybridization buffer consisting of 1x Denhardt's solution and 1 mg/ml salmon sperm DNA as blocking reagent. Membranes were then hybridized with ³²P-labeled internal probe (10⁷ cpm per bag). After hybridization and washing to remove unbound probe, the membranes were exposed to autoradiography film for 1- 2 days at -70°C (19, 20).

Statistics

Subjects' demographic characteristics were assessed using descriptive statistics and students' t-tests (independent samples test, t-test for equality of means, 2-tailed). The 95% confidence interval was chosen. Pearson Chi-Square test and logistic regression odds ratio analysis (Logit) were performed to compare prevalence data between patients and control subjects. Additionally, sex differences were investigated using Pearson Chi-Square test. Illness survey data were statistically analyzed using Spearman Rank correlation and Mann-Whitney tests.

RESULTS

As found in previous studies (9,16), veterans of the Gulf War with chronic illnesses (Gulf War Illness) exhibited multiple signs and symptoms (Figures 1A, B). Upon examination, the signs and symptoms of Gulf War Illness were indistinguishable from civilian patients diagnosed with CFS/ME (Figures 1A, 1B c.f. ref. 9). Examination of the signs and symptoms of approximately equal numbers of male and female Gulf War Illness patients indicated that there were no significant differences between their general signs and symptoms (data not shown). When family members who were symptomatic with chronic signs and symptoms and diagnosed with CFS/ME were examined (Table 1, age range 4-49 years), they also appeared to have signs and symptoms indistinguishable from veterans with Gulf War Illness (Figures 1A, 1B). When CFS-symptomatic Gulf War Illness family members were grouped into male/female or adults/children, there were no significant differences between the signs and symptoms found in these groups. The only signs/symptoms differences found were in symptomatic family members who did not test positive for mycoplasmal infections (Figures 1A, 1B).

Similar to previous studies (6-10) 45 of 110 Gulf War Illness patients or ~41% had mycoplasmal infections (Table 2), and almost all of these (37 out of 45 or ~82%) were single infections (one species of mycoplasma) (Table 3). *M. fermentans* was found in ~85% of these single infection cases. When the few multiple infection cases were examined, most were found to have combinations of *M. fermentans* plus either *M. pneumoniae* or *M. genitalium* (Table 3). In contrast, in healthy control subjects only 6 of 70 subjects (8.5%) were positive for mycoplasmal infections, and all of these were single species infections of various types (Table 4). Comparing Gulf War Illness patients and non-symptomatic control subjects, there was a nine-fold increase in the incidence of mycoplasmal infections and an ~18-fold increase in the incidence of *M. fermentans* infections. These differences were highly significant ($P<0.001$). However, significant differences in infection incidence or species of mycoplasmal infection between male and female Gulf War Illness patients or control subjects were not seen in these patient groups.

In family members of Gulf War veterans with Gulf War Illness there was evidence of transmission of the illness. These families were not randomly chosen; they were families in which one or more veteran members were found to be positive for a mycoplasmal infection and showed symptoms of Gulf War Illness and one or more family members reported illnesses. We found that 57 out of 107 (53.2%) of these members from families showed symptoms of CFS/ME. Among CFS-symptomatic family members, most (40 out of 57 or 70.2%) had mycoplasmal infections compared to the few non-symptomatic family members who had similar mycoplasmal infections (6 out of 50 or 12%) (Table 4), which is a ~17-fold increase. When the incidence of mycoplasmal infection was compared within families, the CFS-symptomatic family members were found to be ~40-fold more likely to have *M. fermentans* infections compared to non-symptomatic family members ($P<0.001$). Although some non-symptomatic family members did have mycoplasmal infections (6 out of 50 or 12%), this was not significantly different from the incidence of mycoplasmal infections in healthy control subjects (6 out of 70 or 8.5%). The mycoplasma infection types were also similar between Gulf War Illness patients and their CFS-symptomatic family members. In 45 mycoplasma-positive CFS-symptomatic family members, most (31 out of 40 or 77.5%) had single species infections, similar to the mycoplasma-positive Gulf War veterans (37 out of 45 or 82%), a 10.5-fold increase. Most of the species found in mycoplasma-positive Gulf War Illness patients as well as in mycoplasma-

positive family members with CFS were *M. fermentans* (Table 4). We did not find differences in the incidence of infection or type of infections between males and females, children versus adults or spouses versus other family members (data not shown). However, similar to previous reports, the time of onset of CFS illness after the Gulf War tended to be shorter in spouses than other family members, but these differences did not achieve significance (data not shown). However, similar to previous reports, the time of onset of CFS illness after the Gulf War tended to be shorter in spouses than other family members, but these differences did not achieve statistical significance.

