Gene-expression Analysis of Pediatric Patients with Multiple Sclerosis Treated with Natalizumab

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Pediatric MS

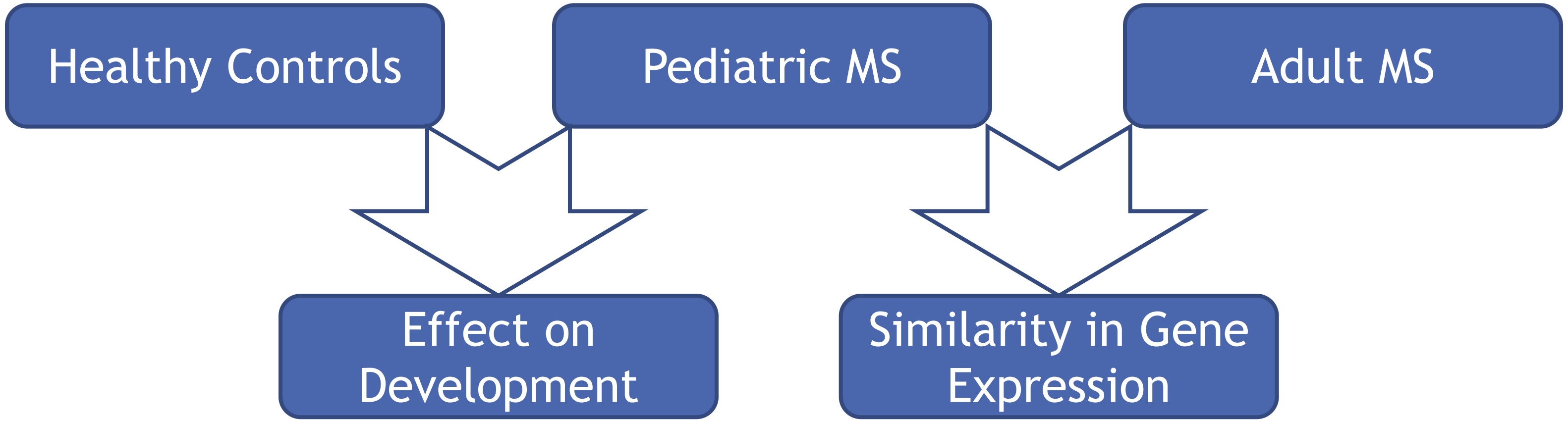
- **Definition of MS**
  - chronic inflammatory autoimmune disease of the CNS
  - Inflammatory brain lesions in both space and time
  - Usually diagnosed at age 20 to 40
- 2-5% of MS cases are pediatric **(onset at age <18 years)**
- Majority of cases are relapsing-remitting
- Although adults and children share basic aspects of the disorder, children have different clinical, neuroimaging, laboratory features, and courses of the disease.
- Treatment for pediatric MS is based on adult therapies, but their **efficacy in children is still being determined through clinical trials**

Natalizumab

- Humanized monoclonal Antibody targeting a4 subunit of a4b1-integrin
- FDA approved therapy for adults
- Clinical trials have shown that Natalizumab works well in children
  - Fewer relapses
  - No enhancing lesions on MRI
  - No association with PML (unlike adults)
  - Similar efficacy to adults
- **Effect on childhood development is not fully understood**

Our Goal

- To determine whether Natalizumab treatment affects the gene expression of essential childhood and adolescent developmental genes
- To determine whether Natalizumab induces changes in gene expression in pediatric MS similar to adult MS patients

