



The challenge of integrating disparate high-content data: epidemiological, clinical and laboratory data collected during an in-hospital study of chronic fatigue syndrome

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Chronic fatigue syndrome (CFS) is a debilitating illness characterized by multiple unexplained symptoms including fatigue, cognitive impairment and pain. People with CFS have no characteristic physical signs or diagnostic laboratory abnormalities, and the etiology and pathophysiology remain unknown. CFS represents a complex illness that includes alterations in homeostatic systems, involves multiple body systems and results from the combined action of many genes, environmental factors and risk-conferring behavior. In order to achieve understanding of complex illnesses, such as CFS, studies must collect relevant epidemiological, clinical and laboratory data and then integrate, analyze and interpret the information so as to obtain meaningful clinical and biological insight. This issue of *Pharmacogenomics* represents such an approach to CFS. Data was collected during a 2-day in-hospital study of persons with CFS, other medically and psychiatrically unexplained fatiguing illnesses and nonfatigued controls identified from the general population of Wichita, KS, USA. While in the hospital, the participants' psychiatric status, sleep characteristics and cognitive functioning was evaluated, and biological samples were collected to measure neuroendocrine status, autonomic nervous system function, systemic cytokines and peripheral blood gene expression. The data generated from these assessments was made available to a multidisciplinary group of 20 investigators from around the world who were challenged with revealing new insight and algorithms for integration of this complex, high-content data and, if possible, identifying molecular markers and elucidating pathophysiology of chronic fatigue. The group was divided into four teams with representation from the disciplines of medicine, mathematics, biology, engineering and computer science. The papers in this issue are the culmination of this 6-month challenge, and demonstrate that data integration and multidisciplinary collaboration can indeed yield novel approaches for handling large, complex datasets, and reveal new insight and relevance to a complex illness such as CFS.

Introduction

Chronic fatigue syndrome (CFS) is an important public health problem. CFS affects 400,000–900,000 adults in the USA [1,2], and at least a quarter of those suffering the illness are unemployed or receiving disability [2,3] with the average affected family forgoing US\$20,000 in lost earnings and wages [4]. Despite the public health burden imposed by CFS, effective diagnostic, treatment and prevention strategies are not available because the etiology, risk factors and pathophysiology remain unknown [5]. Deciphering its pathophysiology will not only advance the public health and medical response to CFS, but can serve as a model for other chronic medically unexplained conditions such as postinfectious fatigue, cancer-related fatigue, and adverse responses to vaccination and Gulf War illness.

The tremendous strides of medicine and public health during the twentieth century, in large part, reflect successful control and prevention strategies for acute infectious diseases where identification of risk factors, etiology and pathogenesis fulfill Koch's postulates. However, the most pressing public health problems are due to chronic diseases. Some are directly caused by infectious agents (for example, AIDS); infectious agents are variously associated with others (for example, gastric ulcers and asthma); and others have no proven infectious association (for example, breast cancer). Regardless of known associations, all are complex illnesses and effective control and prevention strategies require an in-depth understanding of their various causal pathways and associated pathophysiologies. Complex illnesses represent alterations in homeostatic systems arising from the combined

Keywords: CFS, chronic fatigue syndrome, clinical study, data integration, disparate data

future
medicine

action of many genes, environmental factors and risk-conferring behavior. Not only are chronic diseases more complex, but also the molecular tools available to study diseases have become much more sophisticated. High-throughput 'omic' technology is increasingly used in clinical and epidemiological studies; however, our ability to analyze and interpret high-content and complex databases has not kept pace. This is not because the mathematical and computational means do not exist. Rather, it is because optimal understanding of complex diseases requires an integrated perspective of several disciplines.

