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Identification of Molecular Biomarkers for Multiple Sclerosis

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Multiple sclerosis is a demyelinating disease of the central nervous system with a presumed autoimmune etiology. Previous microarray analyses identified conserved gene expression signatures in peripheral blood mononuclear cells of patients with autoimmune diseases. We used quantitative real-time polymerase chain reaction analysis to identify a minimum number of genes of which transcript levels discriminated multiple sclerosis patients from patients with other chronic diseases and from controls. We used a computer program to search quantitative transcript levels to identify optimum ratios that distinguished among the different categories. A combination of a 4-ratio equation using expression levels of five genes segregated the multiple sclerosis cohort ($n = 55$) from the control cohort ($n = 49$) with a sensitivity of 91% and specificity of 98%. When autoimmune and other chronic disease groups were included ($n = 78$), this discriminator still performed with a sensitivity of 79% and a specificity of 87%. This approach may have diagnostic utility not only for multiple sclerosis but also for other clinically complex autoimmune diseases. ***(J Mol Diagn 2007, 9:197–204; DOI: 10.2353/jmoldx.2007.060147)***

Autoimmune diseases are heterogeneous diseases believed to arise from immune-mediated attack against self-antigens. For example, multiple sclerosis (MS) is the most common demyelinating disease of the central nervous system and develops from destruction of myelin sheaths. Both genetic and environmental factors play important roles in the onset and pathogenesis of autoimmune diseases.[1–3] Epidemiological data along with genetic linkage studies clearly support the presence of a genetic contribution to susceptibility to autoimmune disease.[4–12]

Diagnosis of autoimmune diseases may present difficulties to the clinician. For example, there is no single definitive laboratory test for MS; it remains a clinical diagnosis.[13] Abnormal brain magnetic resonance imaging findings and immunological changes in cerebrospinal fluid (elevated IgG index, presence of oligoclonal bands) raise clinical suspicion but are not disease-specific.[14–16] Patients who present with features highly suspicious for MS, or clinically isolated syndromes, present a diagnostic challenge.[17] The identification of biomarkers characteristic of MS aid in its diagnosis.

Investigators have used microarray approaches to analyze gene expression profile differences in affected tissues of individuals with autoimmune diseases, such as the white matter in the brain in MS or synovium in rheumatoid arthritis (RA). Specific gene expression profiles have also been found in peripheral blood mononuclear cells of individuals with different autoimmune diseases.[18–28] For example, an interferon signature has been found in systemic lupus erythematosus that is a function of disease severity.[23] An early disease signature has been found in RA.[28] These are unique to an individual autoimmune disease. In addition, we have described a gene expression signature that is shared among several autoimmune diseases, including RA, systemic lupus erythematosus, type 1 diabetes (insulin-dependent diabetes mellitus), and MS.[18] By analysis of unaffected family members, we found that a portion of this common autoimmune gene expression signature was a family trait not dependent on the presence of an autoimmune disease, and a portion was dependent on the presence of autoimmune disease.[29,30] However, we were unable to identify gene expression signatures that were unique to an individual autoimmune disease using a variety of analytical approaches. Thus, gene expression signatures that are both common to several autoimmune diseases and unique to a given property of an individual autoimmune disease exist.

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Table 1. Clinical Characteristics of Patients Analyzed in This Study

Study group	No. of subjects	Age range (years)	Female:male ratio
Experiment 1			
Controls	46	22 to 58	3.2:1
MS-1	29	26 to 53	3.6:1
RRMS	13		
SPMS	14		
PPMS	1		
Pre-MS (CIS)	1		
Experiment 2			
MS-2	26	29 to 48	2.7:1
RRMS	12		
SPMS	12		
PPMS	1		
Pre-MS (CIS)	1		
RA	35	42 to 69	2.8:1
SLE	21	33 to 57	4.2:1
Other diseases	22	22 to 69	2.6:1
Cumulative	179	22 to 69	3.0:1

RRMS, relapsing-remitting MS; SPMS, secondary progressive MS; PPMS, primary progressive MS; CIS, clinically isolated syndrome; other, optic neuritis ($n = 4$), inflammatory arthritis ($n = 3$), type 1 diabetes ($n = 5$), type 2 diabetes ($n = 5$), and cardiovascular disease ($n = 5$).

