ARTHRITIS & RHEUMATOLOGY
Vol. 66, No. 5, May 2014, pp 1363–1371
DOI 10.1002/art.38341

Whole Blood Gene Expression Profiling Predicts Therapeutic Response at Six Months in Patients With Polyarticular Juvenile Idiopathic Arthritis

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Objective. **To determine whether gene expression profiles identified in peripheral whole blood samples could be used to determine therapeutic outcome in a cohort of children with newly diagnosed polyarticular juvenile idiopathic arthritis (JIA).**

Methods. **Whole blood samples from the Trial of Early Aggressive Therapy (TREAT) in JIA patients were analyzed on Illumina microarrays, and differential gene expression was compared to expression in healthy controls. Microarray results were validated by real-time quantitative polymerase chain reaction in an independent cohort of samples. Pathway analysis software was used to characterize gene expression profiles. Support vector machines were used to develop predictive models for different patient classes.**

Results. **Differential gene expression profiles for rheumatoid factor (RF)–positive and RF-negative patients were remarkably similar. Pathway analysis revealed a broad range of affected pathways, consistent with current mechanistic theories. Modeling showed that the prognosis at 6 months was strongly linked to gene expression at presentation, irrespective of treatment.**

Conclusion. **Gene expression is linked to therapeutic outcome, and gene expression in the peripheral blood may be a suitable target for a prognostic test.**

The completion of sequencing of the human genome was lauded as the necessary first step toward developing specific, patient-tailored treatments for many complex diseases (1). The development of "personalized medicine" is considered highly desirable because, for many of the most vexing diseases in industrialized societies, there is a broad spectrum of individual therapeutic responses to any given empirically derived treatment approach. We know, for example, that some patients with rheumatoid arthritis (RA) will have an excellent and sustained response to methotrexate (MTX), while others will fail to have satisfactory functional outcomes until biologic agents, usually anti–tumor necrosis factor (anti-TNF) therapies, are initiated (2,3). It would be highly desirable to know which patients are going to need more-aggressive therapies from the outset so that we can minimize the human and economic toll that diseases such as RA carry with them.

To date, numerous attempts have been made to develop predictive biomarkers of therapeutic response in human illnesses, that is, to develop strategies for implementing the "personalized medicine," which has been a 10-year goal of physicians and scientists. Among the most promising tools that have been used toward this goal is gene expression profiling, the survey of genes expressed or suppressed in a particular cell type, tissue,

Supported by grants from the NIH (R01-AR-049762 to Dr. Wallace and R01-AI-084200 and R01-AR-060604 to Dr. Jarvis).

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Dr. Wallace has received consulting fees, speaking fees, and/or honoraria from Genentech and Novartis (less than $10,000 each) and research grants from Amgen and Pfizer.

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Submitted for publication February 4, 2013; accepted in revised form December 26, 2013.

or clinical sample. While there has been some success in developing specific chemotherapeutic strategies for cancer using this approach (4,5), similar attempts for rheumatic diseases (typically using mixed cells from the peripheral blood) have yielded disappointing results, largely because the initial findings were not corroborated in independent cohorts.

Until now, no attempt has been made to develop therapeutic biomarkers for the childhood forms of arthritis using gene expression profiling. The importance of doing so is illustrated by the fact that these diseases are among the most common chronic ones in children (6–8) and continue to result in serious functional limitations. Like adult RA, which it resembles phenotypically, the polyarticular form of juvenile idiopathic arthritis (JIA) displays considerable heterogeneity in terms of response to standard therapies (9–11). Thus, in the field of pediatrics, finding biomarkers that can predict therapeutic response at presentation or early in therapy is expected to have an important effect on our ability to treat the disease and restore/preserve function and normal childhood activities.

The Trial of Early Aggressive Therapy (TREAT) in JIA patients is a recently completed, NIH-funded clinical trial (12) comparing 2 therapeutic regimens for the treatment of newly diagnosed polyarticular JIA: one arm used subcutaneous (SC) MTX as initial therapy, and the other arm used a combined regimen of subcutaneous MTX, a TNF inhibitor (etanercept), and oral prednisolone (tapered to 0 by 17 weeks). As part of the TREAT in JIA trial, whole blood was collected from consenting participants for RNA expression studies at specific time points during the course of the first year of therapy. We report here the results of the expression profile analysis using whole-genome microarrays, as confirmed by the study of an independent cohort derived from the Children's Rheumatology Clinic at the University of Oklahoma.