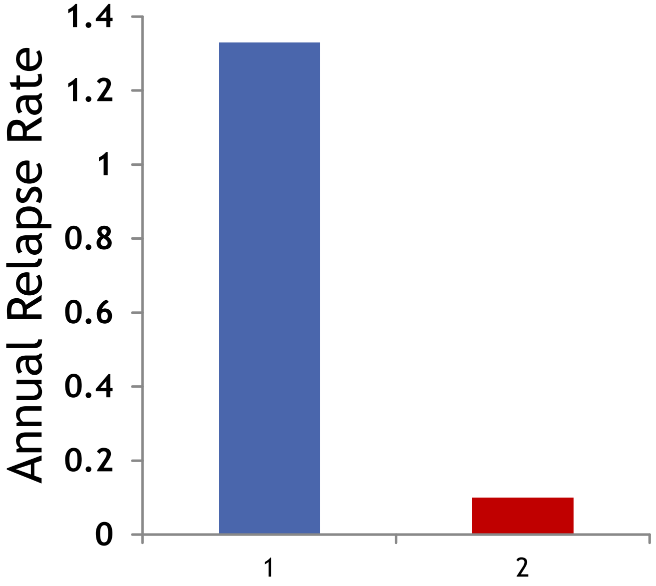


Patient Characteristics

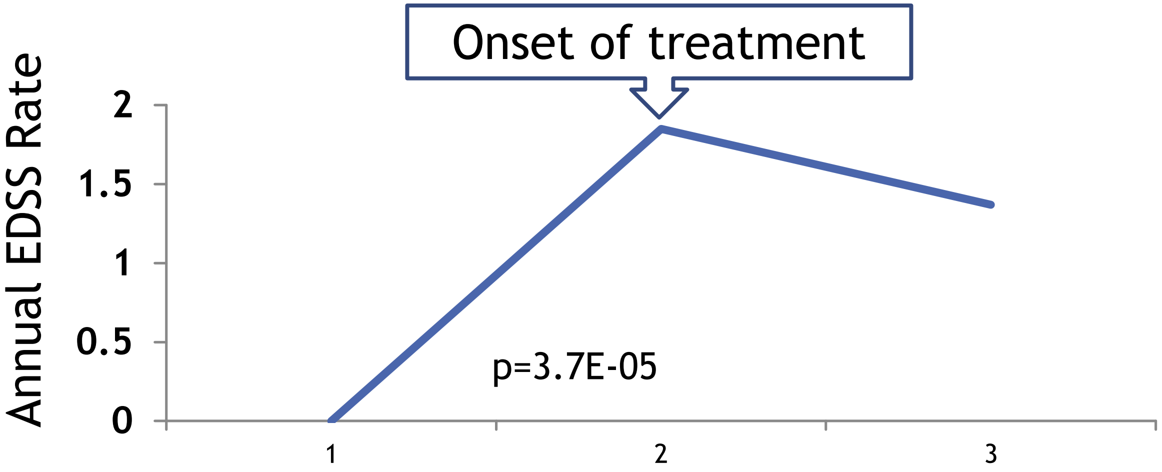
- 18 Patients treated with Natalizumab
  - Of these, 10 patients were sampled for gene expression analysis

	Total n=18	**Female n=10**	**Male n=8**
Age MS onset	13.9±0.8	13.8±1.0	14.1±1.2
Age Tx start	14.8±0.6	14.5±0.8	15.1±1.1
EDSS before Tx	1.67±0.27	1.95±0.36	1.31±0.38
Annual Relapse Rate before Tx	1.33±0.26	1.20±0.31	1.48±0.45

