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Comparing Genomic Profiles of Women With and Without Fibromyalgia

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Abstract

Background—Fibromyalgia syndrome (FMS), a chronic musculoskeletal condition characterized by diffuse pain, fatigue, sleep impairment, and cognitive dysfunction, is associated with significant functional disability. Its underlying biological mechanisms are unknown. This study investigated differentially expressed genes between women with FMS and healthy volunteers.

Methods—Women who met the 1990 or 2010 American College of Rheumatology fibromyalgia criteria were compared to age- and race-matched pain-free healthy women. Peripheral blood samples were collected, and a full genome microarray gene expression analysis was performed. One-way analysis of variance was used to identify differentially expressed genes using the filtering criterion of 1% false discovery rate. Analysis of canonical pathways associated with these genes was performed. Confirmatory quantitative real-time polymerase chain reaction and enzyme-linked immunosorbent assay verified microarray results. Independent *t*-tests compared gene and protein expression between groups.

Result—Participants were 54 women with FMS and 25 controls. Expression arrays from a subset of women with FMS ($n = 29$) and controls ($n = 20$) showed upregulation of 12 genes (>1.8 -fold change, $p < .05$) in the FMS sample. Differentially expressed genes were related to B-cell

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development, primary immunodeficiency signaling, and mitotic roles of polo-like kinase. *CENPK* and *HSP90AA1* were the most differentially expressed genes ($p < .01$).

Conclusion—Activity of interrelated pathways related to immune response, and homeostasis appears to be relevant to the experience of FMS. Replication and exploration of the relationship between gene expression and symptom severity will help determine clinical relevance of these findings.

Keywords

genomic profile; fibromyalgia; pain; fatigue

Fibromyalgia syndrome (FMS) is a chronic condition characterized by chronic, persistent widespread muscular pain and tenderness, fatigue, sleep disturbance, cognitive dysfunction, and other somatic symptoms such as headache, gastrointestinal discomfort, stiffness, bladder symptoms, diarrhea, constipation, and paresthesias (Wolfe et al., 2010). It affects about 2–4% of the U.S. population, mostly middle-aged women (Branco et al., 2010; Lawrence et al., 2008).

Unfortunately, the essential biologic mechanisms that cause and maintain FMS symptoms remain elusive (Bellato et al., 2012). Many assume that fibromyalgia is caused by interactions between multiple etiological factors, including inherited susceptibilities; traumatic, stressful, environmental exposures; and sociocultural influences (Ablin, Cohen, & Buskila, 2006). Although previous studies have provided insight into essential risk factors, they have not revealed the functional biological pathways of FMS. An understanding of how these risk factors are represented in functional biologic pathways would be of great scientific value.

Gene expression analysis is able to provide a sense of the biological moment by measuring the concentrations of specific mRNA at a single point in time. Prior gene expression studies in FMS have focused on the expression of preselected candidate genes, such as those associated with serotonergic pathways (Y. H. Lee, Choi, Ji, & Song, 2012), adrenergic nervous pathways (Gursoy et al., 2003), glucocorticoid and mineralocorticoid receptors (Lukkahatai, Majors, Reddy, Walitt, & Saligan, 2013), dopamine receptor (Malt, Olafsson, Aakvaag, Lund, & Ursin, 2003), and ion channels (A. R. Light et al., 2012), mostly using real-time PCR techniques. Such studies have reported associations between FMS and gene polymorphisms related to the modulation of analgesic pathways (*COMT*, GTP cyclohydrazase, Types 2 and 3 adrenergic receptors, P2X7 receptor pore, *TAAR1*, *RGS4*, *CNR1*, and *GRIA4*; Diatchenko, Fillingim, Smith, & Maixner, 2013; Diatchenko, Nackley, Tchivileva, Shabalina, & Maixner, 2007). To date, researchers have not made an attempt to broadly understand gene expression in thousands of known physiologic pathways.

In the present study, we employed full genome microarray analysis to explore the relative expression level of more than 54,000 probe sets of about 47,400 transcripts with more than 38,000 well-characterized human genes (Affymetrix, 2014). We compared differential expressions of genes from whole blood RNA between Caucasian women with FMS and Caucasian healthy female volunteers and selected the two most differentially expressed

genes, as determined by fold change, for gene and protein verification. We also identified the associated functional pathways of the differentially expressed genes.