The purpose of the studies presented here was to use a different experimental approach and method of analysis or scoring to identify genes in which transcript levels in whole blood discriminated MS from the other autoimmune diseases. We chose to focus on MS because it is one of the more difficult autoimmune diseases to diagnose. We elected to use quantitative real-time polymerase chain reaction (Q-RT-PCR) to measure transcript levels of genes identified from our microarray data that were either control genes (equivalent transcript levels in patients with autoimmune disease and control individuals) or test genes (different transcript levels between autoimmune patients and controls). We developed a new algorithm that would give each gene in the analysis equal weight but would also provide more accurate weight to quantitative differences in transcript levels. Using this analysis, we were able to distinguish patients with MS from control patients and from patients with other diseases, including autoimmune disease, in a retrospective analysis.

Materials and Methods

Patients

A total of 179 patients were analyzed in this study (Table 1). The control group was age-/gender-matched and ascertained for absence of diagnosed autoimmune disease or symptoms by interview. To examine an unbiased cohort of patients, we used the single criterion of diagnosis of a disease using established methods by a specialist in the field for inclusion in the study. The MS patients were further classified into relapsing-remitting MS, primary progressive MS, secondary progressive MS, and pre-MS (clinically isolated syndrome) disease subtypes. We analyzed an initial group (experiment 1) of 29 patients with MS and a second independent group (experiment 2) of 26 patients with MS. The Vanderbilt University Institutional Review Board approved this protocol, and each patient provided written consent.

Procedures

Peripheral blood was collected into PAXgene tubes (Qiagen Inc., Valencia, CA). Total RNA was isolated using the Versagene PAXgene-compatible isolation kit according to the manufacturer's recommendations (Gentra Systems, Inc., Minneapolis, MN). Two μg of RNA was reverse-transcribed using the SuperScript III first-strand cDNA synthesis kit (Invitrogen Corporation, Carlsbad, CA). For every sample, cDNA equivalent to 100 ng of total RNA was used in replicate Q-RT-PCR reactions for each gene assay. Patient clinical history was blinded during processing and data collection.

The relative expression levels of nine genes were determined using TaqMan gene expression assays (Applied Biosystems, Inc., Foster City, CA) and detected on an ABI7700/SDS platform (Applied Biosystems, Inc.). The nine genes and corresponding TaqMan assay catalogue numbers were ACTR1A (Hs00194913_ml), BRCA1 (Hs00173237_ml), CTSS (Hs00175403_ml), EPHX2 (Hs00157403_ml), LLGL2 (Hs00189729_ml), SPIB (Hs00194753_ml), TAF11 (Hs00194573_ml), TGM2 (Hs00190278_ml), and TP53 (Hs00153340_ml). Relative expression levels were determined from the observed C_T data. Each C_T was subtracted from 30, such that $(30 - C_T) = X$, and 2^X calculated the linear expression value.

Statistical Analysis

A computer program was designed to identify the most discriminatory combination of ratios (ranging between 1 and 5). We exhaustively searched all gene expression ratios (eg, ACTR1A/BRCA1, TAF11/ACTR1A, and so forth) in the control and MS data sets to first find an optimal ratio. The search was entirely deterministic because every possible ratio using two gene expression levels was computed. The value of a test ratio was used to separate the MS data set from the control set. For each control individual and MS individual, the test ratio was computed. We let $\{C_1, C_2, \ldots, C_n\}$ denote the test ratios for each of the n control individuals and $\{MS_1, MS_2, \ldots, MS_k\}$ denote the test ratios for each of the k MS individuals. Perfect separation of the two sets (an optimal ratio) would occur if the largest test ratio for the control individuals was less than the smallest test ratio for the MS individuals, but we were unable to identity this optimal ratio. However, we did identity test ratios in which the majority of the MS individuals scored greater than the second largest control individual. Therefore, our optimal test ratio separated the two data sets such that second largest ratio in $\{C_1, C_2, \ldots, C_n\}$ was less than the largest number of ratios in $\{MS_1, MS_2, \ldots, MS_k\}$. This optimal ratio was used to identify a cutoff value that produced the highest sensitivity and specificity for the two data sets. This was accomplished by plotting sensitivity and specificity curves as functions of the cutoff value and identi-