PATIENTS AND METHODS

Samples from patients in the TREAT in JIA study. Eighty-five patients were recruited into the TREAT in JIA trial between October 2007 and November 2009 (12). All children met the international criteria for polyarticular-onset JIA (13). Sixty-two parents of these children gave written, informed consent for providing these samples for translational uses, and children 7 years of age or older gave assent to participate in the study. Approval for use of the specimens was given by the TREAT in JIA study oversight committee. The patients submitting samples for this current study consisted of 19 boys and 43 girls, ages 2–14 years. Four of the boys and 17 of the girls were rheumatoid factor (RF) positive. At the time of enrollment (month 0) and prior to treatment, 2.5 ml of blood was collected into a PAXgene tube (PreAnalytiX). Samples were stored at −80°C. (A summary of patient characteristics is available upon request from the corresponding author.)

Patients were randomly assigned to 1 of 2 blinded, aggressive treatment arms of the study. Arm 1 consisted of treatment with MTX 0.5 mg/kg/week SC plus etanercept 0.8 mg/kg/week SC (maximum dosage 50 mg/week) in combination with oral prednisolone (0.5 mg/kg/day; maximum dosage 60 mg/day) for 16 weeks. Arm 2 consisted of MTX 0.5 mg/kg/week SC (40 mg maximum) plus placebo etanercept SC weekly and placebo oral prednisolone tapered to 0 by 17 weeks. At 4 months, patients who did not achieve American College of Rheumatology (ACR) Pediatric 70 (Pedi 70) improvement from baseline were treated (or retreated) with open-label MTX, etanercept, and prednisolone. At 6 months, patients who did not achieve clinically inactive disease were changed to treatment with open-label MTX, etanercept, and prednisolone, if they were not already receiving this treatment.

Further specimens were collected during visits at 4 months, 6 months, and 12 months after enrollment (month 0). For purposes of the TREAT in JIA study, inactive disease was defined as no evidence of synovitis, absence of fever, rash, lymphadenopathy, and splenomegaly, no active uveitis, normal erythrocyte sedimentation rate or C-reactive protein level, and a physician's global assessment score indicating no active disease.

Samples from healthy control subjects. Controls consisted of 8 healthy female and 11 healthy male children between the ages of 7 and 13 years who were recruited from the University of Oklahoma Children's Physicians General Pediatrics Clinic. The protocol for obtaining these specimens was approved by the University of Oklahoma Institutional Review Board (no. 13205). Anesthesia for the phlebotomy was provided using topical lidocaine/prilocaine solution.

RNA processing. RNA was purified from whole blood PAXgene specimens using a PAXgene blood RNA kit (Qiagen) as recommended by the manufacturer, including a DNase (Qiagen) step to remove genomic DNA. Globin transcripts, which reduce labeling efficiency of whole blood cell RNA and decrease signal-to-noise ratios on microarrays (14), were reduced using GlobinClear (human; Ambion). Final RNA preparations were suspended in RNase-free water, quantified spectrophotometrically, and analyzed for RNA integrity by capillary gel electrophoresis (Agilent 2100 Bioanalyzer).

Due to technical issues such as RNA degradation, not all 62 samples from the TREAT in JIA study were available for microarray analysis at both month 0 and month 4. Figure 1 shows a schematic representation of the study, including which samples were analyzed at each time point. A total of 44 samples were available for microarray analysis at month 0 and 49 samples at month 4.

Microarray analysis. Complementary RNA was produced from reverse-transcribed complementary DNA using an Illumina TotalPrep RNA amplification kit (Ambion), hybridized to Illumina WG-6 v3 or Illumina HT-12 v4 human whole genome microarrays, and stained according to the manufacturer's directions. Microarray hybridizations were undertaken in 2 separate batches. The first batch consisted of samples from the 19 healthy controls as well as samples from the JIA

