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Gene Expression Factor Analysis to Differentiate Pathways Linked to Fibromyalgia, Chronic Fatigue Syndrome, and Depression in a Diverse Patient Sample

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Objective. **To determine if independent candidate genes can be grouped into meaningful biologic factors, and whether these factors are associated with the diagnosis of chronic fatigue syndrome (CFS) and fibromyalgia syndrome (FMS), while controlling for comorbid depression, sex, and age.**

Methods. **We included leukocyte messenger RNA gene expression from a total of 261 individuals, including healthy controls (n = 61), patients with FMS only (n = 15), with CFS only (n = 33), with comorbid CFS and FMS (n = 79), and with medication-resistant (n = 42) or medication-responsive (n = 31) depression. We used exploratory factor analysis (EFA) on 34 candidate genes to determine factor scores and regression analysis to examine whether these factors were associated with specific diagnoses.**

Results. **EFA resulted in 4 independent factors with minimal overlap of genes between factors, explaining 51% of the variance. We labeled these factors by function as 1) purinergic and cellular modulators, 2) neuronal growth and immune function, 3) nociception and stress mediators, and 4) energy and mitochondrial function. Regression analysis predicting these biologic factors using FMS, CFS, depression severity, age, and sex revealed that greater expression in factors 1 and 3 was positively associated with CFS and negatively associated with depression severity (Quick Inventory for Depression Symptomatology score), but not associated with FMS.**

Conclusion. **Expression of candidate genes can be grouped into meaningful clusters, and CFS and depression are associated with the same 2 clusters, but in opposite directions, when controlling for comorbid FMS. Given high comorbid disease and interrelationships between biomarkers, EFA may help determine patient subgroups in this population based on gene expression.**

INTRODUCTION

Fibromyalgia syndrome (FMS) and chronic fatigue syndrome/myalgic encephalomyelitis (CFS) affect 1–5 million Americans (1). Both conditions are multisymptom syndromes, with symptoms that include muscle and joint pain, fatigue, sleep disturbances, and mood dysfunction (2,3). These 2 syndromes frequently co-occur; nearly 70% of individuals with FMS meet criteria for comorbid CFS (4–6). Mood disorders also co-occur with FMS and CFS, with approximately 50% of patients reporting significant depression (7,8).

It is well documented that the presence of depression is associated with worsening of pain, functional impairment, poor sleep, and poor health outcomes in general (5). Although no conclusive causal relationships have been delineated, patients with a depression history are more likely to develop chronic pain and fatigue-related conditions later in life and, conversely, those experiencing pain are more likely to develop depression (5,9). Close association among CFS, FMS, and depression suggests the presence of shared mechanisms contributing to these conditions (10). Other evidence suggests, however, that FMS and CFS show peripheral dysfunction compared to healthy people in pain levels (11), nerve and muscle fibers (12,13), immune markers (14), and messenger RNA (mRNA) gene expression patterns (15,16), which cannot be fully explained by depression.

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Significance & Innovations

- Differences between groups can be examined by messenger RNA gene expression for clusters of intercorrelated candidate genes related to immune function, pain, and fatigue.
- Exploratory factor analysis revealed that expression of 34 genes clustered into 4 meaningful biologic factors, and higher expression of these factors was associated with specific aspects of disease, including chronic fatigue syndrome diagnosis and lower depression severity, but not fibromyalgia syndrome.
- Future studies should examine whether factors, and not independent genes, are altered in these populations following an experimental challenge, such as exercise or a treatment intervention.

Leukocyte gene expression (mRNA) is one relatively noninvasive method to assess the functional status of multiple neural and immune pathways simultaneously from a blood sample. Our previous work indicated that following moderate exercise, gene expression is increased in CFS and FMS patients at baseline for 1 cytokine, IL10, together with acid-sensing (ASIC3), transient vanilloid (TRPV1), and purinergic (P2RX4) ion channel genes (15,16). In animal models, these same ASIC3, TRPV1 and P2RX4 receptors have been shown to work in concert, activated differentially by metabolites at levels that correspond to those evoked by fatiguing versus painful exercise (17). Interestingly, several of the same genes altered in CFS following exercise were found to be upregulated in depressed people (18–20). Importantly, most prior studies examined expression of each gene individually rather than as gene groups, and studied CFS/FMS or depression groups separately, ignoring the effects of comorbidity on gene expression.