Table 2. Average Relative Linear Expression Values

Gene symbol	Subject cohort		MS/C	*P* value†
	Control	MS		
ACTR1A	127 ± 48*	71 ± 31	0.56	$4e^{-8}$
BRCA1	7 ± 4	5 ± 4	0.69	0.03
CTSS	3882 ± 3102	4340 ± 3935	1.12	NS
EPHX2	24 ± 11	12 ± 7	0.51	$2e^{-7}$
LLGL2	91 ± 41	78 ± 75	0.85	NS
SPIB	126 ± 63	96 ± 52	0.76	0.02
TAF11	38 ± 18	14 ± 11	0.38	$5e^{-10}$
TGM2	36 ± 45	31 ± 41	0.85	NS
TP53	610 ± 250	487 ± 416	0.80	NS

*Results are average linear expression values ± SD from a total of 46 control subjects and 29 MS subjects.
†*P* values were determined by the Mann-Whitney test.
NS, not significant.

fying the intersection of the curves. The cutoff value at this intersection was designated the optimal ratio discriminator.

Sensitivity and specificity of the discriminators were determined using standard calculations. Overall test accuracy was calculated as follows: let TP = true positives; FP = false positives; TN = true negatives; FN = false negatives, then:

$$\text{Accuracy} = \frac{TP + TN}{TP + FP + TN + FN}$$

Receiver-operating characteristics curves were used to examine performance characteristics of the tests. In brief, the number of true positives (TP) and false positives (FP) were determined for a range of cutoff scores. The fraction of TP was determined by dividing the TP by the total number of cases, and the fraction of FP was determined by dividing the FP by the total number of controls. A nonlinear regression curve was calculated using a function in Mathematica's Statistics' NonlinearFit package. The nonlinear regression was integrated to determine the area under the curve (AUC). The significance of observed differences (*P*) was determined using the Mann-Whitney test.

Results

Initial Discriminant Analysis

Initial analysis focused on identifying a pattern of gene expression that discriminated MS and control patients. The data set comprised 29 patients with different clinical forms of MS (Table 1) and 49 control patients. We selected nine genes from the microarray data set for analysis (see Materials and Methods) and examined their expression profile using TaqMan gene expression assays. Genes were selected from the microarray data set in which expression level did not vary (control genes: LLGL2, CTSS, TGM2) and those in which expression level varied significantly among control and autoimmune patients (test genes: ACTR1A, BRCA1, EPHX2, SPIB, TAF11, TP53). Initially, we used two approaches to determine relative gene expression levels. The first was by plasmid standard curve analysis. The second was to determine the relative expression level of a given gene from the C_T. Because consistent patterns of normalized gene expression were observed with each method of quantitation (plasmid standard curve analysis versus linear), linear expression values were used in all subsequent analyses because this approach removed one variable from the overall analysis. The average expression values were determined for each gene in the MS and control group (Table 2). A wide range of individual gene expression levels was observed from the lowest value of 0.35 (BRCA1) to the highest value of 22,851 (CTSS). The average expression values were significantly different between the MS and control groups for five genes (Table 2).

Linear expression values were entered into a computer program (see Materials and Methods for details), which searched all possible 1-, 2-, 3-, and 4-ratio combinations of gene expression levels. These ratio combinations, or discriminators, generated individual scores, which were then analyzed to determine sensitivity and specificity (Table 3). The sensitivity and specificity of discriminators increased with the number of ratios. For example, a 1-ratio combination correctly identified 86% of the patients with MS with a specificity of 91%; the best 2-ratio combination identified 83% of the MS patients with a specificity of 93%. The best discriminator was derived from a 4-ratio combination ($CTSS \times LLGL2^2 \times TGM2$)/($TAF11^2 \times TP53^2$). With this combination, the average MS score was 17.5 ± 15.0, and the average control score was 2.2 ± 4.1 ($P < 0.0001$). Discriminators with five ratios produced identical specificity and sensitivity to the best 4-ratio discriminator.

At each stage of the analysis, the computer program identified more than one 1-, 2-, 3-, or 4-ratio discriminator that performed with equal sensitivity and specificity. For example, at the 1-component stage, several genes functioned equally well in the numerator, but TAF11 was always in the denominator, consistent with its low expression value in the MS cohort. Several 4-ratio discriminators also performed equally well, and these varied in two ways. Several genes in the numerator yielded equivalent sensitivity and specificity. The two genes in the denominator were always TAF11 and TP53. However, we identified ratios that used ($TAF11^3 \times TP53$) and ratios that