DISCUSSION

There is growing awareness that Gulf War Illness is not a unique, new syndrome caused by exposures that occurred during the Gulf War (5,9,21-24). Gulf War Illness is characterized by a diverse collection of overlapping, persistent signs and symptoms from which several distinct subclasses of illness have been identified (3,4,21-24). Chronic infections are suspected as an important source of morbidity in a rather large subset of these patients (6-10,22-26). The most common infections found were intracellular mycoplasmal infections (6-10), but a few other chronic infections have been documented in Gulf War Illness patients (25-27). For example, parasitic infections, such as Malaria, Leishmaniasis and Schistosomiasis, have been found in some Gulf War veterans. These infections cause characteristic signs and symptoms and diagnostic tests are available (27). However, the prevalence of Leishmaniasis has been estimated at less than 100 cases in Gulf War veterans (28). Chronic infections may also cause other problems in Gulf War Illness patients. Activation of the coagulation system seen in Gulf War Illness patients could be related to chronic infections that cause vasculitis and coagulation disturbances (29). In addition, approximately one-half of Gulf War Illness patients were found to have fragile chromosomes that are more easily degraded by cellular nucleases, resulting in release of characteristic nucleotide fragments (30). This might be due to the action of intracellular *Mycoplasma* that are known to release chromosome-damaging chemicals (31).

Similar to previous results (6-10), mycoplasma infections were found in ~41% of Gulf War Illness patients and an odds ratio of 9.0. This has now been confirmed in a U. S. Department of Veterans' Affairs study of 1,600 veterans (28). Although mycoplasmal infections were previously thought to produce relatively mild diseases limited to particular tissues or organs, such as urinary tract or respiratory system (13,14,28), the intracellular *Mycoplasma* detected in Gulf War Illness patients with molecular techniques, primarily *Mycoplasma fermentans*, are highly virulent, colonize a wide variety of organs and tissues, and are difficult to treat (13,14). Such intracellular mycoplasmal infections have been successfully treated with long-term antibiotics, such as doxycycline, along with immune and nutritional support (6-9,16).

Although the results presented here document that the chronic infections found in Gulf War veterans with Gulf War Illness can be found in symptomatic family members, we cannot extrapolate our results to the entire Gulf War Illness patient population or their family members. First, our patient sample was not randomly selected. The presence of a positive mycoplasma test result on a veteran with Gulf War Illness who reported illness in his/her immediate family formed the criteria for inclusion in the study. Although CFS illnesses in immediate family members were commonly seen in our study, which examined families of mycoplasma-positive Gulf War Illness patients, these illnesses are expected to be more difficult to find in the general Gulf War Illness population where chemical, radiological and environmental exposures probably account for the majority of cases. Non-biological exposures should not be transmitted to family members, so studies on the entire Gulf War Illness population may not yield significant results on transmission of illnesses to family members. Second, Gulf War Illness patients and their family members were recruited from veterans groups, word of mouth, physician referrals and the Institute for Molecular Medicine website (www.immed.org); they were not recruited from specific military units. Therefore, information on presumed exposures of veterans with respect to unit locations cannot be determined. Testing was performed using coded samples. After testing, all patients and control subjects completed patient Illness Survey Forms. Although some of these patients were examined by physicians at our associated clinics, most were seen by their own private physicians. Fourth, the validity of PCR techniques for *Mycoplasma* species detection has been questioned. In our studies, however, the sensitivity and specificity of the PCR method for *Mycoplasma* species detection were determined by examining serial dilutions of purified DNA from *M. fermentans*, *M. pneumoniae*, *M. hominis* and *M. genitalium*. The primers produced the expected amplification product size in all test species, which was confirmed by hybridization using the appropriate ³²P-labeled internal probe. Amounts as low as a few fg of purified DNA were detectable for all species with the specific internal probes. There was no cross-reactivity between the internal probes of one species and the PCR product from another species. In other experiments *Mycoplasma* species were added to whole blood at various concentrations. Specific PCR products down to 10 ccu/ml of blood could be detected (20).