Methods

Participants

We recruited participants as part of an institutional review board (IRB)-approved Medstar Health Research Institute protocol. Self-referred women were screened from March 2012 to December 2013 for study eligibility on their first outpatient visit. The inclusion criteria included age 18 years or older, an FMS diagnosis after a physical assessment by an evaluating rheumatologist (B.W.), and the meeting of either the 1990 or the 2010 American College of Rheumatology (ACR) criteria for FMS. The 1990 ACR criteria required the self-report of widespread pain in all four quadrants of the body that had persisted for at least 3 months, exhibition of 11/18 tender points on an examination, and the lack of any other reasonable explanation for the symptoms (Wolfe et al., 1990). In 2010, the ACR published preliminary diagnostic criteria that included subjective measurements of fatigue, cognitive dys-function, sleep problems, and other somatic symptoms and removed the need for measuring tender points (Wolfe et al., 2010). These later criteria utilize the Widespread Pain Index (WPI), which sums the number of nonarticular somatic areas (of 19) that caused pain in the last week, and the Somatic Severity Scale (SSS), which quantifies the number and severity of nonpainful FMS symptoms on a 0–12 scale. Patients are diagnosed with FMS if either the WPI ≥ 7 and the SSS ≥ 5 or the WPI is 3–6 and the SSS ≥ 9 (Wolfe et al., 2010). We also assessed participants for other FMS-associated symptoms including anxiety, irritable bladder, irritable bowel, pelvic pain, vulvodynia, headache, chest pain, and paresthesia. All participants were enrolled and evaluated at one of the two hospitals in the northwest of Washington, DC. The rheumatologist saw all participants with FMS once a week in a central outpatient FMS research clinic. Participants with FMS were not compensated for their study participation.

Healthy women matched with participants with FMS on race and age were used as control subjects. These women were participating in an actively enrolling National Institutes of Health (NIH), IRB-approved protocol (NCT00888563). We did not evaluate these healthy volunteers using either the 1990 or the 2010 ACR criteria, and they were excluded from the study if they reported clinically significant pain or fatigue, had significant medical comorbidity, were taking medications known to cause fatigue, had worked late evening and/or night shifts within the past month, had a severe psychiatric condition, were pregnant or lactating, reported consuming >300 mg of caffeine-containing beverages or >1 lb of chocolate a day, reported consuming >2 servings of alcohol-containing beverages every day, or had a detectable blood alcohol content. The protocol was developed to compare genotypic and phenotypic characteristics of healthy volunteers to those of a clinical population suffering from pain and fatigue. We obtained informed consent from all participants before administering study measures. All study measures were completed in one out-patient clinic visit. Healthy volunteers were compensated for their participation based on NIH guidelines.

Measures

We obtained demographic and clinical data (gender, age, WPI score, and symptom severity) via medical chart review. We assessed participants' symptom experiences with the following questionnaires:

The Brief Pain Inventory (BPI) was used to assess pain intensity and the level to which pain interfered with daily life (i.e., pain interference). This self-administered questionnaire addresses pain over the last 24 hr, on average, and at the present moment. Pain severity is rated from 0 (*no pain*) to 10 (*worse pain possible*). Pain interference is also rated from 0 (*does not interfere*) to 10 (*greatest possible interference*), according to how much the respondent feels that pain interferes with her general activity, mood, walking ability, work, relations with others, sleep, and enjoyment of life. Authors have reported strong internal consistency reliability for the BPI in patients with chronic pain (Cronbach's α s of .85 for pain intensity and .88 for pain interference; Tan, Jensen, Thornby, & Shanti, 2004).

The Hospital Anxiety and Depression Scale (HADS) was used to assess depression and anxiety. It is a 14-item instrument, with each item rated using a 0–3 scale. Total score ranges from 0 to 21. Higher scores on the depression and anxiety sub-scales indicate more symptoms. The HADS showed good internal consistency reliability in prior research (Cronbach's coefficient .80 for anxiety and .76 for depression; Mykletun, Stordal, & Dahl, 2001).