Table 3. Discriminator Performance with Increasing Components

Ratios	Test equation	Average score		Sensitivity	Specificity	*P* value
		Control	MS			
1-ratio	CTSS TAF11	19 ± 11	35 ± 10	86%	91%	<0.0001
2-ratio	BRCA1 × CTSS TAF11^2	20 ± 17	131 ± 107	83%	93%	<0.0001
3-ratio	CTSS × LLGL2 × SPIB TAF11^3 (1000)	1.2 ± 2.8	19.9 ± 23.5	93%	93%	0.0002
4-ratio	CTSS × LLGL2^2 × TGM2 TAF11^2 × TP53^2	2.2 ± 4.1	17.5 ± 15.0	93%	98%	<0.0001

Scores were determined for each test equation as outlined in Materials and Methods and are expressed as average score ± SD. Sensitivity and specificity were determined by standard calculations as described in the Materials and Methods section. *P* values were determined using the Mann-Whitney test.

used (TAF11^2 × TP53^2). We have elected to show results from only one ratio at each stage of the selection.

MS scores ranged from 0.6 to 69.1, and control scores ranged between 0.2 and 27.7 (Figure 1A, MS-1). One control subject received a score of 27.7, which was 5.6 times higher than the next highest control score of 4.9- and 12.6-fold higher than the average control score of 2.2. We validated the initial results using only the best 4-ratio discriminator (CTSS × LLGL2^2 × TGM2)/(TAF11^2 × TP53^2) by determining gene expression levels in whole blood from an independent cohort of 26 MS patients (Figure 1A, MS-2) and performing the same analysis to produce scores. The average score of the second group was 14.4 ± 13.6, and the sensitivity was 88%. Combined sensitivity of the test and validation groups (MS-1 and MS-2) was 91%. Different parameters were evaluated by varying the threshold or cutoff score. A cutoff or threshold of 5.2 classified 48 of 49 controls as non-MS (98% specificity) and correctly identified 50 of 55 MS patients as disease-positive (91% sensitivity). The highest overall accuracy (96%, Figure 1C) was reached with a cutoff or threshold of 5.2.

Analysis of the 4-Ratio Test Equation in Different Autoimmune Diseases

The 4-ratio discriminator (CTSS × LLGL2^2 × TGM2)/(TAF11^2 × TP53^2) was applied to individuals with different autoimmune and other chronic diseases. The same parameters of performance were evaluated. The scores of the different disease groups showed a greater degree of overlap with each other (Figure 2A). Using a designated cutoff of 5.2, 31% of RA patients (11 of 35), 24% of systemic lupus erythematosus patients (5 of 21), and 9% of patients with other diseases (2 of 23) scored positive in the test. All patients scoring positive in the test had a known autoimmune disease. Therefore, the overall specificity was reduced to 85% using a cutoff of 5.2 (Figure 2B). The overall accuracy was 85% when all patients were included in the analysis (Figure 2C).

The performance of the 4-ratio discriminator (CTSS × LLGL2^2 × TGM2)/(TAF11^2 × TP53^2) was also evaluated using the receiver-operating characteristics curve. To determine receiver-operating characteristic curves, we first compared the fraction of TP and the fraction of FP using