Symptomatic family members of Gulf War Illness patients were diagnosed with CSF/ME or a related fatiguing illness, Fibromyalgia Syndrome (FMS). In other studies, CFS and/or FMS patients were examined for systemic mycoplasmal infections, and about 50-60% of these patients were positive for any species of mycoplasma (9,19,20,32-34), indicating another link between mycoplasma-positive Gulf War Illness patients and CFS-symptomatic family members. In contrast to mycoplasma-positive Gulf War Illness patients and their mycoplasma-positive family members with CFS, several species of mycoplasmas in addition to *M. fermentans* were found in CSF/ME and FMS from non-military families (19,34). This

further supports the hypothesis that mycoplasmal infections were transmitted from Gulf War Illness patients to immediate family members who then presented with CFS/ME and for the most part had the same species of mycoplasmal infection.

The source of the mycoplasmal infections in Gulf War Illness patients remains undetermined (28,35). A possible source for Gulf War Illness patients is the multiple vaccines that were administered during the time of deployment to the Persian Gulf. A strong association has been found between Gulf War Illness and the multiple vaccines that were administered (36-38). For example, Steele (4) found a three-fold increased incidence of Gulf War Illness in non-deployed veterans who had been vaccinated in preparation for deployment, compared to non-deployed, non-vaccinated veterans, and Mahan et al. (38) found a two-times higher incidence of Gulf War Illness signs and symptoms in veterans who recalled receiving anthrax vaccinations versus those who thought they had not. Although the mycoplasmal infections found in Gulf War Illness patients could have come from several sources, including offensive Biological Warfare attacks (35), we consider the most likely sources of the mycoplasmal infections in Gulf War Illness patients were the multiple vaccines administered during deployment. Indeed, the signs and symptoms that have developed in Armed Forces personnel who recently received the anthrax vaccine are similar to those found in Gulf War Illness patients. On some military bases this has resulted in chronic illnesses in as many as 7-10% of personnel receiving the vaccine (12,39). Although the chronic signs and symptoms associated with anthrax vaccination are similar to those found in Gulf War Illness patients, it is unlikely that all of the chronic illnesses reported by Gulf War veterans were caused by vaccines (39). Undetectable microorganism contaminants in vaccines could have resulted in illness, and this may have been more likely in individuals with compromised immune systems caused by chemical and other exposures (28). Contamination with mycoplasmas has been found in commercial vaccines (40). Thus the vaccines used in the Gulf War should be considered as a possible source of the chronic infections found in mycoplasma-positive Gulf War Illness patients and by airborne transmission in their mycoplasma-positive, CFS-symptomatic family members.