Investigation of gene expression associated with FMS, CFS, and depression may help us better understand what pathways these conditions share. There are many candidate genes, but examining a large number of genes poses analytical challenges and makes the interpretation of the results cumbersome. Identification of coherent subgroups of genes should help lead to better understanding of the gene expressions that may play an important role in these conditions. In this study, we present an alternative strategy using exploratory factor analysis (EFA), based on the premise that a set of candidate genes can be grouped into a much simpler set of cluster factors. We can then apply conventional analysis to a small number of "super-variables," rather than a much larger set of individual genes with high variability. Our study aimed at examining 2 research questions: 1) can candidate genes be separated into meaningful autonomous clusters using factor analysis? and 2) do FMS, CFS, and depression severity have unique or overlapping associations with specific gene expression clusters or factors when controlling for comorbidities?

PATIENTS AND METHODS

Participants. This is a cross-sectional study examining leukocyte mRNA gene expression from several concurrent case–control studies. Characteristics of the groups are listed in Table 1. Participants were recruited via flyers and physician referrals and included both men and nonpregnant women between ages 18–73 years. These include 33 individuals with CFS, using Centers for Disease Control and Prevention criteria (2), 15 individuals with FMS, using American College Rheumatology criteria (3), 79 patients with comorbid CFS and FMS (met both criteria sets), 31 with medication-responsive depression (prior practitioner diagnosis, symptoms currently controlled by medications), 42 with medication-refractory depression (REF; prior prac-

Table 1. Demographic and descriptive parameters for sample population*

Variables	CON (n = 61)	RESP (n = 31)	REF (n = 42)	CFS (n = 33)	FMS (n = 15)	CFM (n = 79)
Female sex†	33 (54.1)	22 (71.0)	23 (54.8)	14 (42.4)	15 (100)	65 (82.3)
Age, mean ± SD years‡	40.8 ± 14.5	39.5 ± 12.3	44.1 ± 14.5	44.7 ± 13.9	44.2 ± 14.5	48.1 ± 13.4
Depression	0 (0)	31 (100)	42 (100)	22 (66.7)	7 (46.7)	42 (53.2)
QIDS, mean ± SD†	2.3 ± 2.2	5.16 ± 3.5	19.2 ± 4.0	5.5 ± 2.8	8.8 ± 6.3	6.7 ± 3.6
None <5	56 (91.8)	19 (61.3)	0 (0)	20 (60.6)	6 (40)	35 (44.3)
Mild 6–10	5 (8.2)	8 (25.8)	0 (0)	4 (12.1)	4 (26.7)	25 (31.6)
Moderate 11–15	0 (0)	4 (12.9)	7 (16.7)	9 (27.3)	2 (13.3)	19 (24.1)
Severe 16–20	0 (0)	0 (0)	21 (50.0)	0 (0)	2 (13.3)	0 (0)
Very severe >20	0 (0)	0 (0)	14 (33.3)	0 (0)	1 (6.7)	0 (0)
Medications						
Antidepressants	0 (0)	23 (74.2)	33 (78.6)	15 (45.5)	6 (40.0)	52 (65.8)
Anticonvulsants	1 (1.6)	8 (25.8)	33 (78.6)	7 (21.2)	7 (46.7)	35 (44.3)
Pain opiates	1 (1.6)	3 (9.7)	7 (16.7)	1 (3)	1 (6.7)	22 (27.8)
Sleep medications	2 (3.2)	6 (19.4)	16 (38.1)	14 (42.4)	4 (26.7)	31 (39.2)

* Values are the number of individuals (percentage) unless indicated otherwise. CON = healthy controls; RESP = medication-responsive depression group; REF = medication-refractory depression group; CFS = chronic fatigue syndrome; FMS = fibromyalgia syndrome; CFM = meeting criteria for both CFS and FMS; QIDS = Quick Inventory for Depression Symptomatology.
† $P \leq 0.01$.
‡ $P < 0.05$.

titioner diagnosis, currently in a refractory depressive state and not responding to medications), and 61 healthy controls with no prior diagnosis of pain, fatigue, or depression. Inclusion and exclusion criteria for these individuals have been previously described (15,16,18). Exclusion criteria included active viral or upper respiratory infections, chronic cardiovascular or pulmonary disorders, or other chronic conditions, such as anemia or cancer.