The papers on CFS in this special issue of *Pharmacogenomics* are a proof-of-concept, applying the more complex logic model to a dataset derived from an in-hospital study of CFS. Four teams of investigators, representing medicine, molecular biology, engineering, mathematics, physics and computer science, participated in a 6-month challenge to analyze and interpret this data. The in-hospital study enrolled 227 people with CFS, other unexplained fatiguing illness and nonfatigued controls identified from the general population of Wichita, KS, USA. During the study we collected basic clinical data (including disability, medication use, standardized measures of fatigue and accompanying symptoms), measured cognitive function, conducted sleep laboratory studies (including electroencephalogram [EEG], electrocardiogram [ECG] and electromyography [EMG]), assessed neuroendocrine and immune system status (including measures of the hypothalamic–pituitary–adrenal [HPA] axis and autonomic nervous system), and measured peripheral blood cell expression levels of 20,000 genes. The teams were provided with the same basic data set and were challenged to identify biologically- and clinically-meaningful information relevant to classification, diagnosis and treatment of CFS. This paper describes the dataset that was distributed, the makeup of each team, and the overall approach the teams used for analysis. The remaining papers in this issue demonstrate that a multidisciplinary approach to decipher complex diseases such as CFS yields novel algorithms and insights that no one expertise could have achieved.

Methods

Recruitment

This study adhered to human experimentation guidelines of the US Department of Health and Human Services and the Helsinki Declaration. The Centers for Disease Control and Prevention (CDC) Institutional Review Board approved

study protocols. All participants were volunteers who gave informed consent. The 2-day in-hospital study was conducted from December 2002 to July 2003, and enrolled participants 18–69 years of age who had been identified from 1997 through to 2000 in a longitudinal surveillance study of CFS in Wichita [2]. **Figure 1** illustrates how subjects were screened and identified for enrollment in this study. In brief, longitudinal surveillance began in 1997 by screening 56,151 residents of Wichita who were between 18–69 years of age. The surveillance study followed a cohort of 7162 people representative of the fatigued and nonfatigued Wichita population at 12-, 24-, and 36-month intervals with telephone interviews and clinical evaluations. Clinical evaluations identified medical and psychiatric conditions considered exclusionary for CFS and (with the exception of melancholic depression) people with exclusions were dropped from the cohort. The surveillance cohort included 70 people classified as CFS, according to the 1994 CFS Case Definition [6], at least once during 4 years of surveillance and, of these, 58 (83%) agreed to participate in the in-hospital study. Each CFS case was matched to a control, based on sex, race/ethnicity, age, and body mass index (BMI). Matched-controls were selected from the cohort who participated in surveillance at the baseline and all follow-up periods and did not report fatigue of at least 1-month duration; 55 controls participated. We also invited 70 (randomly selected) of the 158 participants identified with unexplained chronic fatigue that did not meet criteria for CFS; 59 (84%) agreed to participate. This group was termed 'insufficient symptoms or fatigue' (ISF). Finally, although melancholic depression is considered exclusionary for CFS, we invited all 41 longitudinal surveillance participants who met criteria for CFS, except for concurrent melancholic depression, and all 39 with ISF and melancholic depression; 27 (66%) and 28 (72%), respectively, enrolled in the study. A total of 227 people were successfully enrolled in the 2-day in-hospital study.