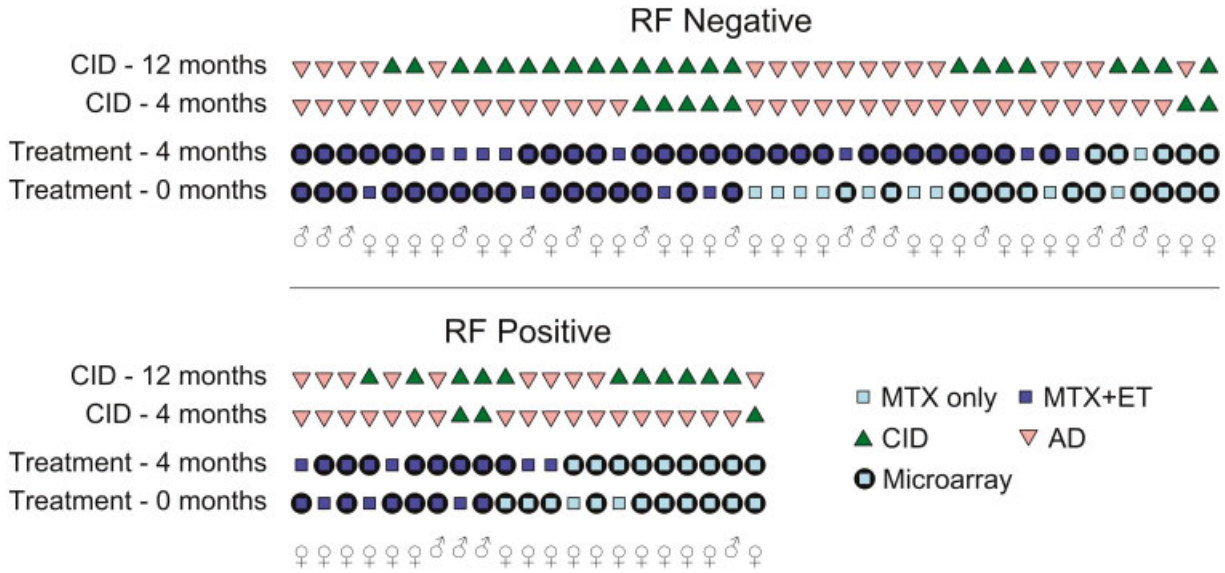

Figure 1. Schematic representation of treatment regimens at month 0 (baseline) and month 4, with clinical outcomes at month 6 and month 12, in patients with juvenile idiopathic arthritis (JIA), according to the presence or absence of rheumatoid factor (RF). Each column of symbols represents a single patient at different stages of the Trial of Early Aggressive Therapy (TREAT) in JIA study. Study arm 1 consisted of treatment with methotrexate (MTX) plus etanercept (ET), as well as prednisolone. Study arm 2 consisted of MTX only. At months 6 and 12, patients were assessed for the presence of clinically inactive disease (CID) or active disease (AD).

patients: 26 obtained at month 0, 2 at month 4, and 1 at month 12. These samples were hybridized on Illumina WG-6 v3 arrays. The second batch consisted of the remaining 18 JIA patient samples from month 0 and 47 JIA patient samples from month 4. These samples were hybridized on Illumina HT-12 v4 arrays. Complementary RNA preparation and hybridizations of the second batch were carried out 12 months after the first batch.

Validation of differential gene expression by real-time quantitative reverse transcription–polymerase chain reaction (qRT-PCR) in an independent patient cohort. An additional cohort of samples was collected for qRT-PCR analysis in order to provide independent validation of the results of gene expression analyses carried out with the TREAT in JIA samples. These whole blood PAXgene specimens were obtained from an independent cohort of 8 children with untreated, RF− polyarticular JIA recruited from the University of Oklahoma Health Sciences Center Pediatric Rheumatology Clinic. These children ranged in age from 2 to 11 years and consisted of 2 boys and 6 girls. These samples were collected at month 0, prior to treatment. Nine genes that showed significant differential expression in the microarray results and that are known to be associated with rheumatoid disease were analyzed by qRT-PCR.

Total RNA (0.9 μg) was reverse transcribed with the use of an iScript cDNA synthesis kit (Bio-Rad) according to the manufacturer's instructions. Relative levels of target gene transcripts were assayed in triplicate using real-time qRT-PCR with SYBR Green reagents and a StepOne Plus PCR system (Applied Biosystems). The temperature profile consisted of an initial step at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds, 60°C for 1 minute, and then a final melting curve analysis with a ramp from 60°C to 95°C over 20 minutes. Gene-specific amplification was confirmed by a single peak in the ABI Dissociation Curve software. The relative abundance of transcript expression data was normalized to GAPDH expression. Results are presented as the ratio of the concentration of messenger RNA (mRNA) relative to GAPDH mRNA ($2^{-\Delta C_t}$). Statistical analysis was performed on the ΔC_t value using unpaired *t*-tests. Primers were synthesized by Integrated DNA Technologies.