Assessments. Participants provided basic medical information, including health history, comorbidities, and current medications. Depression severity was assessed using the Quick Inventory of Depression Symptomatology (QIDS) self-report, which is a validated 16-item questionnaire (21). For the participants in the REF group, the Hamilton Rating Scale of Depression (HRSD)−24 was also obtained and scored by a psychiatrist or trained staff during the clinical consult (22). For those REF participants from whom a QIDS score was not obtained (n = 9), we used patients that had both QIDS and HRSD (n = 29) scores to determine a regression equation to impute the missing QIDS values. Results matched those shown in other studies (see http://www.ids-qids.org/index2.html#table1) when matching depression test scores, including the QIDS and HRSD tests.

mRNA leukocyte gene expression. All samples were obtained and processed January 2011–March 2014, with the majority of samples (88%) processed during 2012–2013. Blood was collected, processed, and analyzed using real-time quantitative polymerase chain reaction (qPCR) as previously described (18). Briefly, blood was collected in EDTA tubes, centrifuged at 3,200 rpm for 12 minutes, plasma was removed, and the white layer (leukocytes) was carefully collected in RLT + β-mercapto-ethanol, quickly frozen using a methanol dry ice slurry, and stored at −80°C. RNA was extracted using RNeasy Mini kits (Qiagen) and converted to complementary DNA (cDNA) library using the ABI High Capacity cDNA Archive Kit (Applied Biosystems/Life Technologies). The cDNA libraries were analyzed using the ABI real-time qPCR system on the ABI Prism 7900 Sequence Detection System (SDS) 2.4.1 (Applied Biosystems), using ABI TaqMan Master Mix. Primer-probes and gene descriptions for the 34 candidate and TF2B reference control genes are listed in Supplementary Table 1 (available on the *Arthritis Care & Research* web site at http://onlinelibrary.wiley.com/doi/10.1002/acr.22639/abstract). Each targeted gene was run in duplicate, with TF2B standards run in quadruplicate. qPCR data was processed using the SDS program 2.4.1, with count values for genes computed in the curve log-linear range using a standard 0.2 threshold. Gene expression amounts were determined using the $2^{-\Delta T}$ method, where ΔT is the count difference of the candidate gene from TF2B. Gene expression data were log10 transformed with genes displaying acceptable Q–Q plots and nonsignificant swilk tests. To avoid listwise deletion, we imputed missing gene expression values with the population mean. Of the 261 individual values per gene, 21 genes had no missing value, 12 had 2 or less, and PPARA had 12 missing. Because gene variables have different scales, we conducted factor analysis using standardized Z scores for each gene.

Statistical methods. We used analyses of variance for continuous variables and chi-square tests for dichotomous variables to examine demographic group differences. Based on significant differences in age and sex, these variables were retained in all regression analyses.

Factor analysis. EFA permits examination of how unmeasured latent variables (factors) summarize patterns of correlations found in the measured relationships between genes. The Pearson correlation matrix (data not shown) revealed that many of the 34 genes have significant intercorrelations. EFA has 4 steps as follows: 1) extraction of factors such that the minimal number of uncorrelated latent factors explains the greatest proportion of common variance among these $(34 \times 33)/2$ pairwise correlations. A scree plot of eigenvalues (factor variances) versus number extracted provides a guide; 2) "rotation" until each factor is defined by a relatively few variables with high "loadings" (large standardized regression coefficients of the gene on the factor), a mathematically equivalent simple structure that facilitates interpretation; 3) biologic interpretation of the factors based on the strength of the gene loadings. EFA was conducted using STATA 13.0. To assist with biologic interpretation of the factors, we segregated genes based on factor loadings $\geq |0.4|$ (i.e., at least 15% of shared variance between the variable and summary factor). We restricted consideration to models that had at least 4 genes per factor with a minimal occurrence of non-unique genes; and 4) we estimated the latent factors by means of the factor score regression procedure, which calculates scores from a linear composite of all 34 genes. The factor score determinacy coefficient, the theoretical correlation between score and factor, yields the reliability and validity of the scores as measures. Coefficients >0.9 indicate excellent agreement.