Clinical Results: Natalizumab induces clinical efficacy



1 – before treatment
2 – one year after Natalizumab treatment

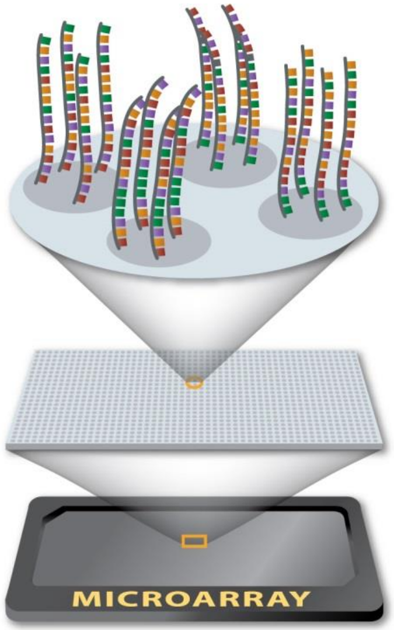


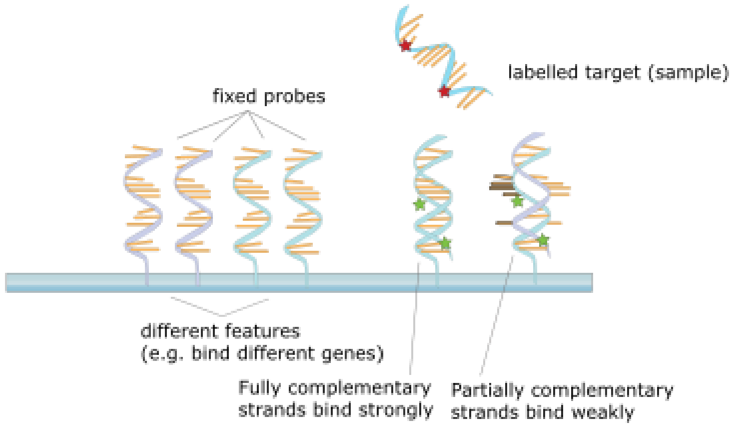
Switch of Annual EDSS rate by Natalizumab treatment

We now know Natalizumab is an effective treatment in pediatric patients. How does Natalizumab effect gene expression?

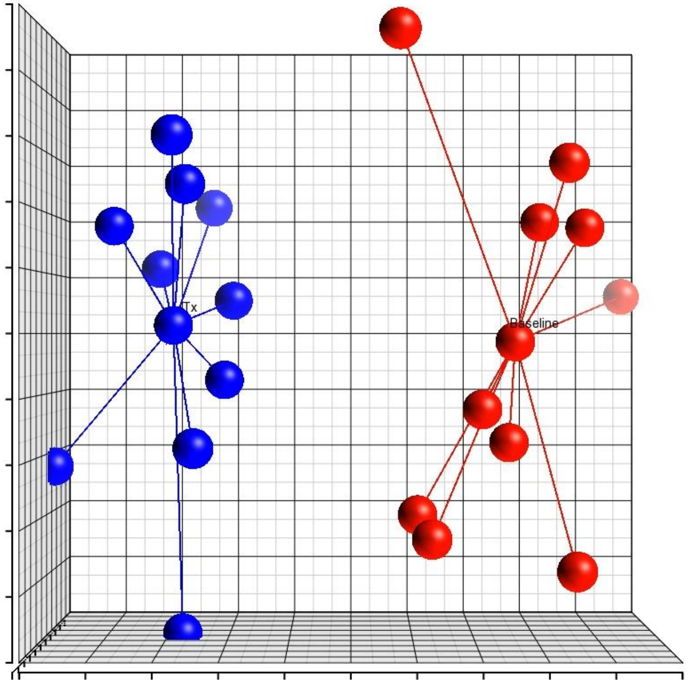
Genetic Microarray Analysis

- Microarrays are microchips that contain genetic information
- They can be used to determine expression of all genes simultaneously in a patient





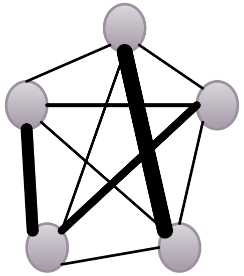
PCA Analysis of Natalizumab-affected gene analysis: two distinct clusters



Red = Before treatment
Blue = After treatment

Weighted Gene Co-expression Network Analysis (WGCNA)

- Developed by Steve Horvath and Peter Langfelder of UCLA in 2008
- This method creates clusters of highly correlated genes which can be related to external traits