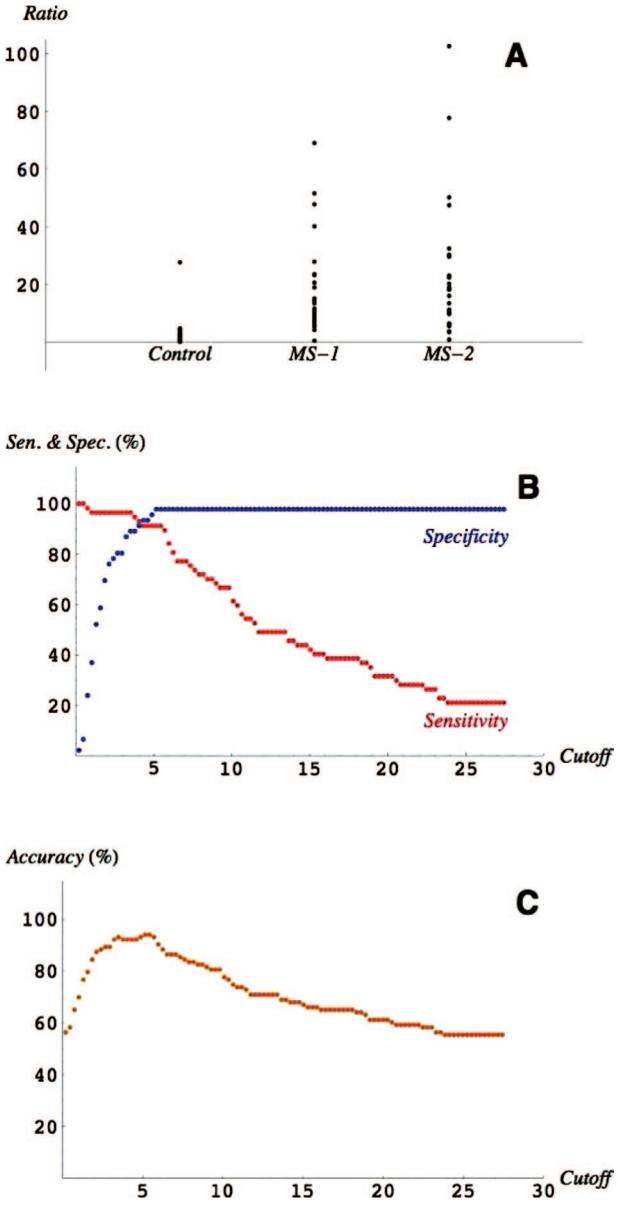

Figure 1. Analysis of the 4-ratio discriminator in the MS and control groups. **A:** Individual test scores within each cohort were determined using the expression equation: (CTSS × LLGL2^2 × TGM2)/(TAF11^2 × TP53^2) as detailed in Materials and Methods. **B:** Sensitivity (red) and specificity (blue) of the test as scoring threshold increases. **C:** Accuracy of test results with varying threshold.

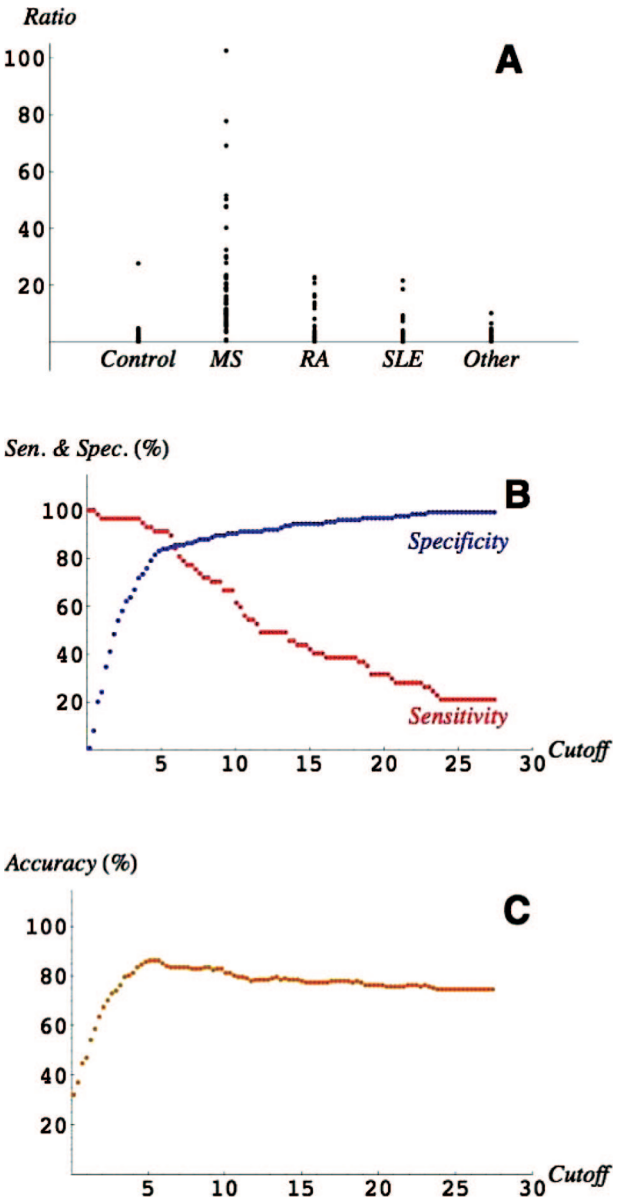

Figure 2. Analysis of the 4-ratio discriminator in different autoimmune and chronic diseases. **A:** Individual test scores within each cohort were determined using the expression equation: $(CTSS \times LLGL2^2 \times TGM2)/(TAF11^2 \times TP53^2)$ as detailed in Materials and Methods. The solid line is drawn through the highest control score. **B:** Sensitivity (red) and specificity (blue) of the test as scoring threshold increases. **C:** Accuracy of test results with varying threshold.