Linear regression. Once the factors were established by the EFA, we used multivariate linear regression models to see if the factors could be predicted by demographic/diagnostic variables. Models simultaneously included 1) FMS diagnosis, 2) CFS diagnosis, 3) standardized QIDS depression severity, 4) standardized age, and 5) sex. We conducted linear regression analysis for the entire sample set and the sample set obtained by removing the REF group. For this exploratory analysis, we chose P values less than 0.05 as the significance level in order to minimize type II errors. More stringent criteria should be used when doing a confirmatory factor analysis.

RESULTS

Sample description. Table 1 provides descriptors for the different population sample groups based on their primary diagnostic criteria. There were significant diagnostic group differences for age ($F = 2.55$, $P = 0.03$) and sex ($\chi^2 = 32.67$, $P < 0.001$); in general, those with CFM or FMS tended to be older and predominantly female. Therefore, both age and sex were retained as covariates. As expected, QIDS depression severity was significantly different between diagnostic groups ($F = 122.24$, $P = 0.00$).

The remainder of the study will consider individuals as a combination of binary and continuous variables, rather than single diagnostic starting groups. For example, some-

one in the CFM comorbid group would have 2 diagnoses, FMS as well as CFS, and a QIDS score indicating how severe his or her current depressive symptoms may be. For the purposes of this exploratory study, these variables were considered to be additive, and interactions between variables were ignored.

EFA results. Factor analysis uses gene–gene correlations to separate genes into relatively independent latent factors. Organizing genes by the factor grouping revealed that 4 factors for this data set were optimal by providing simple structure: loadings either high or near zero, at least 4 genes per factor, and a minimal number of genes with high loadings on multiple factors. Table 2 displays the simple structure EFA results for the 4-factor model. The first factor accounts for 15.7% of the variance, with second and third adding 12.3%, and the fourth 10.3%. Cumulative variance for the 4 factors is therefore 50.7%. Seven genes had high ($>|0.4|$) loadings on 2 factors: HSPA2, PPARA, SULT1A1, LTA, SIRT1, TRPV1, and TLR4. There were 3 genes that did not load significantly (i.e., $<|0.4|$) on any factors: ADR2A, OXTR, and SPARC.

Factor analysis biologic groups. Factor 1 is characterized primarily by genes involved in purinergic and cellular modulator pathways, including purinergic ion channels P2RX4 and P2RX7 (both associated with neuropathic pain states), as well as cellular/immune modulators NFKB1, DBI, TNFA, and IL10. Factor 2 is characterized by top loading genes, including glucocorticoid receptor NR3C1, neuregulin (NRG)−1, chemokine receptor CXCR4, amyloid precursor protein (APP), and toll-like receptor 4 (TLR4), all of which have roles in neuronal growth and immune function. Factor 2 shares loadings for SULT1A1, LTA, SIRT1, and TLR4 with factor 3 and HSPA2A with factor 4. The third factor is characterized primarily by the ASIC3–TRPV1 complex, the NR3C2 mineralocorticoid receptor, and cytokines LTA and IL6, and therefore we classified this set as those belonging to nociception and stress mediators. The fourth factor extracted is characterized by high loadings for genes important for mitochondrial function (HSPA2, NDUFS5, ATP5E, and COX5B), and the GPCR purinergic receptors P2RY1 and P2RY2 that respond to metabolites generated by the mitochondrial machinery, and therefore tentatively classified as those pertaining to energy and mitochondrial function.

Relationship between factors and diagnostic characteristics using linear regression. Having identified 4 biologically meaningful factors from the gene expression data, we next computed factor scores that are a linear combination of all genes. Factor score determinacy coefficients all exceeded 0.95, indicating excellent reliability and validity for the factor scores as measures of latent factors. Because of the high comorbidity of CFS, FMS, and depression, it is important to control for these variables simultaneously. We used linear regression with standardized β coefficients to predict the factor scores using FMS, CFS, depression severity, age, and sex.

Furthermore, because the REF group members were in a state of extreme depression with significantly higher QIDS scores than other patient groups, we also examined the same regression models in a reduced sample excluding the REF group. Results for both models are shown in Table 3.