The nucleotide sequences of the primers were as follows: for CD44, 5′-CATCCAACACCTCCCAGTATG-3′ (sense) and 5′-CTGCTCACGTCATCATCAGTAG-3′ (antisense); for exocyst complex component 4 (Exo-4), 5′-TTGATGTTACAAACCTCCCTACTC-3′ (sense) and 5′-CCAAGCCCTTAATGAGGATACC-3′ (antisense); for macrophage migration inhibitory factor (MIF), 5′-GTCCCGGACCAGCTCAT-3′ (sense) and 5′-GCCGCGTTCATGTCGTAATA-3′ (antisense); for NF-κB1, 5′-CTGCTGTGCAGGATGAGAAT-3′ (sense) and 5′-AAATCCTCCACCACATCTTCC-3′ (antisense); for peptidylarginine deiminase 4 (PADI-4), 5′-CCAGGTCTGAGATGGACAAAG-3′ (sense) and 5′-AGGGAGATGGTGAGGGTAAT-3′ (antisense); for poly(ADP-ribose) polymerase (PARP-1), 5′-GTAGCAACAAACTGGAACAGATG-3′ (sense) and 5'-GGACTTGGTGCCAGGATTTA-3′ (antisense); for protein tyrosine phosphatase receptor type C (PTPRC), 5′-CGTAATGGAAGTGCTGCAATG-3′ (sense) and 5′-TGCGACTCATTTCTAACCAGAG-3′ (antisense); for serpin A, 5′-AATGCCACCGCCATCTT-3′ (sense) and 5′-CCCATTGCTGAAGACCTTAGT-3′ (antisense); and for STAT-6, 5′-CAAGTTTAAGACAGGCT-

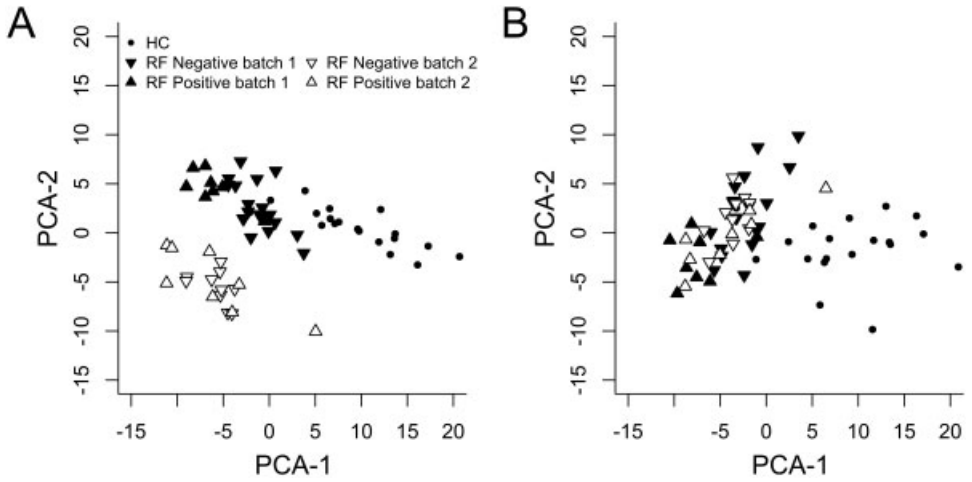

Figure 2. Scatterplots of data from principal components analyses (PCAs), showing the distribution of the healthy control (HC) samples and the Trial of Early Aggressive Therapy (TREAT) in juvenile idiopathic arthritis (JIA) patient samples, whether positive or negative for rheumatoid factor (RF), through the first 2 principal components. **A,** After normalization of raw data, the healthy controls and patient samples show a good degree of separation, but a strong batch effect is apparent between the 2 array batches in the patient samples. **B,** After the ComBat algorithm was applied to the data, the batch effect was removed.

TGCG-3′ (sense) and 5′-TCTTCAGCACTAGGGCTTTG-3′ (antisense). All primers were tested to display an efficiency of ~95% (±SD 2%).

Support vector machine (SVM). Models were constructed to predict clinically inactive disease (CID) or active disease (AD) at 6 and 12 months from gene expression at presentation (month 0) or gene expression at 4 months (month 4). Models were built using data for all patients and separately for RF− patients only. Specifically, for each model, the relevant samples were randomly divided into a training group (two-thirds of available samples) and a test group (one-third of available samples), using a randomized block method to ensure even division between the groups of CID and AD samples. The test groups were then reserved for testing models, which were trained using only data from the training groups. Linear models were applied to each training group as described above and used to identify genes that were differentially expressed between the AD and CID groups in the training groups. Subsets of these differentially expressed gene lists were used to train the models to predict AD or CID in patients based on differential expression between patients and controls. The *e107* package (15) in R was used as an interface for the LIBSVM library (16). Optimization of SVM parameters and the gene subset was done using a 10-fold cross-validation method on the training group. The subsets of each gene list to be used in each model were determined by starting with the 10 most significantly differentially expressed genes (by adjusted *P* value) in the relevant training group and sequentially adding genes up to a total of 200 genes. The performance of each model was assessed using receiver operator characteristic curves. Optimized SVM parameters and gene lists were then used to build models. The resulting models were then used to predict outcome (CID or AD) for the test groups, and the predictions were analyzed for accuracy and rates of false-positive results.