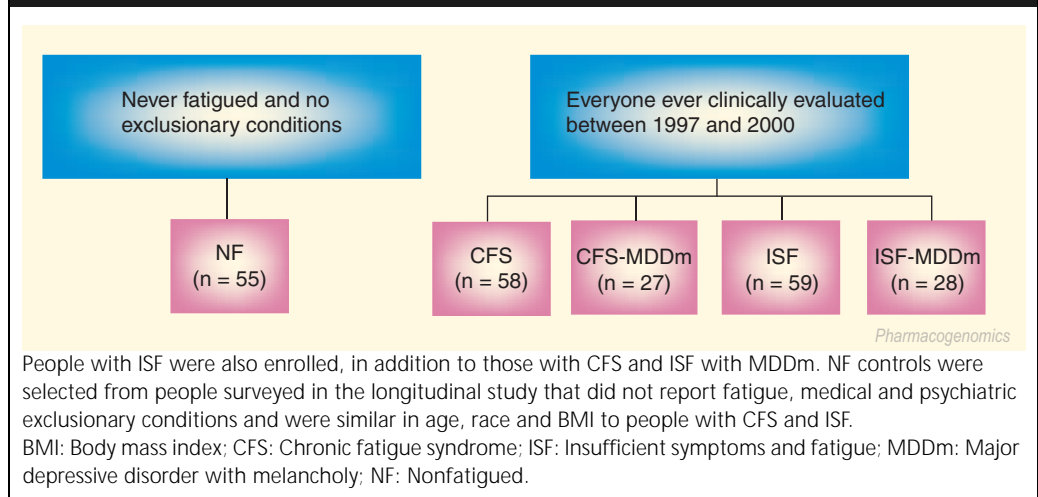
Clinical assessment

Participants completed a variety of tests and questionnaires as summarized in **Table 1** and discussed below.

Medical evaluation

Those who agreed to participate were admitted for 2 days to a research unit established in the Wesley Medical Center in Wichita. Hospital

Figure 1. This in-hospital study enrolled people who were identified with CFS according to the 1994 CFS case definition as described by Fukuda and colleagues during the 4-year longitudinal study of CFS in Wichita, KS, USA.



staff were unaware of subjects' enrollment status, as were the subjects. To identify exclusionary medical conditions [6,7], subjects provided a standardized past medical history and review of systems, which they completed at home. During admission, a study nurse reviewed this, resolved missing items and answered patients' questions. Study participants brought all current prescribed and over-the-counter medications and dietary supplements to the hospital. These were reviewed, catalogued by a study nurse and their use during the study was monitored.

At the time of admission, subjects underwent a standardized physical examination conducted by a specifically trained physician. The physician was unaware of participants' enrollment classification. The physical exam included measurement of basal temperature, weight, height, and waist:hip ratio. The physician also reviewed past medical history, review of systems and medications. Following the standardized physical exam, specific systems were evaluated in more detail as warranted. Patients provided blood and urine for routine analyses. Clinical laboratory tests included a complete blood count (CBC) with differential, c-reactive protein (CRP), alanine aminotransferase (ALT, or serum glutamic pyruvic transaminase [SGPT]), albumin, alkaline phosphatase (AP), aspartate aminotransferase (AST), serum glutamic aminotransferase (SGOT), total bilirubin, calcium, carbon dioxide, chloride, creatinine, glucose, potassium, total protein (TP), sodium, and blood urea

nitrogen (BUN). All blood work and urinalysis were performed at the clinical laboratory in Wesley Medical Center, Wichita.

Psychiatric evaluation

The Diagnostic Interview Schedule (DIS) administered by licensed and specifically trained psychiatric interviewers was used to identify current psychiatric disorders [8]. As noted, melancholic depression is considered exclusionary for CFS and these persons were included for comparison purposes. The existence of melancholic depression was identified in the analysis data set. All study subjects were administered the Zung self-rating depression scale [9], which contains 20 items designed to assess core symptoms of major depression in the past week. Subjects responses to the Zung scale were provided to investigators.

Medical & psychiatric exclusions

A total of 63 study participants had a medical or psychiatric condition exclusionary for CFS identified at the time of the hospital study [7]. These individuals were identified and included in the dataset so that the teams could include or exclude them for comparison purposes.

Evaluation of functional impairment, fatigue & accompanying symptoms

Subjects completed the Medical Outcomes Survey Short Form-36 (SF-36) [10], the multi-dimensional fatigue inventory (MFI) [11], and the CDC symptom inventory [12], before arriving at the hospital at which time the study

Table 1. Data from the following instruments was collected from all subjects and provided to investigators.