Table 2. Results of exploratory factor analysis: factors and factor loadings*

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Uniqueness
P2RX4	0.78				0.32
P2RX7	0.76				0.40
TNFA	0.67				0.53
PPARA	0.61		0.41		0.41
HCN2	0.61				0.58
COMT	0.61				0.57
NFKB1	0.57				0.59
VEGFA	0.56	0.33	−0.37		0.43
ADRB2	0.55			0.33	0.59
TRPV4	0.50		−0.32		0.62
DBI	0.45				0.71
IL10	0.44	0.27	−0.26		0.65
NR3C1		0.84			0.25
CXCR4		0.80			0.33
APP		0.78			0.28
NRG1		0.75			0.42
SULT1A		0.46	0.42		0.51
NR3C2			0.83		0.27
LTA		0.42	0.77		0.21
ASIC3	0.37		0.65	−0.28	0.36
TRPV1	0.45	0.37	0.57		0.32
IL6		0.25	0.53		0.65
SIRT1	0.39	0.40	0.46		0.47
SOD2			−0.58		0.60
TLR4		0.41	−0.58		0.46
NDUFS5				0.90	0.16
ATP5E				0.84	0.26
HSPA2	−0.31	0.40		0.63	0.34
COX5B	0.27			0.62	0.54
P2RY1				0.61	0.59
P2RY2		0.27		0.43	0.67
ADR2A				0.35	0.82
OXTR					0.93
SPARC					0.93
Total variance, %	15.7	12.3	12.3	10.3	
Cumulative variance, %	15.7	28.0	40.4	50.7	

* Factor loadings for 34 genes using exploratory factor analysis from a mixed population of 261 subjects. Genes are organized based on decreasing loading magnitude, with groupings demarcating simple structure by inclusion of gene loadings of $>|0.4|$. Several genes displayed high loadings on 2 factors. Loadings <0.25 are left blank for ease of interpretation. Genes *ADR2A*, *OXTR*, and *SPARC* did not load significantly ($<|0.4|$) on any factors.

In the full sample set, we found that factor 1, representing purinergic receptors and cellular modulators, had significant positive association for CFS ($\beta = 0.34$, $P = 0.03$), age ($\beta = 0.16$, $P < 0.01$), and sex ($\beta = 0.57$, $P < 0.01$). Without the REF group, similar or strengthened associations were seen for CFS, age, and sex with a negative QIDS association ($\beta = -0.23$, $P = 0.05$). Factor 2, representing immune function and growth factors, had only a negative association for age ($\beta = -0.20$, $P < 0.01$) with no differences when the REF group was removed. Factor 3, representing genes related to

nociception and stress mediators, had a negative association for age ($\beta = -0.27$, $P < 0.01$). Without the REF group, there was also a positive association for CFS ($\beta = 0.39$, $P = 0.02$), a negative association with QIDS ($\beta = -0.27$, $P = 0.02$), and continued negative association with age. Finally, factor 4, representing energy and mitochondrial function, showed a negative association for depression severity ($\beta = -0.16$, $P = 0.01$). This relationship was abolished when the REF group was excluded. However, given the model's low R^2 value of 0.03, the association is likely spurious. Contrary to expectations, FMS was not significantly associated with any of the factors, either in the full sample or when the REF group was excluded.

Table 3. Regression analysis results of factor scores with demographic characteristics as predictor variables*

	Full sample			Without REF		
	β	P	R^2	β	P	R^2
F1						
FMS	−0.17	0.28	0.13	−0.02	0.93	0.14
CFS	0.34	0.02†		0.34	0.04†	
QIDS	−0.09	0.13		−0.23	0.05†	
Age	0.16	0.01†		0.15	0.03†	
Sex	0.56	0.00†		0.67	0.00†	
F2						
FMS	−0.04	0.80	0.07	−0.10	0.62	0.08
CFS	−0.22	0.17		−0.23	0.19	
QIDS	−0.10	0.09		−0.08	0.54	
Age	−0.20	0.00‡		−0.02	0.00‡	
Sex	−0.15	0.28		−0.20	0.23	
F3						
FMS	−0.24	0.16	0.08	−0.06	0.73	0.11
CFS	0.25	0.11		0.39	0.02†	
QIDS	0.04	0.48		−0.27	0.02†	
Age	−0.27	0.00‡		−0.27	0.00‡	
Sex	−0.13	0.35		−0.16	0.29	
F4						
FMS	0.07	0.68	0.03	0.00	0.99	0.01
CFS	−0.00	1.00		−0.07	0.70	
QIDS	−0.16	0.01†		0.00	1.00	
Age	0.00	0.99		−0.02	0.82	
Sex	−0.16	0.25		−0.14	0.40	