Gene Expression in Peripheral Blood

We collected peripheral whole blood from each participant using RNA PAXgene tubes (Qiagen, Frederick, MD). We then isolated total RNA from the whole blood following the PAXgene blood RNA kit procedure. All extracted RNA was treated with DNase (Qiagen) during purification. Input RNA quality was tested using NanoDrop (ND-1000; Wilmington, DE) and Bioanalyzer (Agilent 2100 bioanalyzer; Agilent Technologies, Santa Clara, CA). A total of 100 ng of RNA from each sample with 1.9–2.1 A_{260}/A_{280} ratio and 6.5–9.0 RNA integrity number was used for the microarray experiment. The cDNA and cRNA synthesis, amplification, hybridization, and scanning were performed following the GeneChip® 3' IVT Express kit protocol (Affymetrix, Santa Clara, CA). Probe set summarization and initial data quality examination of 49 Affymetrix microarray chips (HG U133 plus 2.0, Santa Clara, CA) were performed using the Affymetrix Expression Console (AGCC, 3.0 V). The Partek Genomics Suite, version 6.6 (Partek, St Louis, MO), was used for subsequent data analysis.

We calculated the power analysis for this experiment based on information from a prior study (Hsiao, Wang, Kaushal, & Saligan, 2013), which required 15 subjects to obtain 80% power to detect differences in gene expression using the microarray platform. The microarray portion of the present study compares the gene profiles of a subset of 29 women with FMS and 20 healthy women drawn from the larger sample.

Real-Time Polymerase Chain Reaction (qPCR) Array

To confirm the differential gene expression observed from the microarray data, we performed qPCR using the same RNA used in the microarray experiment. First-strand

cDNAs were synthesized using RT2 First Strand Kit (Qiagen), with 100 ng of total RNA from each sample and subsequently diluted 10-fold with deionized water (ddH₂O). The qPCR amplification mixers (10 µl) contained 1 µl of diluted first-strand cDNA, 10 µl of 2× RT2 Real-Time SYBR Green/Rox PCR Master Mix (Qiagen) and 400 nM of forward and reverse primers. We used the commercially available qPCR primer assays for the two most differentially expressed genes (Qiagen) in the present experiment. Reactions were carried out using the ABI PRISM 7900HT Sequence Detection System and were subjected to an initial 10-min denaturation at 95°C and 40 cycles each at 95°C for 15 s and at 60°C for 60 s. Two reference genes (*GAPDH* and *ACTB*) were selected for normalization of the data. These two genes are commonly used as reference genes because they are expressed at relatively high levels with low levels of variance in expression across tissue types and experimental conditions (de Jonge et al., 2007). When calculating for delta quantification cycle (ΔC_q) values, we used the geometric means of C_q values of the two reference genes.

Protein Confirmation

We confirmed the concentration of proteins encoded by the selected differentially expressed genes using enzyme-linked immunosorbent assay (ELISA). The commercially available human ELISA kits for the proteins encoded by the two most differentially expressed genes (MyBiosource, San Diego, CA) were used to validate their expression using plasma samples separated from whole blood collected from the same study participants as in the microarray experiment using EDTA tubes and stored at –80°C. Frozen plasma samples were thawed on ice and centrifuged at 1,000 × g for 10 min at 4°C. ELISA was performed using 100 µl of nondiluted plasma samples according to manufacturer's guide. The plates were read using the VICTOR3 microplate reader at 450 nm. To control the quality of the experiment, all samples were tested in duplicate and run by one technical staff member experienced in conducting ELISA. The intra-assay variations (CVs) were below 10%.

Power analysis for the protein validation was calculated. A prior study required 21 subjects to obtain 80% power to detect differences in the expression of the protein of interest using ELISA (Molvarec et al., 2007). The validation portion of the present study compares the gene and protein expressions of 54 women with FMS and 25 healthy women.

Statistical Analysis

We calculated descriptive statistics to describe the participants' demographic characteristics. We imported 49 Affymetrix CEL files (files contained the intensity calculation of the pixel value of the scanned image from microarray chips) into Partek® Genomic Suite™ (Partek Inc., St. Louis, MO) using the Partek default normalization parameters. Probe-level data or transcripts were pre-processed, background corrected, normalized, and summarized with the robust multi-array average method (Wu, Williams, & Koch, 2005). Differential gene expression analysis using one-way analysis of variance was performed between the microarray chips of women with FMS and healthy women. The differentially expressed genes were selected based on a filtering criteria of greater or less than 1.8-fold change and 1% false discovery rate with a p value of < .05. Quality assurance and quality control of the microarray data were confirmed by examining outliers from the histograms of the microarray data generated. Outliers were excluded from the analysis.

Ingenuity pathway analysis (IPA; Ingenuity® Systems, www.ingenuity.com, Redwood City, CA) was used to identify functional networks of the differentially expressed probe sets from Ingenuity's knowledge base. Right-tailed Fisher's exact test was used to calculate the *p* values determining the probability that each biological function and/or disease assigned to these networks was due to chance alone.