Instrument	Assessment	Mode of administration	Mode of monitoring responses	Administration	Ref.
Symptom checklist	Empirical data related to CFS case definition	Self-administered	Paper and pencil	Arrival day	[12]
SF-36	General health/well-being	Self-administered	Paper and pencil	Arrival day	[10]
Multidimensional fatigue inventory	Quantifies fatigue	Self-administered	Paper and pencil	Arrival day	[11]
Epworth sleepiness scale	Screening for sleep pathologies	Self-administered	Paper and pencil	Arrival day	[13]
CANTAB	Neurocognitive screening	Computer-based assessment	NA	Clinic day 1	[14]
Abbreviated Wechsler adult intelligence scale	IQ	Trained psychiatric interviewer	Paper and pencil manual, stimulus booklet, record forms, and set of nine blocks	Clinic day 1	[16]
Zung self-rating depression scale	Quantifies depression	Self-administered	Computerized	Clinic day 1	[9]
Gynecological history	Menstrual cycle	Self-administered	Paper and pencil	Arrival day	NA
Pre/post sleep scale	Measures sleepiness and degree to which sleep is refreshing	Self-administered	Computerized	Arrival day, clinic days 1, clinic day 2	NA
Medical history	Information on subject's medical history	Clinician-administered	Paper and pencil items	Arrival day	NA
Medication diary	Captures medications that may affect laboratory tests and sleep	Clinician-administered	Paper and pencil	From arrival to departure	NA
Tobacco diary	Capture tobacco consumption that may affect laboratory tests	Clinician-administered	Paper and pencil	From arrival to departure	NA

CANTAB: Cambridge Neuropsychological Test Automated Battery; IQ: Intelligence quotient; NA: Not applicable; SF-36: Short Form-36.

manager, who resolved missing responses, reviewed them. The SF-36 assesses functional impairment in eight areas:

- Limitations in physical activities (physical function)
- Limitations in usual role activities due to physical health problems (role physical)
- Limitations in usual role activities due to emotional problems (role emotional)
- Bodily pain
- General health perceptions (general health)
- Vitality (energy and fatigue)
- Social function
- General mental health

Scores in each area reflect function and well-being, with lower values indicating greater impairment. The MFI assesses general fatigue, physical fatigue, mental fatigue, reduced motivation, and reduced activity. The score in each

dimension reflects fatigue severity with higher values indicating more severe fatigue. The symptom inventory measures the occurrence, frequency and intensity of 19 symptoms over the preceding month.

Illness classification

As study participants had been classified as CFS, ISF, or nonfatigued during surveillance up to 6 years previously, and because CFS is cyclic in the occurrence and severity of its symptoms, participants' illness status was classified at the time they participated in the clinical study. Current illness classification by empirical criteria [7] was included in the data set for use at the teams' discretion.

Illness classification by empiric criteria

Information from the SF-36, MFI, and symptom inventory was used to empirically classify subjects according to the three main dimensions

Table 2. Laboratory assays conducted to assess endocrine status.

Test	Collection day	Collection time
ACTH	Clinic day 1	07:30
Aldosterone	Clinic day 1	07:30
Renin (PRA)	Clinic day 1	07:30
Catecholamines (epinephrine and norepinephrine)	Clinic day 1	07:30
Hirsutism test group II (androstenedione, DHEA-S, testosterone, free testosterone, % free testosterone and SHBG)	Clinic day 1	07:30
DHEA	Clinic day 1	07:30
TSH	Clinic day 1	07:30
Total and free T ₄	Clinic day 1	07:30
Total and free T ₃	Clinic day 1	07:30
Reverse T ₃	Clinic day 1	07:30
Estradiol (women)	Clinic day 1	07:30
Progesterone(women)	Clinic day 1	07:30
IGF-1	Clinic day 1	07:30
Salivary cortisol	Clinic days 1 and 2	07:00 (at awakening) 22:00 (at bed time)
Urinary free cortisol	Clinic day 1 to clinic day 2	07:00 to 07:00
Metanephrine and normetanephrine	Clinic day 1	07:30
Neuropeptide Y	Clinic day 1	07:30

ACTH: Adrenocorticotrophic hormone; DHEA: Dehydroepiandrosterone; DHEA-S: DHEA-sulfate; IGF-1: Insulin-like growth factor 1; PRA: Plasma renin activity; SHBG: Sex hormone-binding globulin; TSH: Thyroid stimulating hormone.