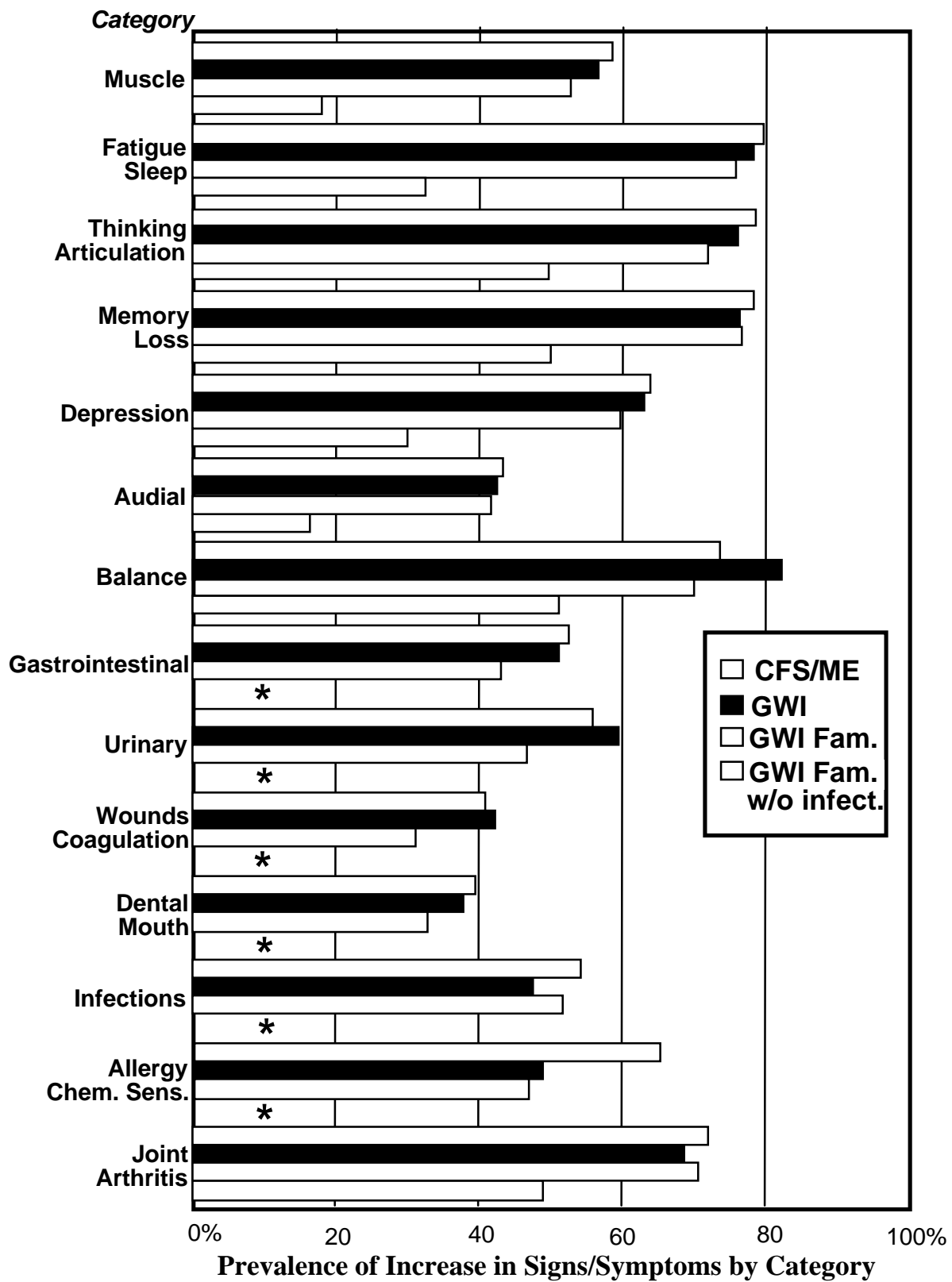
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Figure Legends

Figures 1A and 1B (same legend). Incidence of increase in severity of signs and symptoms in Gulf War Illness, Chronic Fatigue Syndrome, Gulf War Illness family members with CFS and Gulf War Illness family members without evidence of infection. Severity of illness was scored using 117 signs and symptoms on a 10-point scale (0, none; 10 extreme) prior to and after the onset of illness. Scores were placed into 29 categories containing 3-9 signs/symptoms and were recorded as the sum of differences between values before and after onset of illness divided by the number of questions in the category. Changes in score values of 2 or more points were considered relevant. Patient groups were CFS/FMS, Gulf War Illness, CFS-symptomatic Gulf War Illness family members and CFS-symptomatic Gulf War Illness family members that did not show evidence of chronic infection. Asterisk (*) indicates score = 0.