The delta quantitative cycle (C_q) method PCR Array Data Analysis Portal (<http://www.sabiosciences.com/pcrarraydataanalysis.php>; SABiosciences Corp., Qiagen) was used to analyze the qPCR data. The quantitative cycle (C_q), or cycle threshold (C_t), method uses the point or cycle number experiment where the fluorescence curve of the sample exceeds the fluorescence curve of the background in the qPCR. The data analysis portal calculated the C_q values from the difference between the C_q of the target gene and the C_q of the reference genes. The C_q was calculated from the difference between the C_q of the women with FMS and C_q of the healthy volunteers of the target genes. The fold change of target gene expression ($2^{-(C_q - C_{q0})}$) was calculated using the normalized gene expression ($2^{-(C_q - C_{q0})}$) in the women with FMS divided by the normalized gene expression ($2^{-(C_q - C_{q0})}$) in the healthy volunteers. The lower average C_q represents the higher gene concentration. A fold change of >1 indicated upregulation of the gene and <1 indicated downregulation of the gene. We used SPSS, version 19, to run independent *t*-tests to compare ELISA plasma results between the women with FMS and healthy women.

Results

We enrolled 54 women with FMS (43.4 ± 7.2 years old) and 25 age-matched controls (38.9 ± 8.7 years old) in the study. Among the women with FMS, 38 met both the 1990 and 2010 diagnostic criteria, while 10 met either the 1990 or the 2010 diagnostic criteria. The mean number of years since diagnosis with FMS was 9.77 ± 6.65. The mean body mass index (BMI) of women with FMS (29.4 ± 9.5) was significantly higher than the mean BMI of the healthy volunteers (26.0 ± 5.8, *p* = .17). As expected, women with FMS reported significantly higher pain intensity (4.6 ± 1.9) and pain interference (4.9 ± 2.8) than healthy controls (pain intensity = 1.4 ± 1.2, *p* < .01; pain interference = 0.0 ± 0.0, *p* < .01). The levels of depression (7.7 ± 4.1) and anxiety (9.1 ± 4.5) in the women with FMS were also significantly higher than they were in our healthy controls (depression = 0.6 ± 1.6, *p* < .01; anxiety = 1.7 ± 2.0, *p* < .01). Using the ACR criteria, the women with FMS reported widespread tenderness with an average tender point score of 14.5 of 18 bodily sites. They also reported moderate-to-high fatigue (2.2 ± 0.8 out of 3) and unrefreshed waking (2.2 ± 0.9 out of 3), a moderate number of somatic symptoms (2.0 ± 0.7 out of 3), and mild cognitive dysfunction (1.2 ± 1.0 out of 3) with a mean symptom severity score of 8.11 ± 2.27 out of 12 (Table 1). A report of a recent survey revealed a similar mean symptom severity score in patients with fibromyalgia (7.4 ± 2.0), which is higher than the mean symptom severity score of the general population (1.7 ± 1.9; Wolfe, Brahler, Hinz, & Hauser, 2013).

We conducted an initial microarray experiment on the RNA collected from a subset of 29 of the women with FMS and 20 of the healthy volunteers. To increase the statistical power, we included 25 additional women with FMS and five healthy volunteers in the confirmation

experiments with the qPCR and ELISA. Neither demographic nor symptom experiences of the subset of women with FMS and healthy controls in the micro-array experiment differed significantly from those in the full cohort. The BMI of the subset of women with FMS ($25.2 \pm 5.0 \text{ kg/m}^2$) in the microarray experiment was significantly lower than that of the women with FMS in the PCR and ELISA experiment (29.4 ± 9.5 ; $p .03$; Table 1).

We found that 12 probe sets were differentially expressed between women with FMS and healthy controls in the microarray experiment (Table 2), with all 12 differentially expressed genes upregulated in the FMS group. Of these 12 genes, 11 are associated with cellular growth and proliferation, in particular cell signaling and molecular transport (Tables 3 and 4). The IPA revealed three functional networks that are closely associated with these differentially expressed genes, including the glucocorticoid receptor signaling, B-cell receptor signaling, and protein ubiquitination pathways.

Confirmation of Two Differentially Expressed Genes

To increase the power of the analysis, we used the same RNA we used in the microarray experiment and added an additional 25 women with FMS and five healthy volunteers in the confirmation experiments for a total sample size of 54 women with FMS and 25 healthy controls. Using qPCR and comparing average Cq, we confirmed the expression of the most differentially expressed gene, *CENPK* (fold change = 1.52), and the most central gene connecting all three functional networks, based on the IPA, *HSP90AA1* (fold change = 1.57; Figure 1), finding a significant overexpression of these two genes in women with FMS compared to healthy controls ($p < .05$; Table 5).