Statistical analysis. All statistical analyses were carried out in R (www.r-project.org). To facilitate statistical analyses relative to healthy controls, it was necessary to combine data from different hybridization batches. Due to the difference in the microarrays, it was necessary to create combined datasets using only the probes that were present on both array formats. Illumina probe identification numbers were used to identify 39,426 common probes. Datasets were variance-stabilized and normalized using robust spline normalization via the Lumi software package (17,18). Batch effects were removed using the ComBat algorithm in the SVA software package (19). Prior to statistical analysis, nonresponding probes were filtered out of the datasets using the detection *P* value provided by the Illumina quality control metrics to eliminate probes not responding at higher than background levels. Analysis of differential gene expression patterns between patients and controls was performed using the Limma software package (20,21). The false discovery rate (FDR) was estimated using the method described by Benjamini and Hochberg (22). Statistical significance of gene expression was determined at an FDR of ≤0.05. Gene lists of interest were exported from R and uploaded to Ingenuity IPA software for further functional analysis.

RESULTS

Segregation of JIA patient samples from healthy control samples. Principle components analysis (PCA) of normalized signal data for month 0 in JIA patients and healthy controls showed that the gene expression profiles clearly separated the patients from the controls. However, although RF− and RF+ samples show some separation on PCA, they were not perfectly segregated (Figure 2A). A strong batch effect between the first and

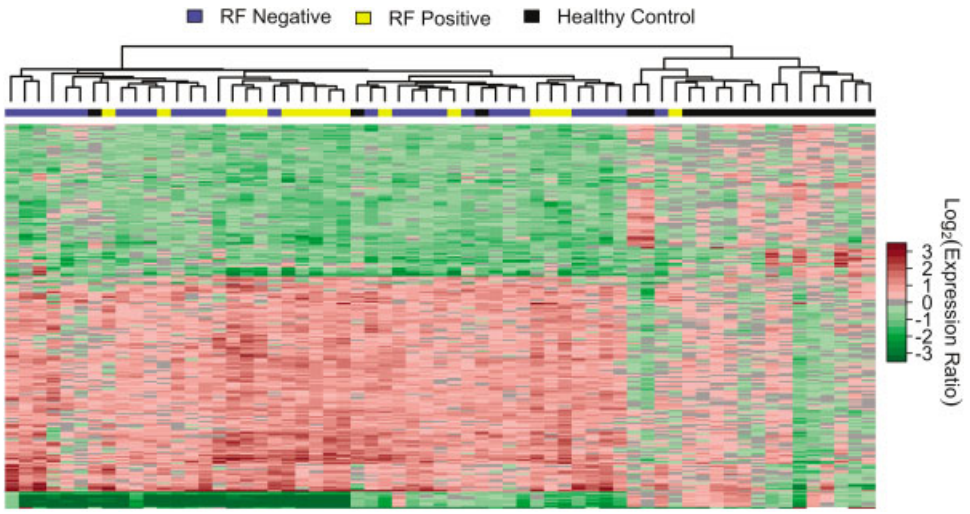

Figure 3. Heatmap showing gene expression levels at month 0 in juvenile idiopathic arthritis patients positive or negative for rheumatoid factor (RF) and in healthy controls, using 250 probes for significantly differentially expressed genes. Data shown are the log ratio for differential expression relative to the mean of the healthy controls (false discovery rate $\leq$0.05; absolute fold change $\geq$1.4). The dendrogram shown is a hierarchical clustering of patient samples using Euclidean distance on the 250 probes.

second batches of the TREAT in JIA samples is also noted in Figure 2A. PCA of data after application of the ComBat algorithm shows that this procedure successfully removed the batch effect (Figure 2B).

Differential gene expression analysis. A heatmap of genes selected at an FDR of 0.05 and a minimum fold change of 1.4 for differential expression in either the RF− or RF+ groups for each patient sample at month 0 and for the healthy control subjects is shown in Figure 3. Differential gene expression was relatively homogenous across the patient samples, and there was no striking difference in differential gene expression between RF− and RF+ patients. The maximum fold change was 10-fold in either direction, but with the majority of genes differentially expressing no more than 3-fold in either direction. (A full list of genes differentially expressed between all month 0 samples and the healthy controls at an FDR of 0.05 irrespective of RF status or fold change is available upon request from the corresponding author).

For further functional analysis, gene lists were selected at an FDR of 0.05 and a minimum fold change of 1.4. While large numbers of genes were significant at a low FDR, absolute fold change levels were low, and filtering by fold change drastically reduced the numbers of gene that were declared significant. At month 0, 125 genes were differentially expressed in RF− JIA samples, while 237 genes were differentially expressed in the RF+ JIA samples. At month 4, 123 genes were differentially expressed in the RF− samples, while 110 genes were differentially expressed in the RF+ samples.

