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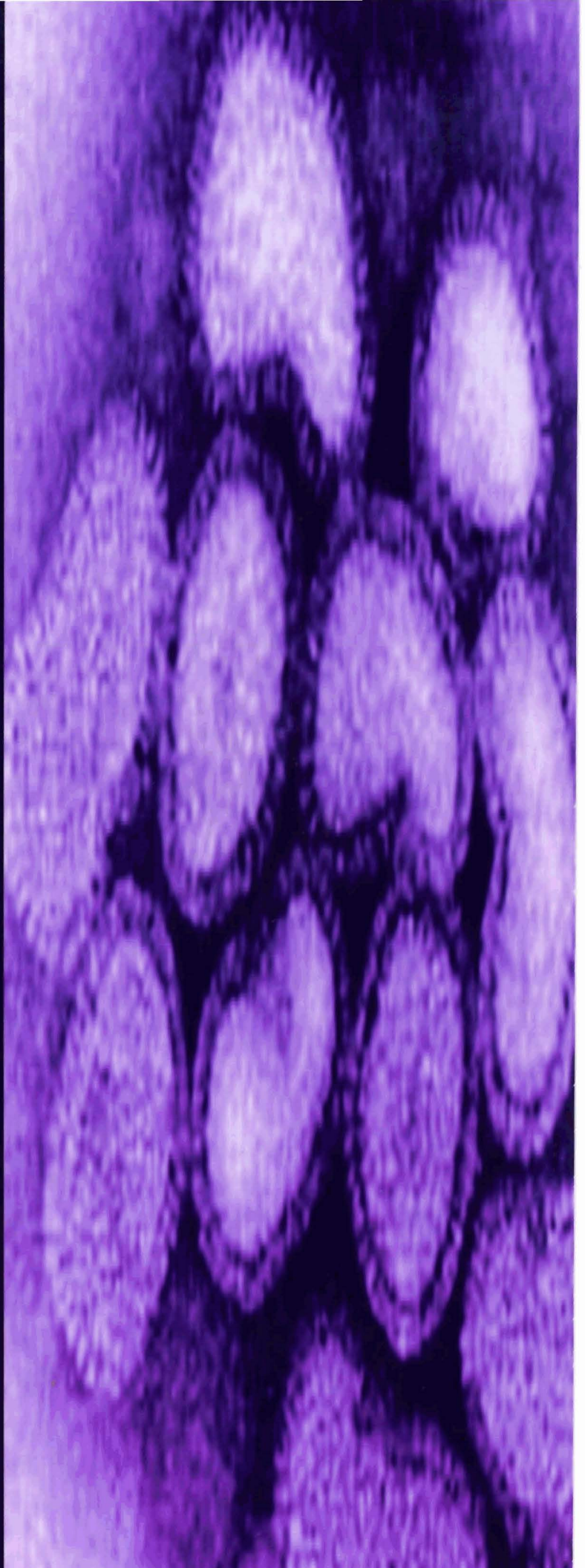

Similarities of Chronic Fatigue Syndrome (CFS) and Autism Spectrum Disorders (ASD): Comparisons of Co-Infections

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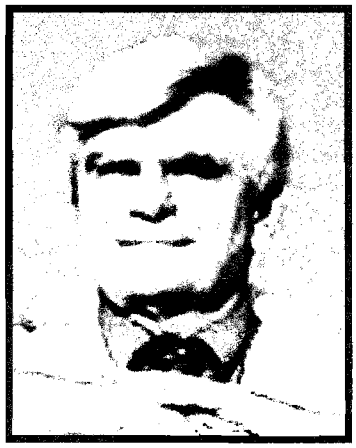
ABSTRACT

Objective:
The majority of neurodegenerative diseases, fatiguing illnesses and neurobehavioral disease patients have chronic infections. Therefore, we examined the presence of certain co-infections in the blood of patients with Autism Spectrum Disorders (ASD) and compared these to CFS patients.

Methods:
North American CFS and ASD patients were examined for various infections by isolation of leukocyte blood fractions and forensic polymerase chain reaction (PCR) to determine various infections.