To determine corresponding upregulation of protein products encoded by *CENPK* and *HSP90AA1*, we conducted ELISA on plasma samples collected from the same 54 women with FMS and 25 healthy, pain-free controls that we used in the qPCR experiment. The results confirmed that centromere protein K (CENPK) and heat shock protein (HSP) 90 kDa alpha (cytosolic) class A member 1 (HSP90AA1) plasma concentrations were significantly higher in women with FMS (CENPK = $46.2 \pm 37.7 \text{ pg/ml}$, HSP90AA1 = $19.5 \pm 14.3 \text{ ng/ml}$) compared to the matched healthy controls (CENPK = $26.4 \pm 16.3 \text{ pg/ml}$, HSP90AA1 = $11.1 \pm 6.7 \text{ ng/ml}$, $p < .05$; Figure 2).

Discussion

We compared the gene expression patterns of thousands of known physiologic pathways between women with FMS and healthy race- and age-matched controls and found statistically significant elevations in the expression of 12 genes that are likely part of linked networks responsible for cellular growth and proliferation. We were able to confirm the elevated gene expressions of both *CENPK* and *HSP90AA1* by qPCR as well as document elevations in the actual protein concentrations with ELISA, demonstrating that these findings are robust. Consistent with previous studies (Bellato et al., 2012; Wolfe, 1999; Wolfe et al., 2010), participants with FMS in the present study reported a cluster of symptoms characterized by higher pain, anxiety, and depressive symptoms compared to healthy controls.

Our results showed that most of the genes that were upregulated in the women with FMS were associated with immune system regulation. Although FMS is considered to be a non-autoimmune disease, patients with FMS experience autoimmune-related symptoms such as pain, photosensitivity, and Raynaud phenomenon (Donmez, Pamuk, Umit, & Top, 2012; Giacomelli, Talarico, Bombardieri, & Bazzichi, 2013). Evidence of a direct link between CENPK and immune function is scant. Some studies have reported that anticentromere protein (CENP) antibodies can serve as a biomarker for autoimmune disease (Jabs et al., 1993; Mahler et al., 2011). Our results suggest that B-cell development and primary immunodeficiency signaling are the two main pathways that are associated with the differentially expressed genes observed in our sample of women with FMS. Evidence of B-cell involvement with FMS has not been reported elsewhere; however, researchers have reported that dysregulation of B-cell activation and increased numbers of B cells play a role in cognitive dysfunction by influencing migration of pro-inflammatory cytokines across the blood-brain barrier (Sternberg, 2006) and producing antibodies to the cerebral myelin antigen, which cause cerebral demyelination (Cercy & Bronson, 2010; Nikbin, Bonab, Khosravi, & Talebian, 2007). The involvement of B cells with sleep disturbance and cognitive dysfunction in women with FMS is unclear because we did not assess the healthy volunteers for their sleep or cognitive function. Further investigation is warranted to determine the role of B cells in the polysymptomatic experience of FMS.

CENPK was the most differentially expressed gene between the women with FMS and the healthy controls (fold change = 1.52 in the women with FMS). *CENPK* is one of the CENP-H-I complex protein subunits, which help with kinetochore formation, effective centromere protein-A (CENPA) synthesis, and chromosome segregation (Okada et al., 2006). Abnormal chromosome segregation can lead to the genomic instability that researchers have found to be associated with infertility, mental disorder, aging, degenerative disease, cancer, and autoimmune disease in humans (Rajput et al., 2011; Zivkovic, Spremo-Potparevic, Plecas-Solarovic, et al., 2010; Zivkovic, Spremo-Potparevic, Siedlak, et al., 2013).

From our data, it does not appear that the mechanism of action of FMS may be reduced to a single molecular cell or pathway. Rather, what is striking about the data is how the gene expression products fit into three closely related networks (Figure 1) with the protein ubiquitination pathway acting as a network hub with *HSP90AA1* sitting in the middle. *HSP90AA1* is a ubiquitous protein found in all cells. It acts as a molecular chaperone, assists in proper folding of several target proteins, and helps to conserve protein structures during cellular stress and regulate cell death pathways (Calderwood, Mambula, Gray, & Theriault, 2007). Increased levels of *HSP90AA1* are not unique to FMS, having been reported in chronic obstructive pulmonary disease (COPD; Hacker et al., 2009), cancer (C. H. Lee, Hong, Chang, & Chang, 2012; Sims, McCready, & Jay, 2011), and systemic lupus erythematosus and other autoimmune diseases (Conroy, Faulds, Williams, Latchman, & Isenberg, 1994; Jarjour et al., 1991; Winfield & Jarjour, 1991). To the best of our knowledge, association of HSPs with behavior responses has not been investigated.