the MS and control samples applying the best 1-, 2-, 3-, and 4-ratio discriminators (from Table 3 and Figure 1A). This comparison yielded an AUC of 0.96 from the 4-ratio discriminator (Figure 3A). AUCs for the 3-, 2-, and 1-ratio discriminators were less than the AUC for the 4-ratio discriminator. Next, we determined the fraction of TP and fraction of FP using the MS samples and all other samples (combined data from Figures 1A and 2A). This comparison produced an AUC of 0.89 for the 4-ratio discriminator (Figure 3B). We also calculated AUC for the 3-, 2-, and 1-ratio discriminators and found the AUC to be less than observed for the 4-ratio discriminator, as were overall sensitivities and specificities (Table 3). Thus, AUC, sensitivity, specificity, and overall accuracy decreased when all samples, controls, and those with other diseases, including other autoimmune diseases, were included in the analysis.

The MS cohort comprised individuals with different MS subtypes (Table 1). However, no scoring pattern could be identified that correlated with a subtype (Figure 4). At a threshold of 5.2, two patients with clinically definite MS received negative scores. One patient with secondary progressive MS received a score of 4.2, and one relapsing-remitting MS patient scored 0.6, one of the lowest scores observed in any group.

Because this was a retrospective analysis, all MS patients were under a clinician's care. Common therapies for MS include β-interferons, glatiramer acetate (Copaxone), methotrexate, and prednisone. Therefore, we asked whether scores varied among MS patients receiving different therapies. Average score ± SD for the control group was compared with all MS patients, and the MS patients were separated into those receiving a β-interferon ($n = 19$), MS patients not on a β-interferon ($n = 33$), and patients receiving glatiramer acetate ($n = 9$) (Figure 5). Average scores in the different treatment groups ranged from 16.6 to 25.9. These differences were not statistically significant ($P > 0.05$). However, the difference between each treatment group and the control group was statistically significant. These results clearly demonstrate that test scores are independent of a specific therapy and therefore support the notion that scores are dependent on the presence or absence of MS or another autoimmune disease.

Discussion

In this study, we used the results from our genome-wide microarray analysis[18] to design a sensitive and specific Q-RT-PCR-based assay capable of distinguishing patients with MS from control patients and patients with other chronic diseases including autoimmune diseases. This assay discriminates individuals with MS from controls with a specificity of 98% and an overall sensitivity of 91% (combined data from the initial test experiment and second validation experiment). This level of specificity and sensitivity is among the highest reported in laboratory testing for MS (specificity ~95% for magnetic resonance imaging[14,17]; ~85% for OGCB[16,17]). Unlike microarray analysis, this Q-RT-PCR assay can easily be adapted into a clinical molecular genetics laboratory.

We designed the search algorithm with three goals in mind. First, we reasoned if we searched for expression ratios of two genes rather than expression levels of a single gene we would control for any sample-to-sample variations such as differences in cDNA amounts or integrity. Second, by multiplying component ratios rather than using another mathematical function, each gene will receive equal weight in the analysis. Third, we wanted the search capable of being performed with a personal computer. We set the parameters so the algorithm would identify optimum 1-, 2-, 3-, 4-, and 5-ratio discriminators based on sensitivity and specificity. At each stage, more