of CFS; functional impairment (SF-36), fatigue (MFI), and accompanying symptoms (symptom inventory) [7]. Substantial reduction in occupational, educational, social, or recreational activities was defined as scores lower than the 25th percentile of the published US population on the physical function (≤ 70), or role physical (≤ 50), or social function (≤ 75), or role emotional (≤ 66.7) subscales of the SF-36. Severe fatigue was defined as greater than or equal to the medians of the MFI general fatigue (≥ 13) or reduced activity (≥ 10) scales. Finally, subjects reporting four or more symptoms and scoring 25 or more on the symptom inventory case definition subscale were considered to have substantial accompanying symptoms. Subjects who met all three criteria (SF-36 and MFI and symptom inventory) when they entered the clinical study were classified as CFS according to standardized clinically empirical criteria; those who met some but not all three criteria were considered as insufficient symptoms or fatigue (ISF); those who met none of the criteria were classified as nonfatigued (NF).

Clinical assessments

Neurocognitive assessments: CANTAB

Patients performed tests of cognitive function on the Cambridge Neuropsychological Test Automated Battery (CANTAB) [14]. The CANTAB is administered on a touch screen portable computer, ensuring a standardized and homogeneous form of testing with instantly available data and detailed recording of accuracy and speed of responses of the subjects. The seven tests included reaction time, stockings of Cambridge, spatial working memory, pattern recognition memory, spatial recognition memory, intra/extradimensional attention shift and rapid visual information processing [15]. To control for intelligence quotient (IQ), participants completed the abbreviated Wechsler adult intelligence scale [16].

Sleep assessment

To diagnose exclusionary sleep disorders and describe clinical and polysomnographic sleep characteristics of study participants, the study conducted nocturnal polysomnography and

subsequent day daytime multiple sleep latency testing in a four-bed laboratory established at Wesley Medical Center. Nocturnal polysomnography was conducted the night that participants arrived, multiple sleep latency testing was conducted the following day and repeated overnight polysomnography the second night. Patients were asked to arrive approximately 3 hours before their typical bedtime on night 1 to allow adequate time for electrode application and standard biocalibrations. 'Lights out' and 'lights on' times were 22:00 and 07:00, respectively. The daytime multiple sleep latency testing schedule was adjusted for other measures being collected; it began at 11:00 and consisted of three additional 20-minute naps at 13:00, 15:00 and 17:00.

Electrophysiological measures of wakefulness and sleep were acquired and recorded with the Flaga/Medcare N7000 digital polysomnographic system on a Windows XP platform using proprietary software (Flaga/Medcare Somnologica Studio, CO, USA). A sampling rate of 256 Hz was employed to allow for fast Fourier transform of EEG signals. Standard gold cup electrodes were employed for recording of EEG, electro-oculogram (EOG), and EMG for sleep staging and appreciation of sleep architecture. Respiration was measured with inductance plethysmography-like belts placed around the chest and abdomen. A pressure transducer, positioned in close approximation to the nares, provided indices of airflow. A pulse oximeter probe was applied to either the right or left index finger, to measure arterial oxygen saturation (SaO₂). Electrocardiogram (ECG) was recorded with standard snap electrodes (NeuroSupplies, CT, USA). The following signals were recorded: central (C₃-A₂/C₄-A₁) and occipital (O₁-A₁/O₂-A₂) EEG, left and right monopolar EOG, surface mentalis EMG, ECG (modified V3), respiratory airflow and effort and surface EMG from the right and left anterior tibialis.

A registered polysomnology technologist manually scored each recording in 30-second epochs was used to score each epoch as wake, nonrapid eye movement (NREM) stages 1–4 sleep, or rapid eye movement (REM) sleep. Criteria for scoring respiratory variables was based upon that described by the Cardiovascular Health Study [17]. Briefly, apnea was scored if airflow decreased to less than or equal to 25% of the immediately preceding baseline for a period of at least 10 seconds. Hypopnea was scored if either airflow or thoracic–abdominal excursion decreased by at least 30% of baseline, for at least 10 seconds, with

a concomitant reduction in SaO₂ of 4% or greater. The respiratory disturbance index (RDI) (apneas plus hypopneas corrected for hour of sleep) was derived from these scored events. To determine the Board of Registered Polysomnographic Technologist's (RPSGT's) level of reproducibility, 12 randomly selected studies were scored twice, with the at least 6-week interval separating the original scoring and the repeat scoring. Polysomnography data were scored and results were interpreted by a registered polysomnology technologist and an American Board of Sleep Medicine certified physician who was blinded to the subjects' fatigue classifications. Sleep pathology data provided to investigators included summary diagnoses for sleep apnea [18], periodic limb movement disorder [19], pathological daytime sleepiness and narcolepsy [20–22], and α EEG sleep disorder.

