



# A Potential Blood Based Diagnostic Marker of Multiple Sclerosis

Benjamin M. Greenberg, MD, MHS\*, Douglas Bigwood, PhD<sup>±</sup>, Eric Eastman, PhD<sup>±</sup>

\*Johns Hopkins School of Medicine, Baltimore, MD 21287; <sup>±</sup>DioGenix, Gaithersburg, MD 20878



## Objective

To determine if there is a gene expression signature specific for patients with multiple sclerosis (MS).

## Background

Although the underlying cause of MS is unknown, there appears to be an autoimmune aspect to the disease with involvement of both genetic and environmental factors that are incompletely understood. Accurate diagnosis of MS is sometimes difficult, expensive and can take months to years, often resulting in an incorrect preliminary diagnosis and administration of inappropriate, expensive and sometimes toxic therapies.

## Methods

Initial Study: In order to investigate the feasibility of developing a clinically viable blood-based MS molecular diagnostic test 11 MS samples, 4 systemic lupus erythematosus (SLE) and 8 normal case controls were selected for statistical analysis. The MS patients were selected because they had no other major complicating diseases or conditions, but they were heterogenous for treatment, length of disease and subtype of MS. Likewise, the control subjects had no known diseases. Isolated RNA from PBMCs was used to examine gene expression profiles on the Affymetrix GeneChip microarray platform (HGU133A/B). A t-test was performed which yielded a number of genes that were significantly differentially expressed between test groups.

Subsequent Study: 62 untreated, early RRMS patients and 64 healthy controls had RNA isolated from whole blood via PaxGene<sup>®</sup> tubes and analyzed on the same Affymetrix arrays.

## Results

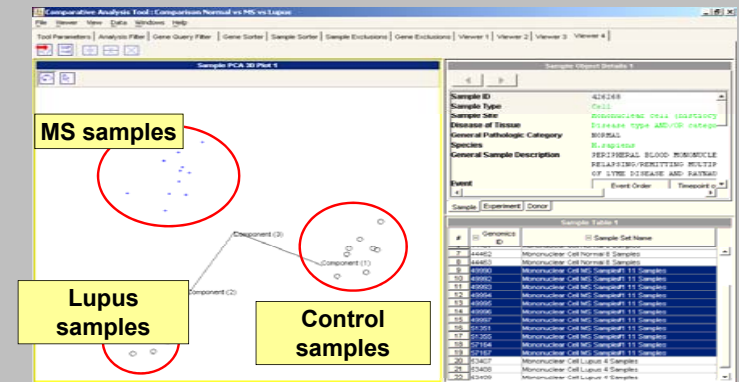
Data from Initial Affymetrix Whole-Genome Human GeneChips<sup>®</sup> (54,000+ gene fragments)

1. We would expect 2700+ gene fragments to be significantly ( $p \leq 0.05$ ) differentially expressed by random chance
2. We actually found 7,525 gene fragments to be significantly ( $p \leq 0.05$ ) differentially expressed
3. False Discovery Rate\* (FDR) correction applied to control for false positives:  
 # of gene fragments at  $p < 0.001 = 339$   
 # of gene fragments at  $p < 0.001$  and Fold Change  $\geq 2 = 104$
4. Bonferroni Adjustment Analysis (the most conservative FDR):  
 # of gene fragments at  $p < 0.01 = 87$   
 # of gene fragments at  $p < 0.01$  and FC  $\geq 2 = 42$

## Partial Gene List

Gene Title	Gene Symbol	p-value	Mean (CONTROL)	Mean (MS)	Fold Change (MS/CONTROL)
guanylate binding protein 1, interferon-inducible, 67kDa	GBP1	0.0073	550.04	1,236.05	2.247
interferon-induced protein with tetratricopeptide repeats 3	IFIT3	0.0091	1,097.07	4,175.94	3.806
myxovirus (influenza virus) resistance 1	MX1	0.0183	579.23	2,816.04	4.862
interferon-induced protein with tetratricopeptide repeats 5	IFIT5	0.0193	92.21	266.31	2.888
poly (ADP-ribose) polymerase family, member 14	PARP14	0.0320	481.23	1,069.58	2.223
2'-5'-oligoadenylate synthetase 2, 69/71kDa	OAS2	0.0332	51.61	214.41	4.154
interferon regulatory factor 7	IRF7	0.0334	752.48	1,694.22	2.252
basic leucine zipper transcription factor, ATF-like 2	BATF2	0.0382	57.86	206.70	3.572
ISG15 ubiquitin-like modifier	ISG15	0.0386	501.68	2,955.58	5.891
suppressor of cytokine signaling 1	SOCS1	0.0482	67.44	142.72	2.116

## Results



Data from Subsequent Whole Blood Study:

1. 10,161 gene fragments were significantly ( $p \leq 0.05$ ) differentially expressed
2. False Discovery Rate\* (FDR) correction applied to control for false positives:  
 # of gene fragments at  $p < 0.001 = 625$   
 # of gene fragments at  $p < 0.001$  and Fold Change  $\geq 1.5 = 101$
3. Bonferroni Adjustment Analysis (the most conservative FDR):  
 # of gene fragments at  $p < 0.01 = 160$   
 # of gene fragments at  $p < 0.01$  and FC  $\geq 1.5 = 39$

## Conclusions/Future Directions

1. There appears to be a gene expression signature for multiple sclerosis
2. Gene expression data changes between using PBMCs and whole blood
3. Confirmatory tests in treatment naïve MS patients, early in disease course, utilizing whole blood is underway and thus far, confirms these initial observations
4. A blood based test, based on differential gene expression may be clinically viable