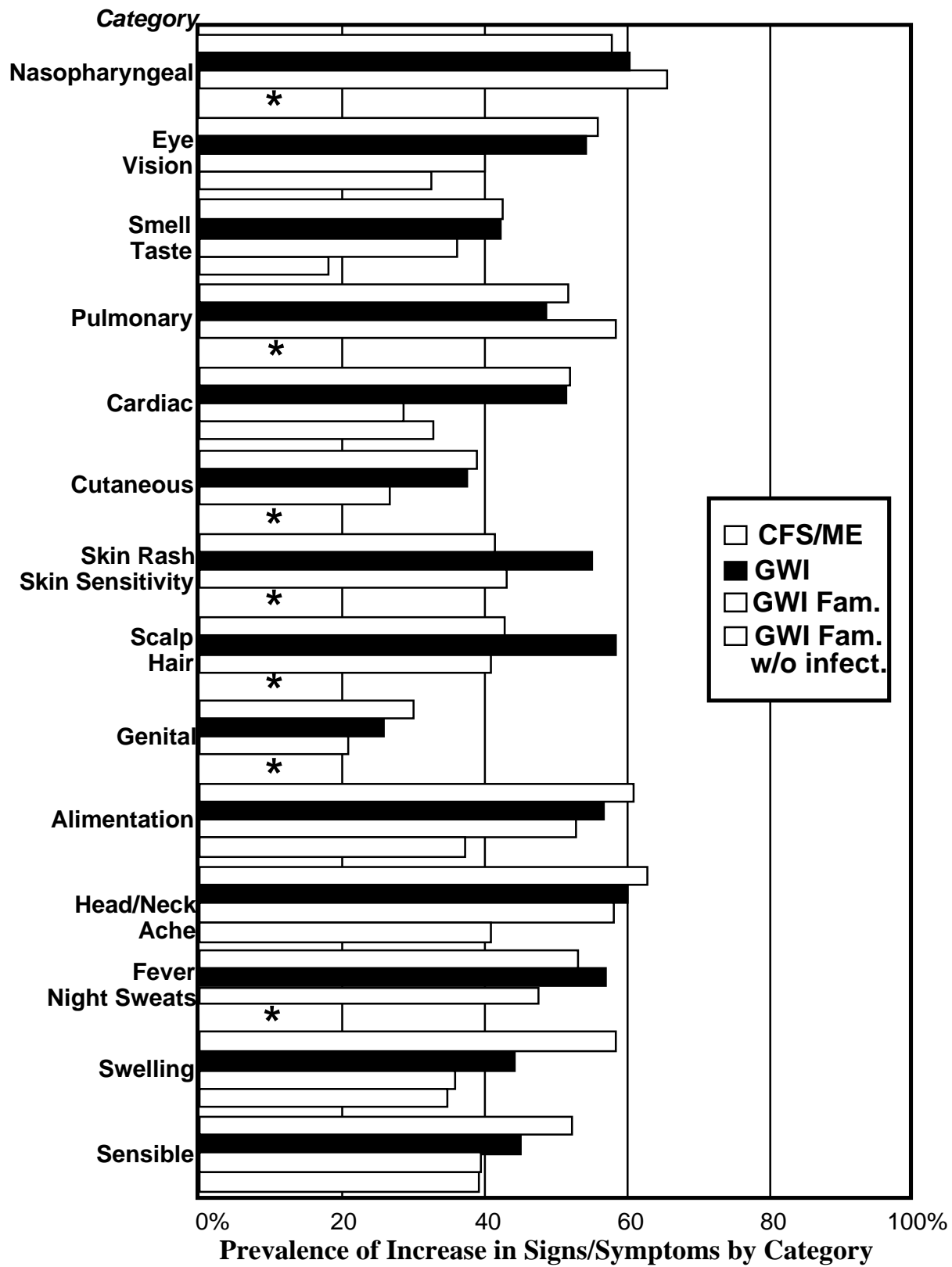


Table 1. Demographic data of the patient sample for the Gulf War Illness family study.

	n	Mean age (SD)	Range	Males (%)	Females (%)
All Patients	149	26.4 (10.8)	4-49	72 (48)	77 (51)
All Controls	70	28.6 (9.9)	8-54	31 (44)	39 (55)
Female patients	77	25.6 (9.8)	5-47	0 (0.0)	77 (100)
Male patients	72	26.9 (10.2)	4-49	72 (100)	0 (0.0)
Male veterans	22	29.5 (5.6)	24-39	22 (100)	0 (0.0)
Female veterans	20	28.1 (6.0)	25-35	0 (0.0)	20 (100)

Table 2. Comparison of *Mycoplasma* species infections between patients and healthy volunteers

	n	Free of mycoplasma (%)	Mycoplasma infected (%)	Sign (2-sided)
All Gulf War Illness patients	110	65 (59)	45 (41)	P<0.001*
Gulf War Illness patients (family study)	42	0 (0.0)	42 (100)	-
CFS patients (family members)	57	17 (29.8)	40 (70.2)	P<0.01†
Healthy family members	50	44 (88)	6 (12)	NS*
Healthy controls	70	64 (91.5)	6 (8.5)	-

*compared to healthy control subjects

†compared to healthy family members

Table 3. Prevalence of *Mycoplasma* species infections among 110 Gulf War Illness patients compared to 70 healthy control subjects.