Results:
CFS patients (n=100, age=39.7±8.9) show evidence of multiple, systemic infections (Odds Ratio = 18.0, 95% CL 8.5-37.9, p< 0.001) that may be important in CFS morbidity. CFS patients had a high prevalence (51%) of 1 of 4 *Mycoplasma* species (OR = 13.8, 95% CL 5.8-32.9, p< 0.001) and often showed evidence of co-infections with different *Mycoplasma* species, *Chlamydia pneumoniae* (OR = 8.6, 95% CL 1.0-71.1, p< 0.01) and/or active Human Herpes Virus-6 (HHV-6) (OR = 4.5, 95% CL 2.0-10.2, p< 0.001). We found that 8% of the CFS patients showed evidence of *C. pn.* and 31% of active HHV-6 infections. Recently we examined ASD patients (n=48, age 8.4±2.8) and found a large subset (58.3%) of ASD patients showed evidence of *Mycoplasma* species infections compared to age-matched control subjects (OR = 13.9, p<0.001). ASD patients also had *C. pn.* (4/48 or 8.3% positive, OR = 5.6, p<0.01) and HHV-6 (14/48 or 29.2%, OR = 4.5, p<0.01) infections in their blood.

Conclusions:
The results indicate that similar to CFS patients a large subset of neurobehavioral (ASD) disease patients show evidence of chronic infections. Although there were significant differences in median age and diagnoses between the two groups of patients, they tended to have similar incidence of three types of chronic infections: *Mycoplasma, Chlamydia* and HHV-6.



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INTRODUCTION

Although no single underlying cause has been established for CFS, there is growing awareness that CFS can have an infectious nature that is either causative for the illness, a cofactor for the illness or appears as an opportunistic infection(s) that aggravate patient morbidity (1, 2). There are several reasons for this, including the nonrandom or clustered appearance of CFS, sometimes in immediate family members (2-4), the presence of certain signs and symptoms associated with infection, the often cyclic course of the illness and its response to anti-microbial therapies (2, 5, 6).

Previously we found that Gulf War veterans with CFS-like illnesses and a positive test for *Mycoplasma fermentans* transmitted their infections to their spouses and children (4). The adults in these families were diagnosed with CFS (7) but the children were subsequently diagnosed with Autism Spectrum Disorders (ASD) (8). The criteria for diagnosis of ASD are, in general terms, the presence of a triad of impairments in social interaction, communication and imagination (9). Examination of ASD patients in civilian families for the presence of *Mycoplasma* species infections revealed that the majority of these patients had one or more infections (10).

In ASD cases there are reports of nonspecific signs and symptoms similar to those seen in CFS, such as fatigue, headaches, gastrointestinal and vision problems and occasional intermittent low-grade fevers and other signs and symptoms that are generally excluded in the diagnosis of ASD. These suggested to some authors that a subset of ASD patients may suffer from bacterial or viral infections (11). Here we examined three commonly found systemic infections in CFS patients, *Mycoplasma* species, *Chlamydia pneumoniae* and HHV-6 (12-14) and compared the incidence to the same infections found in ASD patients (10).

MATERIALS AND METHODS

Patients: All CFS patients (North American, n=100) underwent a medical history, completed a sign/symptom illness survey, had routine laboratory tests and met the Fukuda et al. (15) exclusionary criteria. Control subjects (n=100) had to be free of disease for at least three months prior to data collection, and they had to be free of antibiotic treatment for three months prior to blood collection. All ASD patients (N=48) were randomly recruited from patient support groups in California after diagnosis of ASD according to the *International Classification of Diseases* (ICD-10) and the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV). All ASD patients were assessed by the Autism Diagnostic Interview-Revised (ADI-R) (16) and Childhood Autism Rating Scale (CARS) (17, 18). Most (45/48) had a diagnosis of autism, while 6/48 were diagnosed with ADD (three of which were also diagnosed with autism) and nine autism patients with Asperger's Syndrome.

PCR Analysis of Blood:
Blood was collected in EDTA-containing tubes and immediately brought to ice bath temperature as described previously (12-14). Samples were shipped with wet ice by overnight air courier to the Institute for Molecular Medicine for analysis. All blood samples were blinded. Whole blood (50 µl) was used for preparation of DNA using Chelex (Biorad, Hercules, USA). Aliquots from the centrifuged samples were used immediately for Polymerase Chain Reaction (PCR) as described previously (12-14).