It remains unclear what these observed changes represent. While the pathways with increased gene products include glucocorticoid signaling and B-cell development, there has been no obvious pathologic dysfunction of these pathways noted in FMS. Rather than being

an immunologic failing, the atypical B-cell findings appear to be another aspect of the organism-wide alterations in physiology noted in FMS, such as the reported alterations in pain processing, autonomic and neuroendocrine function, neurotransmitter concentrations, and small-fiber nerve density (Balkarli, Sengul, Tepeli, Balkarli, & Cobankara, 2014; Oaklander, Herzog, Downs, & Klein, 2013; Serra et al., 2014). Understanding the significance of these B-cell differences requires knowing whether such differences precede and predispose people to future symptom development, are causal in creating symptoms, or are just an epiphenomenon that accompanies or instantiates the experience of symptoms. It is not possible to speculate about this using our cross-sectional data. Previous studies of gene expression in FMS focused on specific pre-selected pathways specifically associated with chronic pain, such as on adrenergic receptors and immune genes (A. R. Light et al., 2012; Vargas-Alarcon, Fragoso, Cruz-Robles, Vargas, Lao-Villadoniga, et al., 2007; Vargas-Alarcon, Fragoso, Cruz-Robles, Vargas, Martinez, et al., 2009; White, Light, Hughen, Vanhaitsma, & Light, 2012), serotonin receptors (Cohen, Buskila, Neumann, & Ebstein, 2002), opioid receptors (Salemi et al., 2007), and ion channels (A. R. Light et al., 2012; A. R. Light, White, Hughen, & Light, 2009; K. C. Light, White, Tadler, Jacob, & Light, 2012; White et al., 2012). Interestingly, we did not find differential expression of genes associated with most of the pathways reported in previous pathway-targeted genomic FMS studies.

FMS affects predominantly middle-aged women, making our cohort representative of patients who are seen in most clinical practices that see FMS patients in the United States. However, this study does have some limitations. Our hypothesis-generating findings confirm the association of differentially expressed genes with FMS, but they do not indicate causation. Additionally, collecting data at one time point does not fully describe the variability in symptoms women with FMS experience. We did not collect other variables that would optimize comparison of genomic profiles, such as contraceptive use and comorbid conditions. Our variable regarding the length of time since FMS diagnosis was based on patient report and is, therefore, subject to strong recall bias. Finally, the study findings should be interpreted with caution because the genomic profile presented only includes Caucasian women with FMS. Although we were able to control for gender variability on gene expression, future investigation of men with FMS is warranted.

The primary strength of the present study is the full-genome approach that we took, which avoids potential biases that come with pre-selection of genes or pathways and provides a more complete picture of the physiologic mechanism of FMS. A wider view of how different systems interact and influence each other may be better than reductive approaches in understanding the physiologic mechanisms behind FMS. Prior studies of similar polysymptomatic conditions, such as chronic fatigue syndrome (CFS), have explored the use of whole-genome microarray technology in describing the biologic underpinnings of these conditions (Kerr, 2008; Kerr, Petty, et al., 2008). These reports have described the differential expression of genes associated with immune function, inflammation, apoptosis, and neurological disease and function in CFS patients compared to healthy controls (Kerr, Burke, et al., 2008; Kerr, Petty, et al., 2008). These findings, including ours, provide empirical support that the biologic underpinnings of complex, polysymptomatic conditions such as FMS and CFS can be investigated using unbiased approaches such as microarray

technology to investigate multiple biological markers from different physiological pathways simultaneously.

With the emerging view that FMS represents the end of a continuum of polysymptomatic distress, an understanding of whether the avidity of gene expression correlates with symptom severity would help elucidate the clinical importance of these findings. Although we recently reported differential expression of genes in a small sample of women with FMS with different categories of pain and catastrophizing that identified specific functional pathways delineating different categories of symptom experience (Lukkahatai et al., 2013), there are no other studies yet available that can improve our understanding of the clinical importance of these genomic correlates in explaining the symptom experience reported by individuals with FMS. Despite these issues, the observation that, of the thousands of gene products evaluated, those few that were significantly elevated are part of similar overlapping molecular networks may be an important one.