* Standardized β coefficients and *P* values are shown next to each predictor variable. The R^2 for each model is also included. Linear regression analysis models with factor scores (F1–F4) as the dependent variable and inclusion of fibromyalgia (FMS), chronic fatigue syndrome (CFS), Quick Inventory for Depression Symptomatology (QIDS), age, and sex as the predictor variables. Full sample includes all individuals (n = 261). Without REF (medication-refractory depression group) sample excludes the REF group (n = 219).
† $P \leq 0.05$.
‡ $P < 0.01$.

DISCUSSION

It is well documented that FMS and CFS often co-occur, and comorbid depression is common for both (4–6). Unfortunately, despite well-documented patient heterogeneity within these diseases (6,23–26), research has typically relied on simple group comparisons between patient group and healthy controls while ignoring patient comorbidities. Given the sparsity of studies identifying biologic factors associated with comorbidities and patient heterogeneity, consideration of these factors is critical for the advancement of our knowledge about pathophysiology of these conditions.

The mRNA genetic expression related to FMS, CFS, and depression is complex. These conditions may be maintained by the expression of very large numbers of genes, which almost certainly interact in complicated patterns to produce the diversity of phenotypic comorbidities. The conventional candidate gene approach regards such genes as largely independent and capable of modulating behavior individually. However, even with the relatively small number of 34 candidate genes, total independence is biologically implausible and statistically impossible. The primary aim of the current exploratory study was to present an alternative strategy to examine whether candidate genes from blood leukocytes could be grouped into meaningful biologic clusters in a mixed population of FMS, CFS, depression, and healthy controls. The second aim was to examine whether the factors would segregate with diagnostic variables within this population.

As shown in Figure 1, the data set used a gene panel of 34 genes representing elements from biologic pathways previously implicated in pain, fatigue, and depression, including ion channels, mitochondrial function, immune/inflammation, monoamine receptors, transcription factors, and cellular signaling modulators. Using EFA, we identified 4 independent factors that account for 51% variance, and we have classified these, based on the top loaders of each group, as having characteristics pertaining to factor 1 (purinergic and immune modulators), factor 2 (neuronal growth and immune function), factor 3 (nociception and stress mediators), and factor 4 (energy and mitochondrial function). Not surprisingly, genes from one family, e.g., IL10, IL6, TNFA, and LTA immune function, will appear in different factors. This simply relates to the fact that within the larger class of immune markers, certain subsets are correlated with genes from other families. We next examined if these factor scores were associated with diagnostic variables FMS and CFS, as well as depression severity. Therefore, in our analyses, we have in effect conducted analyses on 4 gene expression super-variables, rather than 34 genes, and therefore are less subject to capitalization on chance from multiple comparisons. At the present we may not fully understand these groupings and can only speculate at their possible relationships and biologic significance. Below we briefly discuss the biologic rationale for each factor and how that factor may be associated with clinical variables from our regression results.

The first factor extracted, factor 1 (purinergic and immune modulators), is characterized by the purinergic ion channels, P2RX4 and P2RX7, and several cellular/immune modulators, including TNFA, NFKB1, DBI, and IL10. The P2 ligand-gated channels are opened by ATP and often form multimeric complexes. They are expressed in many cell types, including immune, microglia, and glial cells, and have roles in immune and inflammation pathways. Recent reviews describe the many functional roles that these receptors could play in mood disorders and pain in animals and humans (27,28). Among the signaling targets are the transcription factor NFKB1 and downstream cytokines such as TNFA and IL10. These have all been implicated in FMS, CFS, and