Of the genes differentially expressed at month 0 relative to the healthy controls, 90 genes represented by 98 probe sets were significantly differentially expressed in both RF+ and RF− patients.

Given the complex composition of whole blood specimens, it is difficult to make testable inferences about disease pathogenesis from the expression profiling patterns. When functional associations of the genes differentially expressed between JIA patients and healthy controls were explored using the Ingenuity software package, predictable numbers of genes associated with immunologic disease (n = 18), inflammatory disease (n = 16), and connective tissue disorders (n = 15) were identified, including 12 genes associated with rheumatoid arthritis (*CD3D*, *CD97*, *CYP4F3*, *FOXO3*, *GNLY*, *GRN*, *HSPA1A/HSPA1B*, *MMP9*, *PADI4*, *SORL1*, *UBE2H*, and *WNK1*). It was interesting to note the number of genes associated with cancer, in light of the emerging data regarding inflammatory signatures in cancer (23,24) and the cancer-like behavior of rheumatoid synovial cells (25). Consistent with recent reports of an "inflammatory signature" in the gene expression profile of many tumors (23,24), 21 differentially expressed genes were related to cancer, while others fell

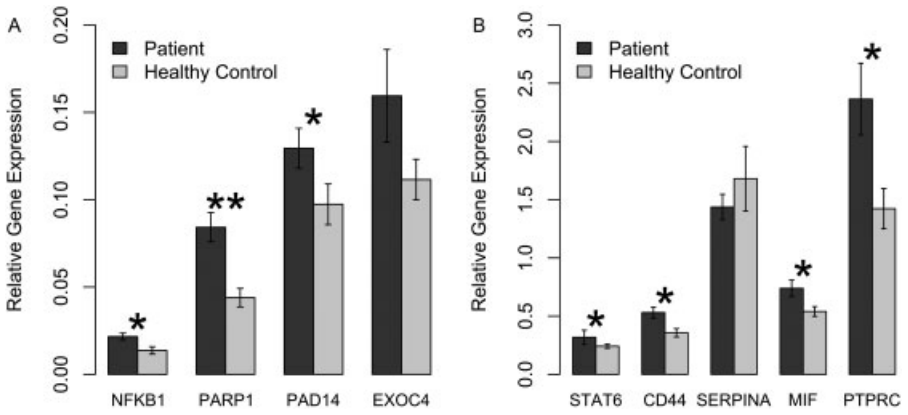

Figure 4. Validation of the microarray results by real-time quantitative reverse transcription–polymerase chain reaction analysis of 9 genes in an independent cohort of 8 children with untreated, rheumatoid factor (RF)–negative polyarticular juvenile idiopathic arthritis (JIA) and in 8 healthy controls from the initial cohort. Statistical analysis was performed on ΔC_t values using unpaired *t*-tests. Values are the mean ± SEM. * = $P < 0.05$; ** = $P < 0.01$ versus healthy controls.

into categories of connective tissue disorders, immunologic disease, and inflammatory disease.

Validation of the gene expression results. To confirm the differences in gene expression between patients and healthy controls observed in the microarray experiments, real-time qRT-PCR was performed. Nine genes known to be associated with rheumatoid disease and shown to be significantly differentially expressed (FDR <0.05) between the RF− patients and the healthy controls in the microarray analysis (*CD44*, *EXOC4*, *MIF*, *NFKB1*, *PADI4*, *PARP1*, *PTPRC*, *SERPINA*, and *STAT6*) were analyzed by real-time qRT-PCR in an independent cohort. Figure 4 shows that 7 of 9 genes differentially expressed in the microarray analysis were also differentially expressed in the real-time qRT-PCR. For *MIF* the real-time qRT-PCR results showed a relative increase in gene expression in the independent cohort, while the microarray results showed a decrease in the expression of *MIF* in the TREAT in JIA cohort, though it was differentially expressed on qRT-PCR.

Prediction of disease status at 6 and 12 months. A schematic representation of the treatment regimens and outcomes in the JIA patients is shown in Figure 1. Consistent with the overall findings in the TREAT in JIA study (12), the findings in the subset of samples analyzed herein strongly suggested that for the RF− patients, there was a relationship between early use of combined treatment and the attainment of a positive outcome at 12 months. However, this pattern was not apparent in the RF+ patients.