Implications for Nursing and Clinical Practice

This study provides initial evidence that genes related to immune regulation are differentially expressed in women with FMS compared to healthy controls. Increasing our understanding of the underlying mechanisms of debilitating conditions such as FMS that are mainly diagnosed via symptom experience is vital for nursing practice and research to advance symptoms science. Further, this type of information provides opportunities for nurse clinicians and researchers to develop innovative interventions or personalize health management to attenuate these distressing symptoms and improve the quality of lives of individuals suffering from these poorly understood conditions.

Our study results demonstrate that three interrelated pathways involved in cellular growth and proliferation appear to be more active in patients with FMS compared to matched controls. These pathways have been found to be associated with other clinical populations with similar polysymptomatic conditions (e.g., arthritis and systematic lupus erythematosus). Exploration of the relationship between gene expression patterns and symptom severity will help determine the clinical relevance of these findings. The hope for an effective pharmacological intervention for the wide-ranging and disabling symptoms of FMS lies with understanding the biologic mechanisms responsible for creating the experience.

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Figure 1.

Functional networks of differentially expressed probe sets. This figure demonstrates the networks of differentially expressed genes as well as the canonical pathways associated with these genes, including glucocorticoid receptor signaling, protein ubiquitination, and B-cell receptor signaling pathways.

Figure 2.

Enzyme linked immunosorbent assay (ELISA) plasma concentrations of centromere protein K and heat shock protein 90alpha in women with fibromyalgia (FMS) and healthy controls.

** $p < .01$.

Demographic and Clinical Characteristics of Women With FMS and Healthy Women.

Table 1

Variable	Microarray Study (A)				Real-Time PCR and ELISA Study (B)				t Test (p Value)	
	FMS (n = 29)		Healthy (n = 20)		FMS (n = 54)		Healthy (n = 25)		Microarray Versus PCR	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	FMS	Healthy
Age, years	30–55	43.3 ± 7.1	28–51	40.3 ± 7.4	30–55	43.4 ± 7.2	28–51	38.9 ± 8.7	.99	.57
Body mass index, kg/m ²	17.3–35.7	25.2 ± 5.0	20.5–42.2	26.8 ± 6.1	17.3–56.2	29.4 ± 9.5	20.5–42.2	26.0 ± 5.8	.03*	.72
Pain intensity ^a	0.50–7.8	4.5 ± 1.9	0–2.5	1.3 ± 1.3	0.5–7.8	4.6 ± 1.9	0–2.5	1.4 ± 1.2	.78	.87
Pain interference ^a	0–9.1	4.7 ± 2.7	0	0.0 ± 0.0	0–9.1	4.9 ± 2.8	0	0.0 ± 0.0	.77	—
Depression ^b	1–13	7.7 ± 3.4	0–5	0.9 ± 1.9	1–16	7.7 ± 4.1	0–5	0.6 ± 1.6	.99	.76
Anxiety ^b	1–19	9.4 ± 4.5	0–4	1.4 ± 1.5	1–19	9.1 ± 4.5	0.6	1.7 ± 2.0	.80	.77
Widespread Pain Index	3–17	11.3 ± 3.9			3–19	10.9 ± 4.3			.75	
Symptom severity										
Fatigue	0–3	2.1 ± 0.9			0–3	2.2 ± 0.8			.88	
Waking unrefreshed	0–3	2.2 ± 0.8			0–3	2.2 ± 0.9			.87	
Cognitive dysfunction	0–3	1.7 ± 1.0			0–3	1.7 ± 1.0			.87	
Somatic symptoms	1–3	2.1 ± 0.7			1–3	2.0 ± 0.7			.89	

Note. ELISA = enzyme linked immunosorbent assay; PCR = polymerase chain reaction; FMS = fibromyalgia syndrome; SD = standard deviation.

^aScore on Brief Pain Inventory.

^bScore on Hospital Anxiety and Depression Scale.

* $p < .05$.

Table 2

Differentially Expressed Genes From Microarray Analysis of RNA From Women With FMS and Healthy Women.