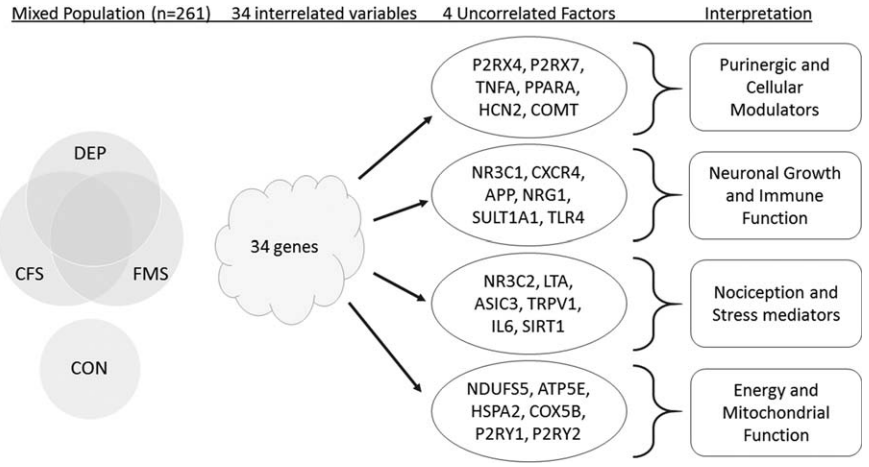

Figure 1. Exploratory factor analysis (EFA) process. Far left shows the overlap of 3 major diagnostic groups (fibromyalgia [FMS], chronic fatigue syndrome [CFS], and depression [DEP]). Data from 34 candidate genes for each of 261 individuals were incorporated into the EFA. The EFA uses the gene–gene correlations to determine a set of functional autonomies, where the elements within a factor are highly correlated but are distinct from those in other factors. We used conventional criteria to determine the number of optimal factors: only loadings of $>|0.4|$ are considered significant, at least 4 genes per factor, and minimized factor models with genes that load onto multiple factors. Results from the EFA resulted in 4 uncorrelated factors, the top loaders of which are shown. Based on the composition of each group, the biologic interpretation of the factor is listed. CON = healthy controls.

depression (29–32). Our results suggest an association of CFS for this factor when controlling for FMS and depression severity. Depression severity had a negative association in the reduced sample without the REF depression group. Our previous research in gene expression following exercise did not reveal differences for CFS at baseline (16). The current results suggest this super variable may be particularly important for CFS. This also may argue that CFS is a distinguishable disease entity from depression, although they may both share dysfunction in these genes, because higher expression was linked to presence of CFS, but also to lower depression severity score. Furthermore, since several studies have shown that exercise is associated with abnormal changes in inflammatory pathways, future research should examine factor scores before and after an exercise challenge (15,16,33,34).

Factor 2 (neuronal growth and immune function) has positive loadings for genes that are involved in neuronal growth and immune function. Compared to factor 1 that has immune cellular modulators, including IL10 and NFKB1, the genes in this group have upstream roles in controlling these modulators and thereby immune cell growth and function. In both models with and without REF depression there was no association of factor 2 with either CFS or FMS. Since growth factors may be altered following an insult or challenge, baseline levels may not show any differences, therefore highlighting the need for multiple time point studies.

Factor 3 (nociception and stress mediators) is characterized by positive loadings for the ion channel receptor complex TRPV1–ASIC3 and mineralocorticoid receptor (MR) NR3C2. As described previously, these receptors have important roles in the sensation of pain, inflammation, and fatigue (35,36), and are associated with postexertional pain and fatigue worsening in CFS patients (15,16). They may also have roles in depression and anxiety (37–39). Blocking MR can decrease pain in animal models (40,41) and may be involved in pain from diabetic neuropathy (42). Factor 3 also has loadings for LTA and IL6, inflammatory cytokines that are known to interact with the TRPV1, ASIC3, and MR receptors. Regression analysis suggests that diagnosis of CFS is positively associated with this factor, showing a positive trend in the full sample and significant association when individuals with severe refractory depression are not included. Furthermore, QIDS score is negatively associated with this factor, but only when the REF group is not included. Given the role of these receptors in pain, fatigue, and depression, these results suggest that CFS patients show dysfunction in this pathway (enhanced expression), and just as with the genes in factor 1, depression is linked to a directionally opposite dysfunction than CFS in the same pathway (decreased expression).

Factor 4 was characterized by genes involved in energy and mitochondrial function. This collection of genes was of great interest given their involvement in muscle function and fatigability in FMS and CFS (43,44). Previous studies have also examined mitochondrial function gene expression in affective disorders (45–49). Despite its potential relevance, our regression models suggest that factor 4 was unrelated to FMS or CFS diagnosis, and was associated with depression only when including patients with severe refractory depression. Rather than concluding that this factor is not dysregulated in CFS or FMS, however, we suggest instead that future studies should examine changes in expression following an energy-related challenge such as sustained exercise.