Eight models were built with support vector machines to predict disease status (CID or AD) at month 6 or month 12 using the gene expression data in patients at month 0 or month 4 and using either all of the samples or only the RF− samples. A total of 28 RF− and 16 RF+ arrays were available at month 0, and 32 RF− and 17 RF+ arrays at month 4. For each model, a different number of genes was found to give the optimum predictive power; this ranged from 12 for the model using month 4 data in RF− patients to predict the month 12 outcome, to 120 for the model using month 0 data in all patients to predict the month 12 outcome. (Details of the genes selected for each model are available upon request from the corresponding author.)

Figure 5 shows the receiver operating characteristic curves, where CID is considered the positive outcome, for the 4 models built with month 0 data, and Table 1 gives the areas under the curve for all 8 models. The model using month 0 gene expression to predict CID at 6 months for RF− patients was able to perfectly classify the 9 samples in the test group (Table 1). The equivalent model for all patients (RF+/RF−) correctly classified 11 of the 14 samples tested, with a false-positive rate of 0.12. The 2 models using month 0 data to predict CID at month 12 were able to achieve accuracies of ~70%; however, the RF− model had a false-positive rate of 0.33, while the RF+/RF− model had a false-positive rate of 0.80. Models using month 4 data were not able to significantly improve on chance, with accuracies between 40% and 60%.

Table 1 also shows the accuracies and false-positive rates achieved when treatment with the combined MTX plus etanercept regimen was used as the predictive feature. Gene expression was the better pre-

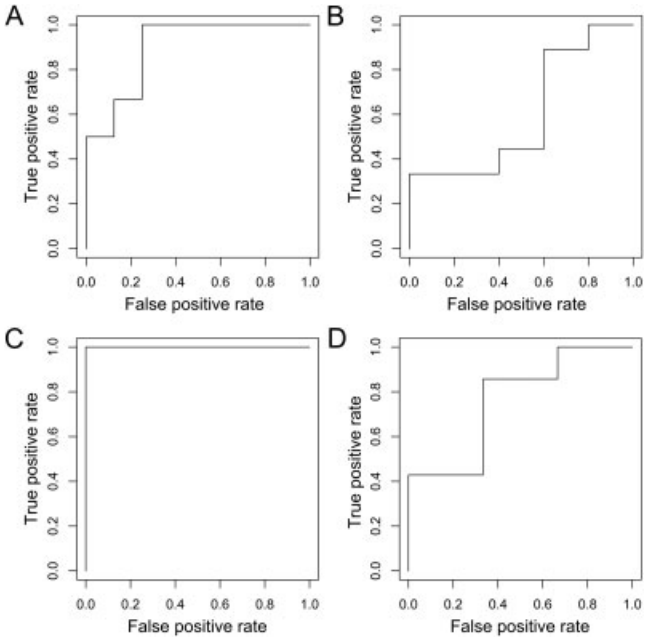

Figure 5. Receiver operating characteristic (ROC) curves for 4 models built using gene expression data obtained at month 0 from juvenile idiopathic arthritis (JIA) patients and healthy controls. Data obtained at month 0 from the entire group of JIA patients, whether positive or negative for rheumatoid factor (RF), were used to predict outcome at month 6 (**A**) and at month 12 (**B**). Data obtained at month 0 from only the RF− JIA patients were used to predict outcome at month 6 (**C**) and month 12 (**D**). The ROC curves show the relationship between changes in the true-positive rate and the false-positive rate as the classification threshold is varied. The better the performance of the classifier the greater the area under the curve. An entirely random classifier would generate a line at y = x. A perfect classifier is typified by the ROC curve shown in **C**.

dictor of outcome at month 6 based on month 0 data for both the RF+/RF− group and the RF− only group. For the prediction of month 12 outcome based on month 0 data in RF+/RF− patients, gene expression provided a more accurate predictor than did MTX plus etanercept therapy. However, for the analysis of only the RF− patients, the accuracies were similar, but using MTX plus etanercept as the predictor resulted in a much lower false-positive rate. As with gene expression, prediction based on treatment at month 4 was no better than chance.

DISCUSSION

The completion of the Human Genome Project was heralded as the beginning of a new era of "personalized medicine." Technological spin-offs from the project, including gene expression profiling, have further added to the promise that individualized therapies can be developed based on genomic data (26). In this study, we used whole blood gene expression profiles to determine whether we could predict therapeutic response in children enrolled in the TREAT in JIA study. We used an independent cohort of patients from Oklahoma to validate the statistical methods used to analyze the TREAT in JIA study samples.