Statistics:
Subjects' demographic characteristics were assessed using descriptive statistics and

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students' t-tests (independent samples test, t-test for equality of means, 2-tailed). The 95% confidence interval was chosen for minimal significance. Odds Ratios were calculated using logistic regression (Logit method) Statistica 5.5 (Statsoft, Tulsa, OK). In some cases, Pearson Chi-Square test was performed to compare prevalence data between patients and control subjects.

RESULTS

Patient Demographic Data: These are shown in Tables 1 and 2.

TABLE 1. CFS Patient demographic data.

	n	Mean age (SD)	Range	Males (%)	Females (%)
Patients	100	39.7 (8.9)	18-66	28 (28)	72 (72)
Controls	100	34.6 (9.1)	21-58	31 (31)	69 (69)
Female patients	72	39.8 (9.8)	18-66	0 (0.0)	72 (100.0)
Male patients	28	39.2 (10.3)	20-60	28 (100.0)	0 (0.0)

TABLE 2. ASD Patient demographic data.

	n	Mean age (SD)	Range	Males (%)	Females (%)
Patients	48	8.4 (2.8)	3-14	36 (75)	12 (25)
Controls	45	7.9 (3.3)	4-11	28 (62.2)	17 (37.8)
Rural patients	18	8.1 (2.9)	3-14	14 (77.7)	4 (22.3)
Urban patients	30	8.6 (3.2)	4-14	22 (73.3)	8 (26.7)

Bacterial and Viral Infections:

Using the blood of CFS patients (n=100) and PCR procedures an overwhelming majority of patients showed evidence of multiple, systemic bacterial and viral infections (Odds Ratio=18.0, $p<0.001$).[17] CFS patients had a high prevalence (51%) of one of four *Mycoplasma* species (Odds Ratio=13.8, $p<0.001$) and often showed evidence of co-infections with different *Mycoplasma* species, *C. pneumoniae* (8%, Odds Ratio=8.6, $p<0.01$) and active HHV6 (30%, Odds Ratio=4.5, $p<0.001$) (Table 3).

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TABLE 3. Prevalence and Odds Ratio Analysis of Systemic Infections Between 100 CFS Patients and 100 Healthy Control Subjects.

Type of infection	CFS Patients n = 100	Control Subjects n = 100	Odds Ratio, 95% CL, *p* or Chi^2
Number Infected	71	12	**18.0**, 8.5-37.9, p< 0.001
HHV-6	31	9	**4.5**, 2.0-10.2, p< 0.001
C. Pneumoniae	8	1	**8.6**, 1.0-71.1, p< 0.01
Mycoplasma spp.	51	7	**13.8**, 5.8-32.9, p< 0.001
M. pneumoniae	29	3	**13.2**, 3.8-45.4, p< 0.001
M. fermentans	22	2	**13.8**, 3.1-61.1, p< 0.001
M. honinis	16	1	**18.8**, 2.4-147.0, p< 0.001
M. penetrans	8	1	**8.6**, 1.0-71.1, p< 0.01
Single mycoplasmal infection	29	7	**13.8**, 5.8-32.9, p< 0.001
Multiple mycoplasmal infections	22	0	Chi^2 = **24.7**, p< 0.001
M. fermentans +*M. pneumoniae*	10	0	Chi^2 = 10.5, p< 0.001
M. fermentans +*M. hominis*	7	0	Chi^2 = 7.3, p< 0.007
M. pneumoniae +*M. hominis*	3	0	Chi^2 = 3.1, p< 0.08
M. fermentans +*M. hominis* + *M. pneumoniae*	2	0	Chi^2 = 2.0, p= 0.16
Mycoplasma + HHV-6	16	0	Chi^2 = **17.4**, p< 0.001
Mycoplasma + *C. pneumoniae*	4	0	Chi^2 = 4.1, p< 0.04
C. pneumoniae + HHV-6	3	0	Chi^2 = 3.1, p< 0.08

Evidence for *Mycoplasma spp.* infections was found in 28/48 or 58.3% of ASD patients and 2/45 (4.7%) age-matched control subjects (Odds Ratio=13.8, p<0.001) (Table 4). *C. pneumoniae* infections were found in 4/48 or 8.3% of ASD patients and in 1/45 or 2.1% of control subjects (Odds Ratio=5.6, p< 0.01) (Table 4).