Laboratory tests

Specimen collection

Participants collected 24-hour urine samples between 07:00 on day 1 until 07:00 on day 2. They also used salivettes to collect saliva immediately after awakening and before lights-out on night 2. Blood for neuroendocrine, immune, and gene expression studies was collected at 07:30 and subjects had fasted overnight. They were awakened at 07:00, an intravenous (IV) line was placed into a forearm vein, and they remained recumbent for 30 minutes prior to blood collection. All endocrine testing was completed by Esoterix, Inc. Laboratory Services (CO, USA) and are detailed in Table 3.

Hypothalamic–pituitary–adrenal axis status

HPA axis activity was assessed by measuring 24-hour urinary free cortisol concentrations. Diurnal variation of cortisol release was assessed by salivary cortisol concentrations at awakening and bedtime. Basal plasma adrenocorticotropin (ACTH) concentrations were measured in the morning. The hypothalamic–pituitary–gonadal axis was evaluated by measuring total and free testosterone serum levels as well as serum dehydroepiandrosterone (DHEA) and DHEA-S. In women, estradiol and progesterone levels were measured in the morning. Thyroid status was assessed by determination of morning serum total and free triiodothyronine (T3) and thyroxine (T4), reverse T3 and TSH concentrations in the morning. Serum insulin-like growth factor-1 (IGF-1) was measured using a morning blood sample. Plasma levels of inflammatory cytokines

including tumor necrosis factor (TNF)- α , interleukin (IL)-6 and its soluble receptor sR-IL-6, as well as serum CRP, were determined in the morning and in the evening. Cytokines were measured using an enzyme-linked immunosorbent assay (ELISA) kit (RayBiotech, GA, USA) and all cytokine assays were run at the CDC.

Autonomic nervous system status

Autonomic nervous system evaluation included measurement of blood pressure and heart rate while lying and standing. Subjects were recumbent for 30 minutes and then stood for 5 minutes. Blood pressure and heart rate were measured at lying and after 5 minutes of standing. Basal catecholamine, metanephrine, normetanephrine and neuropeptide Y concentrations were determined in plasma by Quest Diagnostics (KS, USA). To assess whether the mineralocorticoid axis was intact, plasma renin and serum aldosterone were measured in the morning.

Gene expression profiling

Blood was collected for gene expression profiling studies in three 8 ml vacutainer tubes containing citric acid (BD, NJ, USA). The peripheral blood mononuclear cells (PBMCs) were immediately isolated on lymphocyte separation medium (Organon Teknika, NC, USA) and stored in liquid nitrogen under conditions designed to maintain viability. Total RNA was extracted using Trizol[®] reagent (Invitrogen, CA, USA) and contaminating DNA was removed by incubation with 2U DNaseI (GeneHunter Corp., TN, USA) at 37°C for 15 minutes. Quality and quantity of the RNA was determined using the 2100 Bioanalyzer (Agilent, CA, USA). Each RNA sample was labeled and hybridized to a microarray. 1 μ g of RNA was reverse transcribed to produce a labeled cDNA probe as previously described [23]. Each labeled cDNA was hybridized to a glass microarray containing 20,000 oligonucleotide features representing human genes (MWG Biotech, Ebersberg, Germany). The MWG 40K, which consists of two microarray slides (A and B), each with 20,000 features, was used for this study. Only the A microarray produced results that were quality standards for distribution. Hybridization of the microarrays was performed using the Ventana Discovery system and ChipMap[™] kit (Ventana Medical Systems, AZ, USA). Signal was detected with antibiotin antibodies conjugated to resonance light scattering RLS[™] particles binding to the biotinylated target cDNA hybridized on the

arrays. The slides were archived and images captured using the GSD-501[™] scanner (Genicon Sciences Corporation, CA, USA). Feature intensity was quantified using ArrayVision[™] RLS image analysis software (Genicon Sciences Corporation). Microarray data was provided to investigators as raw intensity values with artifact and background noise subtracted from each feature value.