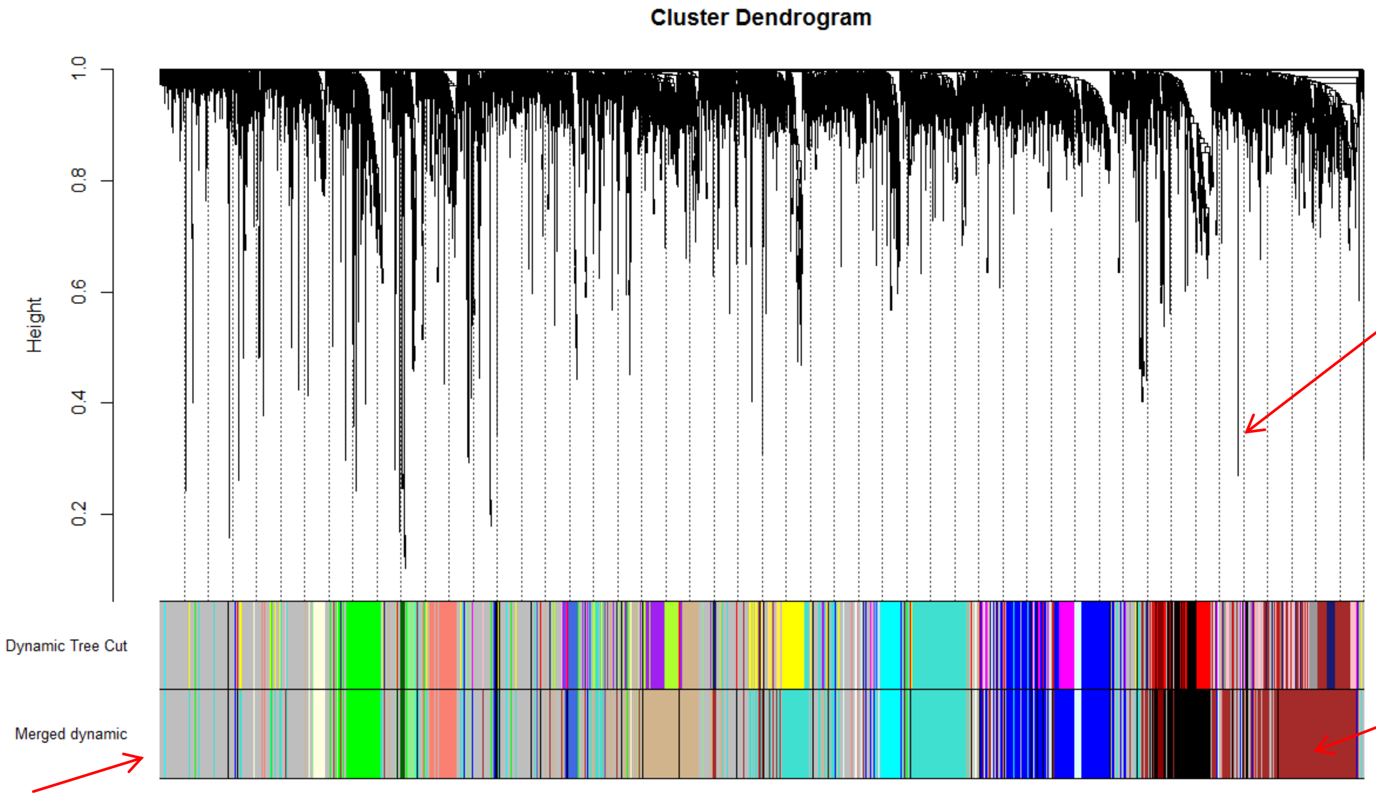


Step 1: Construct a Network
Quantify connection and determine interaction patterns between genes

Step 2: Identify Modules
Find gene clusters

Step 3: Relate Modules to External Information
Find biologically interesting modules