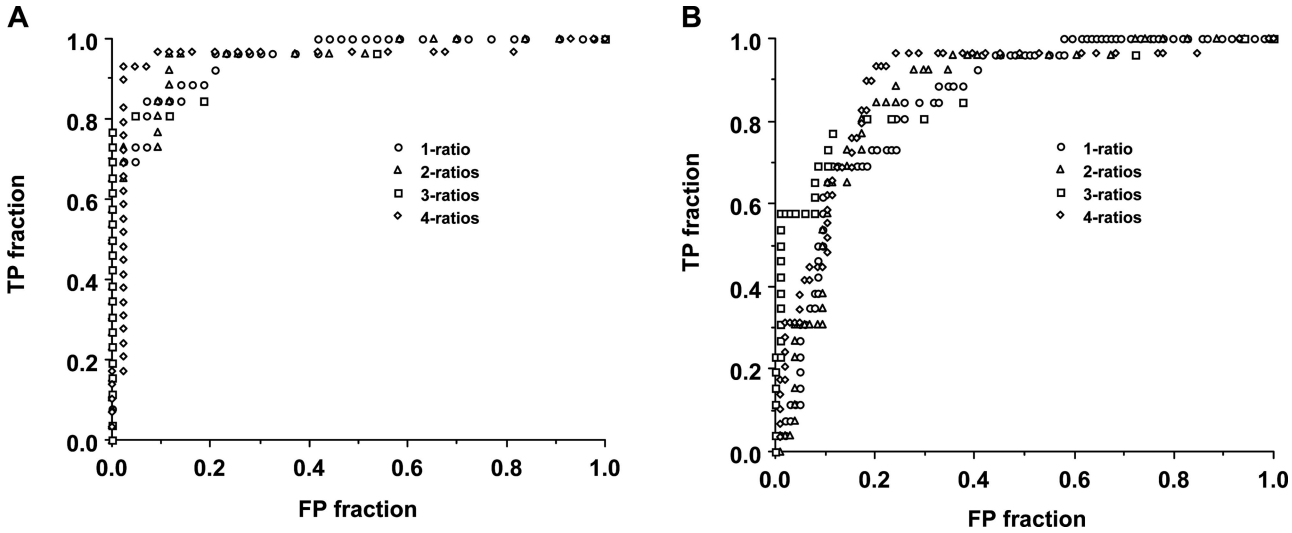

Figure 3. Receiver-operating characteristics curves of 1-, 2-, 3-, and 4-ratio discriminators. **A:** MS versus control samples, TP and FP fractions were calculated as a function of test score (from Figure 1). **B:** As in **A**, except MS was compared with controls, and other disease groups (from Figure 2A), TP and FP fractions were calculated as a function of test score.

than one ratio was identified that yielded equivalent sensitivities, specificities, and *P* values. We elected to report only one ratio at each stage. Several 4-ratio discriminators yield identical sensitivity, specificity, and *P* value, but these only vary slightly by having a different control gene in the numerator or by the function, for example $TAF11^3 \times TP53$ in the denominator works as well as $TAF11^2 \times TP53^2$. We also searched for 5-ratio discriminators, but these ratios do not improve sensitivity or specificity. At this point, it is difficult to conclude that the performance of the 4-ratio discriminator will be superior to the performance of the 3-, 2-, or 1-ratio discriminators. First, a larger sample size will be necessary to clearly establish, for example, whether 93% sensitivity or 98% specificity (4-ratios) is statistically different from 86% sensitivity or 91% specificity (1-ratio), respectively. Second, analysis of additional patient cohorts or prospective rather than retrospective analyses may demonstrate that a 4-ratio discriminator is or is not superior to a 3-ratio discriminator. Nevertheless, using this approach, it is possible to distinguish patients with MS from control patients with a high degree of accuracy based on expression levels of at most five genes in whole blood. These differences in sensitivity and specificity are clinically relevant to a diagnostic test, making it worthwhile to establish whether the 4-ratio discriminator is superior to the 3-, 2-, or 1-ratio discriminators.

In our previous microarray analyses, we identified a conserved pattern of gene expression in individuals with different forms of autoimmune disease.[18] Using these data, we were able to design a scoring system that accurately discriminated patients with an autoimmune disease from control patients or from patients undergoing an immune response after influenza vaccination. However, we were unable to discriminate among patients with different autoimmune diseases. When the data set from the Q-RT-PCR assays was analyzed with different autoimmune patients included, the discriminator still performed with a specificity of 87%, negative predictive

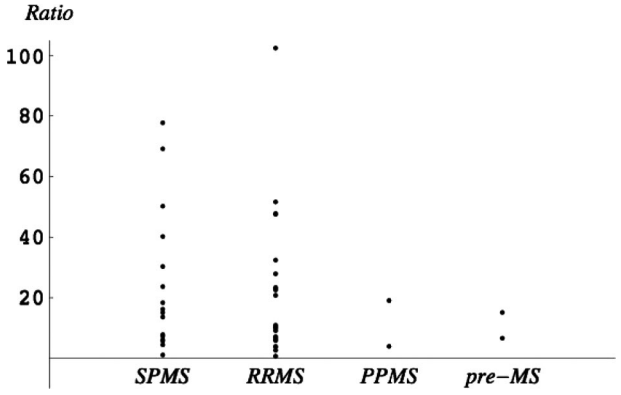

Figure 4. Individual MS test scores within each clinical subtype. Individual test scores within each MS clinical subtype were determined using the expression equation: $(CTSS \times LLGL2^2 \times TGM2)/(TAF11^2 \times TP53^2)$ as detailed in Materials and Methods.