Subgroup or Mycoplasma species	Gulf War Illness n (%)	Healthy controls n (%)	OR, 95% CL, P or Chi²
Positive	45 (41)	5 (7)	9.0 , 3.3-24.3, <i>P</i> <0.001
Single infection	37 (33.6)	5 (7)	6.6 , 2.4-17.9, <i>P</i> <0.001
Multiple infection	8 (7.2)	0	Chi ² = 8.1, <i>P</i> <0.004
<i>M. fermentans</i>	38 (34)	2 (2.8)	17.9 , 4.1-78.1, <i>P</i> <0.001
<i>M. pneumoniae</i>	6 (5.4)	2 (2.8)	1.9 , 0.4-10.1, <i>P</i> = 0.39
<i>M. genitalium</i>	7 (6.3)	0	Chi ² = 7.1, <i>P</i> <0.008
<i>M. hominis</i>	2 (1.8)	1 (1.4)	1.3 , 0.1-14.5, <i>P</i> = 0.89
<i>M. penetrans</i>	1 (0.9)	0	Chi ² = 0.6, <i>P</i> = 0.42
<i>M. fermentans</i> + <i>M. pneumoniae</i>	4 (3.6)	0	Chi ² = 3.5, <i>P</i> <0.05
<i>M. fermentans</i> + <i>M. genitalium</i>	3 (2.7)	0	Chi ² = 2.9, <i>P</i> <0.08
<i>M. fermentans</i> + <i>M. hominis</i>	1 (0.9)	0	Chi ² = 0.6, <i>P</i> = 0.42
<i>M. pneumoniae</i> + <i>M. hominis</i>	0	0	-

Table 5. Prevalence and Odds Ratios of *Mycoplasma* species infections among 107 non-symptomatic and CFS symptomatic spouses, children and other Gulf War Illness family members.

Subgroup or <i>Mycoplasma</i> species	CFS N (%) n=57	non-CFS N (%) n=50	OR, 95% CL, P or Chi ²
Positive	40 (70.2)	6 (12)	16.9 , 6.0-47.6, <i>P</i> <0.001
Single infection	31 (54.4)	5 (10)	10.5 , 3.6-30.7, <i>P</i> <0.001
Multiple infection	9 (15.8)	1 (2.0)	9.0 , 1.1-75.7, <i>P</i> <0.009
<i>M. fermentans</i>	36 (63.2)	2 (4.0)	40.3 , 8.7-186.4, <i>P</i> <0.001
<i>M. hominis</i>	2 (3.5)	1 (2.0)	1.7 , 0.2-20.4, <i>P</i> = 0.65
<i>M. pneumoniae</i>	6 (10.5)	2 (4.0)	2.7 , 0.6-14.7, <i>P</i> = 0.19
<i>M. penetrans</i>	1 (1.8)	1 (2.0)	0.9 , 0.05-14.5, <i>P</i> = 0.91
<i>M. genitalium</i>	6 (10.5)	0	Chi ² = 7.7, <i>P</i> <0.005
<i>M. fermentans</i> + <i>M. hominis</i>	1 (1.8)	0	Chi ² = 0.89, <i>P</i> = 0.34
<i>M. fermentans</i> + <i>M. pneumoniae</i>	4 (7.0)	1 (2.0)	Chi ² = 3.6, <i>P</i> = 0.21
<i>M. pneumoniae</i> + <i>M. hominis</i>	1 (1.8)	0	Chi ² = 0.89, <i>P</i> = 0.34
<i>M. fermentans</i> + <i>M. genitalium</i>	3 (5.3)	0	Chi ² = 3.8, <i>P</i> <0.051

*Not enough power to calculate