WGCNA: Gene Clusters



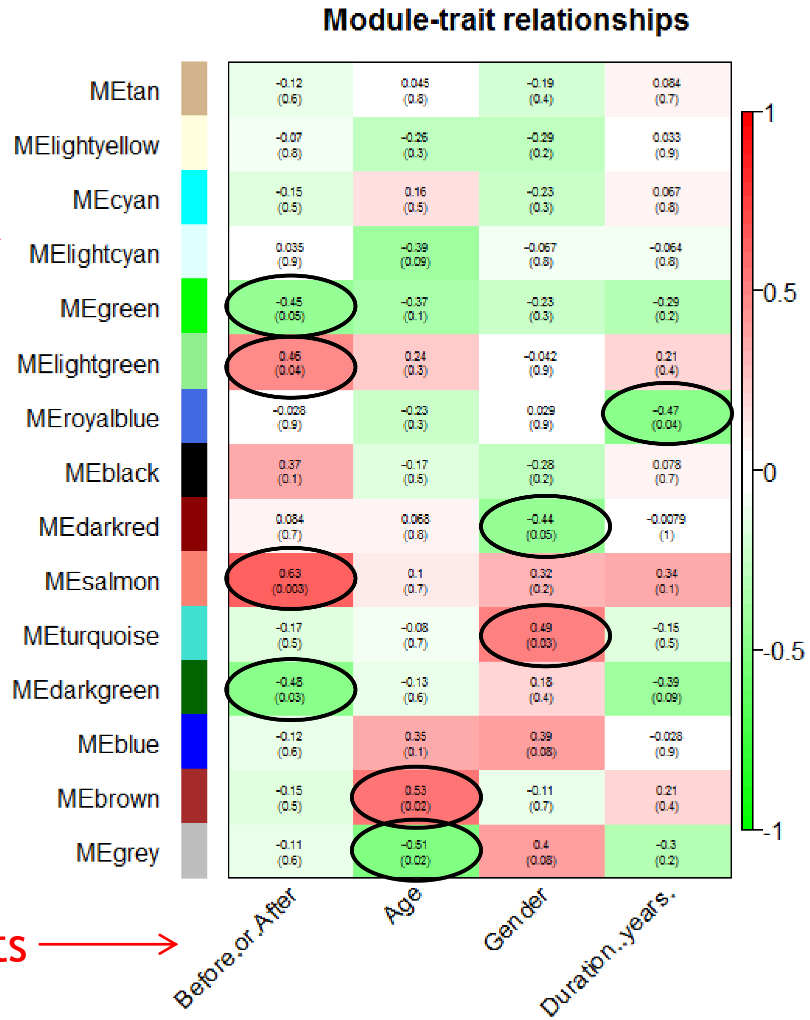
Each black line represents one gene

Each color represents one cluster

We merged highly-related clusters together

WGCNA: Comparison to External Traits

Names of clusters



Module-trait relationships

	Before.or.After	Age	Gender	Duration..years.
MEtan	-0.12 (0.6)	0.045 (0.8)	-0.19 (0.4)	0.084 (0.7)
MElightyellow	-0.07 (0.8)	-0.26 (0.3)	-0.29 (0.2)	0.033 (0.9)
MEcyan	-0.15 (0.5)	0.16 (0.5)	-0.23 (0.3)	0.067 (0.8)
MElightcyan	0.035 (0.9)	-0.39 (0.09)	-0.067 (0.8)	-0.064 (0.8)
MEgreen	-0.45 (0.05)	-0.37 (0.1)	-0.23 (0.3)	-0.29 (0.2)
MElightgreen	0.46 (0.04)	0.24 (0.3)	-0.042 (0.9)	0.21 (0.4)
MEroyalblue	-0.028 (0.9)	-0.23 (0.3)	0.029 (0.9)	-0.47 (0.04)
MEblack	0.37 (0.1)	-0.17 (0.5)	-0.28 (0.2)	0.078 (0.7)
MEdarkred	0.084 (0.7)	0.068 (0.8)	-0.44 (0.05)	-0.0079 (1)
MEsalmon	0.63 (0.003)	0.1 (0.7)	0.32 (0.2)	0.34 (0.1)
MEturquoise	-0.17 (0.5)	-0.08 (0.7)	0.49 (0.03)	-0.15 (0.5)
MEdarkgreen	-0.48 (0.03)	-0.13 (0.6)	0.18 (0.4)	-0.39 (0.09)
MEblue	-0.12 (0.6)	0.35 (0.1)	0.39 (0.08)	-0.028 (0.9)
MEbrown	-0.15 (0.5)	0.53 (0.02)	-0.11 (0.7)	0.21 (0.4)
MEgrey	-0.11 (0.6)	-0.51 (0.02)	0.4 (0.08)	-0.3 (0.2)

