**Bioinformatics Analyses Determined the CNS and Peripheral Lymphoid Surrogate Biomarker Candidates Between Two Distinct EAE Models for Progressive Multiple Sclerosis**

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**Abstract**

Background: Histologically, multiple sclerosis (MS) is heterogeneous with four patterns even among patients with the same (homogeneous) clinical course. We have established two distinct experimental autoimmune encephalomyelitis (EAE) models for primary progressive MS, mimicking two H-2R mouse strains with myelin oligodendrocyte glycoprotein (MOG) peptide. While the two models have a similar stimulating T helper cell (Th) type, the EAE induction with MOG developed primary progressive (PP) disease. In PP-EAE, we found markers were up- or downregulated in the blood from MS patients on Gene 'genefilter'. EE induction:

Data mining was conducted to determine whether mouse peripheral surrogate with MOG developed primary progressive (PP) disease. In PP-EAE, we found markers were up- or downregulated in the blood from MS patients on Gene 'genefilter'.

**Materials & Methods**

**EAE induction:** ASW and SJL mice were injected with MOG peptide subcutaneously and curdlan intraperitoneally to induce PP-EAE. RR-EAE was induced in SJL mice with PLP-139. Polyclonal IgG was transduced into SJL mice, using the IndoImmuno® transfer system (Promega). Using 50 μg of cDNA, real-time PCR was conducted with the MyiQ™2 Real-Time PCR Detection System (Bio-Rad). Fold changes were calculated using the Relative Quantitation Average in the Expression Console (Affymetrix).

**Microarray bioinformatics analyses:** RNA samples from brains and spleens of PP-EAE and control mice at disease peak were processed to labeled cDNA microarray bioinformatics analyses: 'prcomp'.

To find the differences of gene expression profiles between the strains or organs, we conducted a k-means clustering. A radial chart visualizing the expression patterns of 35 clusters (left); among 12 clusters showed differently expressed patterns (right). The number at each vertex is the cluster number (1, 2, 3, 4, 5, 10, 13, 15, 18, 19, 20) whereas the numbers along the axis (4 to 10) are log ratios, compared with controls. 9 typical genes included in 6 clusters were correlated significantly with brain PC1.

**Results**

Heat maps: the expression patterns of highly up- or down-regulated genes in the brain and spleens from both mouse strains. PP-EAE mice, compared with control mice, at disease peak. White areas indicate that the expression ratio was less than 0.5 or more than 2.0 and P values were less than 0.05. The number of upregulated genes was more than that of downregulated genes in the brains of both ASW and SJL/J mice with EAE, while upregulated genes were less than downregulated genes in the spleens. Red and blue boxes indicate positive and negative log2 FC, shown in heat maps, respectively.

**Conclusions**

1. IFN-γ and IL-17 might be used as the biomarkers for progressive EAE in the brain.
2. Stfa2l1 and immunoglobulin might be useful as the biomarkers for progressive EAE in the brain.
3. PCA ranked IgG-complement-related pathway in the brain from ASW mice as a top pathway (A), while both EAE and control pathway were ranked in SJL/J mouse brain. Pathobiological molecules, such as Fkbp5, Igk, Mmp9 and Nos2 were downregulated in ASW mice (B), while prototypical molecules were upregulated and Th1-associated molecules, Il4, Il5, and Gata3 were downregulated in SJL/J mice (C). Downregulation of cyclin A-related pathway was ranked in ASW mouse spleen (D), while downregulation of ion transport-related pathway was ranked in SJL/J mouse spleen (E).

To identify the peripheral surrogate markers, which reflect the transcription changes in the brain, we conducted pattern matching analysis, using brain PCA values and spleen microarray data. Fkbp5 and Ppap2 were correlated significantly with brain PCA. Among them, Fkbp5 was used as a biomarker candidate in the spleen PCA of EAE mice. In a data mining on GEO databases, Fkbp5 and Ppap2 were upregulated in blood samples from MS patients.