Investigators

A total of 20 investigators from around the world agreed to participate in this challenge and were divided into four teams (Table 3). Each team determined how they were going to communicate, analyze and interpret the data and report results.

Results

Classification of subjects

As presented in the methods, empirical criteria were used to classify study participants' fatigue status during the hospital study (Table 4). Investigators could choose to use either or neither of these classifications. It should be noted that classification by the 1994 case definition of these study subjects during longitudinal surveillance was not congruent with the empirical classification at the time of the hospital study. This likely reflects that waxing and waning of the illness in addition to the heterogeneous nature of medically unexplained fatigue. As a result of this, some teams choose to ignore the empirical fatigue classification and derive their own.

Team approaches

This issue of *Pharmacogenomics* presents the detailed approaches each team used to analyze and describe the data. A summary of each team approach is appropriate to guide the reader through the logic and order of these manuscripts. Team 1 hypothesized that CFS is heterogeneous in its nosology, and results from deregulation of several genotype–phenotype relationships that interact with environmental factors to produce different conditions and addresses this hypothesis in four manuscripts. This team chose to ignore all previous CFS classifications and generated their own classes using clinical and epidemiological data. Genetic and gene expression profiles for each of the classes were then examined. Team 2 took the approach of reducing the data complexity while retaining as much biological information of potential importance to CFS as possible. They attempted to identify common patterns of change in

Table 3. Investigators and expertise that participated in computational challenge.

Team	Investigator	Expertise	Institution
1	Peter D White	Medicine/psychiatry	University of London (UK)
	Ute Vollmer-Conna	Psychology/immunology	University of New South Wales (Sydney, Australia)
	Eric Aslakson	Physics	CDC
	Rajeevan Mangalathu	Molecular biology	CDC
	Sol Efroni	Computer science	NIH
	Liran Carmel	Biostatistics	NIH
2	Elizabeth R Unger	Medicine/pathology	CDC
	Nancy Klimas	Medicine/immunology	University of Miami (FL, USA)
	Renee Taylor	Psychology	University of Illinois (IL, USA)
	Richard C Craddock	Bioengineering	CDC
	Toni Whistler	Molecular biology	CDC
	Gordon Broderick	Chemical engineering	University of Alberta (Canada)
3	Andrew Lloyd	Medicine/infectious disease	University of New South Wales
	Jennifer Fostel	Mathematics	NIH
	Weida Tong	Computational chemistry	FDA
	Roumiana Boneva	Medicine/cardiology	CDC
4	James F Jones	Medicine/pediatrics	CDC
	Brian Gurbaxani	Bioinformatics	CDC
	Elizabeth Maloney	Epidemiology	CDC
	Ben Goertzel	Mathematics	Biomind, LLC (MD, USA)

CDC: Centers for Disease Control and Prevention; NIH: National Institutes of Health.

clinical and gene expression data and identify gene expression signatures of measured phenotypes. This team used the CFS empiric classification and their work is presented in three manuscripts. Team 3 chose to adopt an alternative approach for the analysis, which started with the premise that the diagnostic boundaries of CFS are generally arbitrary, and examine symptom domains that span health and disease such as sadness (a normal human experience) to clinical depression. Team 3 used responses to fatigue questionnaires and validated their findings by comparing to the empiric classification. Their work is presented in two papers. Team 4 used a hypothesis-driven approach and examined the physiological, genetic and genomic characteristics of allostatic load in the CFS subjects that had been defined by the empiric classification.