Overall, the factor analysis results support the use of EFA to delineate a smaller set of super-variables using gene–gene intercorrelations from a larger number of individual genes. The current set of candidate genes was chosen based on pre-

vious literature documenting involvement in these disorders. These genes almost certainly do not represent the exhausted list of target genes relevant to the FMS and CFS populations. In fact, the current regression results suggest that at least at baseline, gene expression was related to CFS and depression severity in factors 1 and 3, with no relationship to FMS for any factors. Therefore, it is possible that these genes are more related to fatigue than FMS-pain, which is supported by greater changes following an exercise challenge in the CFS but not FMS population (15). Future studies may consider the inclusion of other genes of interest that expand upon the nominal factor groupings. Furthermore, while this study focused on FMS-pain and CFS-fatigue, these individuals may also have other sources of pain. It is possible and likely that gene expression factors will differ for other pain conditions, including arthritis, lower back pain, neuropathic pain, and cancer pain.

Our study included the entire spectrum of healthy, depressed only, FMS/CFS only, and comorbid groups. This is in contrast to previous studies that compared these disorders to nondepressed, nonmedicated healthy controls (15,16,18,19). Comparisons between patients with chronic pain/mood conditions and healthy controls are difficult to understand because of multiple confounders. Teasing out all potential confounders is a challenging task; however, future confirmatory analysis should include other factors that are likely important, such as life-style fitness, obesity, sleep quality/quantity and disorders, and concurrent medications, all likely to contribute to disease severity and potentially gene expression changes.

There are several noteworthy limitations to this study. First, the data set is cross-sectional and represents only a single time point. Previous research has suggested that individuals with CFS expose their biologic differences compared to controls following an experimental challenge (16). Therefore, it is not surprising that individuals at baseline may show fewer differences related to FMS, CFS, and depression compared to controls. It is critical that future studies examine these factors following an experimental challenge or treatment intervention known to induce symptom improvement. Secondly, this study began with the focus on high comorbidity of CFS and FMS with depression. However, these conditions share high incidence of other disorders, including irritable bowel syndrome, restless leg syndrome, and temporomandibular joint disorders, among others. These comorbidities contribute to disease heterogeneity and may have obscured possible relationships of gene expression with FMS or CFS. Third, we have chosen to name the factors based on the characteristics of the top loading genes. However, these classifiers do not encompass the diverse functions of all the contained genes. Future studies that expand on the candidate genes and in a confirmatory factor analysis setting would benefit from using gene ontology tools to further investigate gene commonalities.

The objective of this study was to identify clusters of genes that displayed functional autonomy at a statistical and measurement level (high correlations within clusters but low correlations across clusters). Capturing this exploratory objective faithfully in a confirmatory setting is challenging (50). When our data are considered in an exploratory structural equation model (confirmatory) context, however, the fit produced is acceptable (root mean square error of approximation [RMSEA] 0.067; 90% confidence interval 0.060–0.075). RMSEAs <0.07 are generally considered acceptable. Other confirmatory indices (Comparative Fit Index 0.944, standardized root mean residual 0.024) were also consistent with acceptable fit. This in no way demonstrates that the clusters identified are uniquely accurate; it merely shows that they provide an acceptable approximation of the correlations among the 34 genes sampled in this study.

The results of this EFA study support the notion that gene expression relevant to FMS, CFS, and depression can be grouped into biologically coherent and meaningful categories. Because the groupings are based on gene–gene correlations and combine genes that are in multiple overlapping pathways, future research studies should further examine the nature of the grouping relationships by using confirmatory factor analysis on a longitudinal data set. We also examined whether gene factors, rather than individual genes, would segregate with specific symptoms. Preliminary exploratory results suggest that CFS and depression severity, but not FMS, are associated with the factor scores when controlling for age, sex, and comorbid symptoms, but that CFS is linked to increased expression, while depression is linked to decreased expression of the clustered genes. As research continues to better understand complex disorders, including pain and fatigue, it is critical to better understand heterogeneous populations and how comorbid conditions can modulate physiology. Given that clinical interventions for CFS and FMS often require multimodal treatments, similarly, research studies should treat these populations as a combination of diseases and symptom presentations rather than singular classifications.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be submitted for publication. Dr. Iacob had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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