Clinical traits

Red = positive correlation

Green = negative correlation

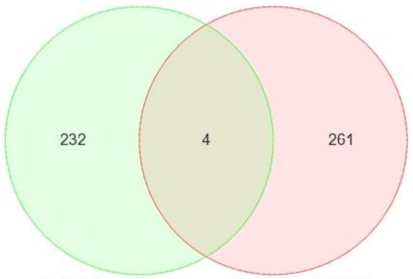
(p-values in parentheses)

Circles = $p \leq 0.05$

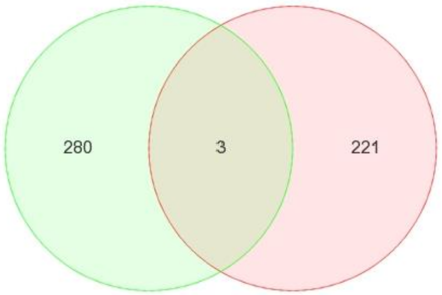
Clusters that are correlated with treatment are not the same clusters that are correlated with age, gender, and duration → Natalizumab does not affect age-related genes

Another approach: analyzing age-related genes using external database

- Using publically available databases, we found a group of healthy controls (n=17). We determined the gene expression that correlates with age.
- The genes most associated with Natalizumab treatment had little similarity (~2%) to the genes most associated with age in these control groups.



Female



Male

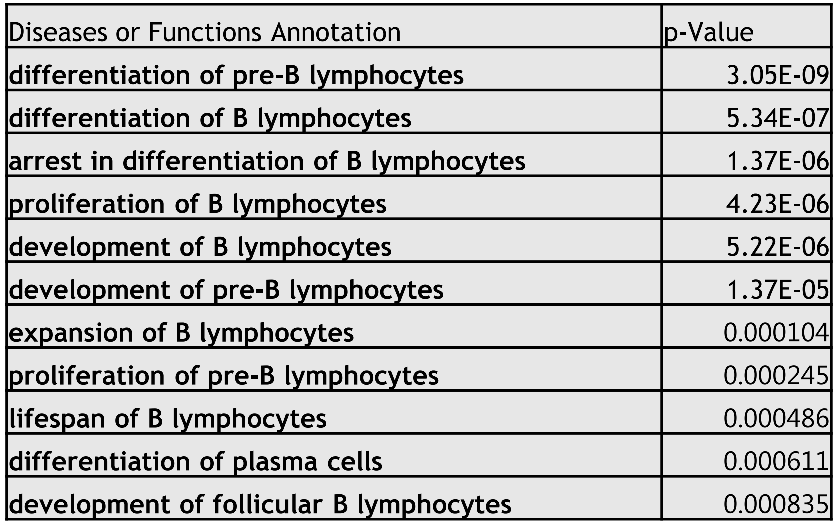
Green = healthy
Red = treated

Future Research Aims

- Evaluation of developmental genes based on age alone has limitations.
- In order to improve our analysis, we plan on obtaining Tanner scores (quantification of puberty) in our control and Natalizumab populations to determine whether Natalizumab-related genes affect pubertal development.

WGCNA: Comparison to Adults

- It has been previously shown that in adults, Natalizumab alters expression of genes related to B cell activity
- We analyzed the clusters of genes that correlate to Natalizumab treatment (722 genes) using ingenuity software → they contain genes related to B cell development



Diseases or Functions Annotation	p-Value
differentiation of pre-B lymphocytes	3.05E-09
differentiation of B lymphocytes	5.34E-07
arrest in differentiation of B lymphocytes	1.37E-06
proliferation of B lymphocytes	4.23E-06
development of B lymphocytes	5.22E-06
development of pre-B lymphocytes	1.37E-05
expansion of B lymphocytes	0.000104
proliferation of pre-B lymphocytes	0.000245
lifespan of B lymphocytes	0.000486
differentiation of plasma cells	0.000611
development of follicular B lymphocytes	0.000835

- **In other words, Natalizumab alters gene expression in children and adults in similar ways**

Conclusion

- Natalizumab treatment is an effective treatment in pediatric patients.
- Natalizumab does not affect genes that are associated with childhood development.
- Natalizumab-related genes are associated with B cell activity, similar to its effects on adults.