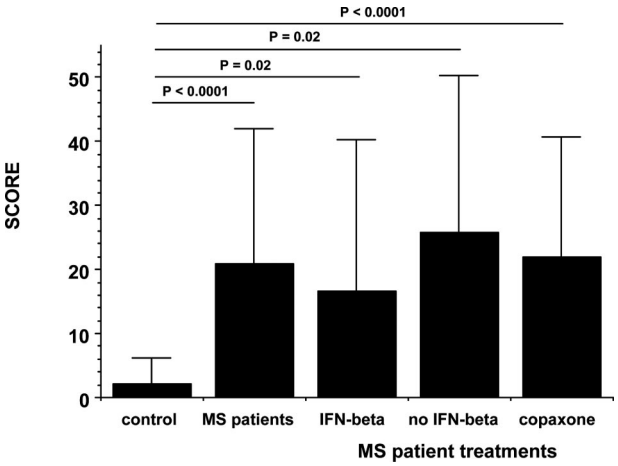

Figure 5. Average test scores as a function of type of therapy. The MS patient samples were segregated into all MS patients, patients receiving a β-interferon, patients not receiving β-interferon, and patients receiving glatiramer acetate (Copaxone). Average scores $\pm$ SD were calculated, and *P* values were calculated using the Mann-Whitney test.

value of 98%, and overall accuracy of 86%. The greatest degree of overlap was identified with RA patients, but it is important to note that distinguishing between MS and RA is not traditionally a clinical dilemma.

In the area of molecular diagnosis, much attention has been directed toward the development of optimal Q-RT-PCR assays and appropriate endogenous or control gene selection, but no standardized method exists to evaluate the data. We selected a group of genes that were either underexpressed (test genes) or did not vary between control and autoimmune patients (control genes) based on microarray results. We did not use standard control genes, such as GAPDH or ACTB, because they showed some degree of statistically significant difference in microarray data sets between the subject groups. Rather, we developed a computer algorithm to search randomly and exhaustively all possible combinations of gene expression ratios and evaluated a series of optimal 1-, 2-, 3-, and 4-ratio discriminators using expression values of two to five genes. In the analysis, TAF11 was the most underexpressed gene in the majority of MS patients and was represented in all of the component ratios. TP53 was moderately underexpressed on average in the MS population, and its inclusion in the discriminator did not improve sensitivity. Rather, its inclusion increased specificity from 93 to 98%.

TAF11 encodes a small subunit of transcription factor IID that is present in all TFIID complexes and interacts with TATA-binding protein.[31,32] Its function in mammalian systems is not well understood. Its function is better understood in yeast. In yeast, promoters of genes have been grouped based on their interactions with TAFs such that deletion or mutation of an individual TAF can alter transcription of a class of genes and change the transcriptional profile of a cell. Therefore, reduced TAF11 expression could have a pleiotropic effect altering normal transcriptional regulation of multiple genes involved in the MS phenotype. *TP53* encodes the tumor suppressor protein, p53, which regulates cell proliferation, DNA damage/repair, and apoptosis.[33] Lymphocytes from RA and MS patients have reduced TP53 transcript levels, p53 protein levels,[34,35] and defects in lymphocyte apoptosis induced by γ radiation, a process known to be dependent on p53. Defects in apoptosis are hypothesized to contribute to autoimmunity.[1]

These alterations in gene expression may result from the disease process or may reflect a family or genetic trait, or other factors may lead to these differences in gene expression. We were interested to ascertain if a particular pattern of scoring correlated with different MS subtypes or treatment status. The MS patients demonstrated similar scoring patterns independent of treatment status or current therapy regimen. Although we had a small number of patients in each category, we did not find scoring patterns that segregated the different subtypes. However, the pre-MS (clinically isolated syndrome) patients did receive positive scores. This finding must be reproduced in a larger cohort, but if assay-positive pre-MS patients develop MS throughout time, these results could contribute to earlier clinical intervention. An early-onset differential pattern of expression could support a contributory role for these identified genes in disease pathogenesis.

In summary, we have designed a sensitive and highly specific Q-RT-PCR assay and have developed a mathematic approach to evaluate the data. Our assay enables us to discriminate patients with MS from controls and other autoimmune diseases. For MS, this approach offers a noninvasive, rapid test that provides diagnostic utility to assist in the clinical decision-making process for this complex and challenging disease.

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