We also examined the incidence of HHV-6 infections in ASD patients and found that 14/48 or 29.2% of ASD patients were positive compared to 4/45 (8.8%) positives in age-matched control subjects (Odds Ratio=4.5, p<0.01). We did not find any multiple co-infections in control subjects. The differences between infections in ASD patients and control subjects were highly significant (Odds Ratio=16.5, p< 0.001). Significant differences were not found in the prevalence of infections in urban and rural patients, in male or female patients or between autism and other ASD diagnoses.

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TABLE 4. Prevalence and Odds Ratio Analysis of Systemic Infections in ASD Patients and Matched Healthy Control Subjects.

Type of infection	ASD Patients n = 48 (%)	Control Subjects n = 45 (%)	Odds Ratio, p or Chi^2
HHV-6	14 (29.2)	4 (8.3)	**4.5**, $p< 0.01$
C. Pneumoniae	4 (8.3)	1 (2.1)	**5.6**, $p< 0.01$
Mycoplasma spp.	28 (58.3)	2 (4.7)	**13.8**, $p< 0.001$
M. pneumoniae	16	2	**9.2**, $p< 0.001$
M. fermentans	17	0	**14.8**, $p< 0.001$
M. hominis	5	0	**11.8**, $p< 0.01$
M. penetrans	1	0	**6.6**, $p< 0.01$
Single mycoplasmal infection	16 (33.3)	2 (4.7)	**13.8**, $p< 0.001$
Multiple mycoplasmal infections	12 (25.0)	0 (0)	Chi^2 = **11.7**, $p< 0.001$
M. fermentans +*M. pneumoniae*	7	0	$Chi^2 = 4.7$, $p< 0.01$
M. fermentans +*M. hominis*	2	0	$Chi^2 = 1.9$, $p< 0.3$
M. pneumoniae +*M. hominis*	1	0	$Chi^2 = 1.4$, $p< 0.2$
M. fermentans +*M. hominis* + *M. pneumoniae*	2	0	$Chi^2 = 1.9$, $p<0.2$
Mycoplasma + HHV-6	8 (16.7)	0 (0)	Chi^2 = **4.4**, $p< 0.01$
Mycoplasma + *C. pneumoniae*	2 (4.2)	0 (0)	$Chi^2 = 2.1$, $p< 0.19$
C. pneumoniae + HHV-6	1 (2.1)	0 (0)	$Chi^2 = 1.6$, $p< 0.3$

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DISCUSSION

In contrast to the ASD children in military families where primarily one species of Mycoplasma was found (usually *M. fermentans*), the majority of ASD patients in Central California were found to have single or multiple mycoplasmal infections involving *M. pneumoniae, M. fermentans, M. hominis* or *M. genitalium*. We also examined two other commonly found infections in CFS patients (4-7), *C. pneumoniae* and HHV-6 (13, 14). The results suggested that infections are a common feature in ASD as well as CFS. Consistent with this hypothesis is the finding that autism occurs at greater prevalence during periods of more frequent hospitalizations for bronchitis or pneumonia (19), and maternal viral infections during the second trimester of pregnancy are associated with increased risk of autism in their offspring (20, 21).

In a separate study on CFS patients the presence of chronic infections has also been statistically related to the number and severity of signs/symptoms seen in CFS patients (14). Although similar studies in ASD patients have no been done, it has been observed that patients with severe ASD are those with systemic infections of the type seen in this study (unpublished observations).

The appearance of infections in children diagnosed with ASD may eventually be linked to the multiple vaccines received during childhood either as a source or from opportunistic infections in immune suppressed recipients of multiple vaccines. Although the etiology of ASD is currently unknown and thought to involve both genetic and environmental factors (22, 23), the infections found in ASD patients should be considered along with other factors in the management of these disorders (24).

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