Discussion

Despite more than 2 decades of basic and clinical research and over 3000 peer-reviewed papers indexed in Medline, there has been little advancement of our understanding of the pathophysiology of CFS. There are at least three reasons that account for this disappointing lack

of progress. First, at the most basic level, most published studies have used patients recruited from various specialty and referral clinics so that recruitment bias and heterogeneity of patient groups within and between centers precludes critical comparison of results. This in-hospital study enrolled persons identified with CFS from the general population and, therefore, results can be generalized to people suffering from CFS with similar demographics. Second, most published studies either have had no controls or controls of convenience, so that it is impossible to critically assess the meaning of associations. This study identified controls from the same general population as the CFS cases and these controls were similar to cases in all respects other than the illness in question. Third, because of the nature of the illness, all studies suffer from ambiguities diagnosing CFS.

CFS is a complex illness representing alterations in complex systems of homeostasis. Such complex illnesses are not the result of a single mutation or single environmental factor; rather they arise from a combined action of many genes, environmental factors, risk-conferring behavior and the patients' response. This is the

Table 4. Empirical fatigue classification and general characteristics of subjects participating in the study.

	CFS (n = 58)	CFS-MDDm (n = 27)	ISF (n = 59)	ISF-MDDm (n = 28)	Control (n = 55)	All (N = 227)
% women	86.2	77.8	71.2	92.9	85.5	81.9
Median age (years)	51.5	48.0	50.0	51.5	51.0	51
% white	94.8	92.6	93.2	92.9	96.4	94.3
BMI (%)						
<18.5: underweight	1.7	0.0	1.7	0.0	1.8	1.3
18.5–24.9: normal	13.8	33.3	20.3	21.4	16.4	19.4
25–29.9: overweight	41.4	29.6	32.2	35.7	36.4	34.7
30–34.9: obese	31.0	29.6	35.6	21.4	34.5	31.7
≥35: morbidly obese	12.1	7.4	10.2	21.4	10.9	11.9

BMI: Body mass index; CFS: Chronic fatigue syndrome; ISF: Insufficient symptoms or fatigue; MDDm: Major depressive disorder with melancholy.

first study to collect information from subjects using internationally standardized and validated instruments (the SF-36, MFI and SI) in order to characterize the major domains of CFS (impairment, fatigue and accompanying symptoms). This is also a unique scientific and computational effort to share an exhaustive list of epidemiological, clinical and laboratory measures and use a multidisciplinary approach to analyze and interpret the resulting complex data.

Thus, if CFS is heterogeneous, the accompanying papers in this issue should capture the heterogeneity and provide the means to reproducibly and empirically classify CFS. Currently, there is not one button, one software program or one investigator that can integrate and interpret disparate, high-content data to provide meaningful insight for complex biological systems in healthy and disease states. However, the papers in this issue demonstrate that with collaborative, multidisciplinary efforts, new perspectives, new algorithms and novel biology can result even for the most complex datasets about one of the most complex illnesses.

Outlook

CFS is an illness that is currently defined by self-reported symptoms. Molecular profiling of CFS has demonstrated several, albeit subtle, perturbations in peripheral blood gene expression, central nervous and immune system function and metabolism. Integration of these multiple body system measures will define the extent of illness heterogeneity and enable identification of the biological and physiological pathway(s) affected in people with CFS. This information will demonstrate that CFS and other illnesses with prominent and disabling fatigue are legitimate illnesses that can be medically explained. Algorithms that capture the heterogeneity and identify perturbations will allow us to identify an objective diagnostic marker, decipher the pathophysiology and customize therapeutic intervention to control and ultimately prevent CFS.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the funding agency.

Highlights

- A 2-day in-hospital study of 227 people with chronic fatigue and nonfatigued controls was conducted. Epidemiological, clinical and biological measures were collected from each subject.
- The data, including over 500 clinical and epidemiological measures and 20,000 gene expression measures, was shared with 20 investigators who were challenged with integrating the data to delineate the heterogeneity of the study population and identify biologic correlates of chronic fatigue syndrome (CFS).
- Investigators were divided into four teams, with medicine, mathematics, molecular biology, engineering and computer science represented.
- Each team took a unique approach to integrating the data.
- Data integration and multidisciplinary collaboration resulted in novel approaches for handling large, complex datasets and revealed new insight and relevance to CFS.

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