We found that, while it might be feasible to develop such predictive assays, the number of samples available from the TREAT in JIA study and the multiple different phenotypes (e.g., RF+ and RF−, as well as the 2 different arms of the protocol with crossover to the "aggressive" side of the protocol for treatment failures at 4 months and at 6 months) made it difficult to develop statistically robust predictive models. Nevertheless, within these constraints, we were able to predict, based on expression profiling alone, the achievement of CID at 6 months in the RF− patients. At 12 months, however, initial therapy with etanercept was a better predictor of disease response than was gene expression in the RF− patients (Table 1). While both treatment arms of the TREAT in JIA protocol demonstrated therapeutic efficacy (12), the use of etanercept as initial therapy, at least in the samples available to us for analysis, exerted the strongest influence on outcome at 12 months. However, the results from the support vector machine indicate that there is also a genomic component to prognosis.

Our findings do not preclude the possibility of

Table 1. Accuracy rates and FPRs for the prediction of clinical outcome at month 6 and month 12, based on either the gene expression model or the combined therapy with MTX plus etanercept initiated at month 0 or month 4*

Patient group, initiation → outcome	Gene expression model			MTX plus etanercept therapy	
	Accuracy	FPR	AUC	Accuracy	FPR
RF+/RF− (all patients)					
Month 0 → month 6	0.79	0.12	0.90	0.62	0.37
Month 4 → month 6	0.56	0.30	0.48	0.36	0.82
Month 0 → month 12	0.64	0.80	0.60	0.60	0.36
Month 4 → month 12	0.44	0.71	0.44	0.47	0.82
RF− patients					
Month 0 → month 6	1.00	0	1.00	0.66	0.36
Month 4 → month 6	0.60	0.17	0.50	0.34	0.92
Month 0 → month 12	0.70	0.33	0.76	0.68	0.28
Month 4 → month 12	0.50	1.00	0.48	0.51	0.89

* Predictions were made for the entire cohort of 62 patients, including those positive and those negative for rheumatoid factor (RF), as well as for the subset of 41 RF− patients. The area under the curve (AUC) provides a quantitative measure of the performance of the model. FPRs = false-positive rates; MTX = methotrexate.

developing a longer-term predictive model of sustainability of therapeutic response based on gene expression patterns if a larger patient cohort, one with more samples from patients in each arm of the protocol, were available. Particularly helpful in this regard was the analysis of the baseline samples. While there are some distinct phenotype differences between RF+ and RF− children with JIA, the expression profiles between RF+ and RF− children with JIA were remarkably similar, with overlap between the groups on hierarchical cluster analysis (Figure 3). These findings seem to support those of earlier studies showing that RF expression in children is more ubiquitous than is typically considered and is dependent more on the assay used to detect RFs than on their actual prevalence in the JIA population (27,28). Under any circumstances, although the TREAT in JIA study may have experienced some recruitment bias in favor of RF+ patients (35% of the TREAT in JIA study subjects were RF+), it appears to be feasible to group RF+ with RF− patients in future attempts to develop expression-based predictive models or assays. Furthermore, it was interesting to note the degree of homogeneity among and between the patient groups at the gene expression level (Figure 2). These findings suggest that a broad range of interpatient variability will not be a serious impediment to developing expression-based predictive assays in the future.

It should also be noted that both arms of the TREAT in JIA protocol were more aggressive than protocols that have previously been used in the routine clinical setting. The MTX dosage of 0.5 mg/kg/week (with a maximum dosage of 40 mg/week) is higher than the more standard dosages of 10–20 mg/m^2 orally with a weekly maximum of 25 mg. Whether the models would be more predictive in the setting of current clinical practice is unknown. Furthermore, the findings of the TREAT in JIA study, and particularly the degree to which the higher doses of MTX were tolerated in children, may provide an impetus to change clinical practice and, thus, obviate the need to test predictive models using lower oral doses of MTX.

As is commonly seen in gene expression profiling in rheumatic diseases, a broad spectrum of functional associations were seen among the differentially expressed genes, including genes associated with inflammation (*NFKB*, *MAPK*), cancer (*BCL2*), and adaptive immunity. The expression profiles do suggest complex interactions between innate and adaptive immunity that are not subsumed under any single prevalent theory concerning the pathogenesis of JIA.

In conclusion, we have provided evidence that it is feasible to develop models of disease pathogenesis based on patterns of gene expression for the purpose of predicting outcome at 6 months. However, in the subset of samples from the TREAT in JIA study with which we worked, the use of etanercept was as good as or better than gene expression as a predictor of a patient's achieving CID by 12 months. Future translational studies will likely require larger numbers of patients in order to develop clinically usable predictive assays.

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 published. Dr. Jarvis 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.

Study conception and design. Jiang, Frank, Wallace, Jarvis.

Acquisition of data. Jiang, Frank, Chen, Wallace, Jarvis.

Analysis and interpretation of data. Jiang, Sawle, Frank, Wallace, Jarvis.

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