Gene Symbol	Gene Title	RefSeq Transcript ID	Function	p Value	Fold Change	Expression in FMS
CENPK	Centromere protein K	NM_001267038//NM_022145	Maintain proper kinetochore function and mitotic progression	0.00068	2.03111	Up
PLXNC1	Plexin C1	NM_005761//NR_037687	Protein binding, receptor activity and binding	9.26E-06	1.98653	Up
C5orf24	Chromosome 5 open reading frame 24	NM_001135586//NM_152409		4.76E-06	1.90018	Up
TPR	Translocated promoter region, nuclear basket protein	NM_003292	ATP binding, heat shock protein binding	6.65E-06	1.87403	Up
RC3H1	Ring finger and CCHC-type domains 1	NM_172071	Ubiquitin-protein ligase activity, mRNA 3'-UTR binding	0.000112	1.85571	Up
MS4A1	Membrane-spanning 4-domains, subfamily A member 1	NM_021950//NM_152866	B-cell activation, immune response	7.69E-05	1.84318	Up
CAPZA1	Capping protein (actin filament) muscle Z-line, alpha 1		Actin binding	0.000246	1.82247	Up
HSP90AA1	Heat shock protein 90 kDa alpha (cytosolic), class A member 1	NM_001017963//NM_005348	ATP binding, ATPase activity, nitric oxide synthase regulator activity	1.59E-08	1.82075	Up
ANXA3	Annexin A3	NM_005139	Calcium iron binding, calcium-dependent phospholipid binding	0.001305	1.81675	Up
PRMT2	Protein arginine methyltransferase 2	NM_001242864//NM_001242865//NM_001242866//NM_001535//NM_206962	Androgen receptor binding, estrogen receptor binding, histone-arginine N-methyltransferase activity	1.68E-05	1.81641	Up
PCBP1-AS1	PCBP1 antisense RNA 1			0.000211	1.81483	Up
PTPRC	Protein tyrosine phosphatase, receptor type, C	NM_001267798//NM_002838//NM_080921//NM_080922//NM_080923//NR_052021	Protein binding, protein tyrosine phosphatase activity	5.43E-05	1.81098	Up

Note. FMS = fibromyalgia syndrome.

Table 3

Gene Networks of Differentially Expressed Genes Between Women With FMS and Healthy Controls.

Network	No. of Focused Genes ^a	Score ^b	Gene Symbol
Cellular growth and proliferation, cell signaling, molecular transport	11	33	<i>ANXA3, C5orf24, CAPZA1, CENPK, HSP90AA1, MS4A1, PLXNC1, PRMT2, PTPRC, RC3H1, TPR</i>

Note. FMS = fibromyalgia syndrome.

^aFocused genes are differentially expressed genes from the experiment.

^bScores are calculated from: [score = $-\log_{10}$ (hypergeometric (Fisher's exact test *p* values))]. A score greater than 3 indicates that the gene network was not generated by random chance with a confidence level of more than 99%.

Table 4

Top Canonical Pathways of Differentially Expressed Genes Between Women With FMS and Healthy Controls.

Canonical Pathway	Ratio ^a	p Value	Gene Symbol
B-cell development	1/36	1.82×10^{-2}	<i>PTPRC</i>
Primary immunodeficiency signaling	1/64	2.85×10^{-2}	<i>PTPRC</i>
Mitotic roles of polo-like kinase	1/74	4.28×10^{-2}	<i>HSP90AA1</i>
Hypoxia signaling in the cardiovascular system	1/68	4.35×10^{-2}	<i>HSP90AA1</i>
Prostate cancer signaling	1/103	5.49×10^{-2}	<i>HSP90AA1</i>

Note. FMS = fibromyalgia syndrome.

^aRatio = number of genes found in participating women with fibromyalgia (FMS)/total number of genes in the human genome in each canonical pathway.

Table 5

Confirmatory Quantitative Real-Time Polymerase Chain Reaction (qPCR) Results for *CENPK* and *HSP90AA1*.

Gene Symbol	FMS Group, <i>n</i> = 54		Healthy Controls, <i>n</i> = 25		Fold Change	95% CI	<i>p</i> Value
	Average Ct	2 ^(-average Ct)	Average Ct	2 ^(-average Ct)			
<i>CENPK</i>	8.69	0.0024	9.29	0.0015	1.518	[1.03, 2.01]	0.012*
<i>HSP90AA1</i>	4.75	0.0372	5.40	0.0236	1.573	[1.06, 2.08]	0.009**

Note: The lower average Ct value = higher gene concentration. FMS = fibromyalgia syndrome; CI = confidence interval; Ct = cycle threshold.

*
p < .05.

**
p < .01.