Blood transcriptional profiling of pediatric multiple sclerosis

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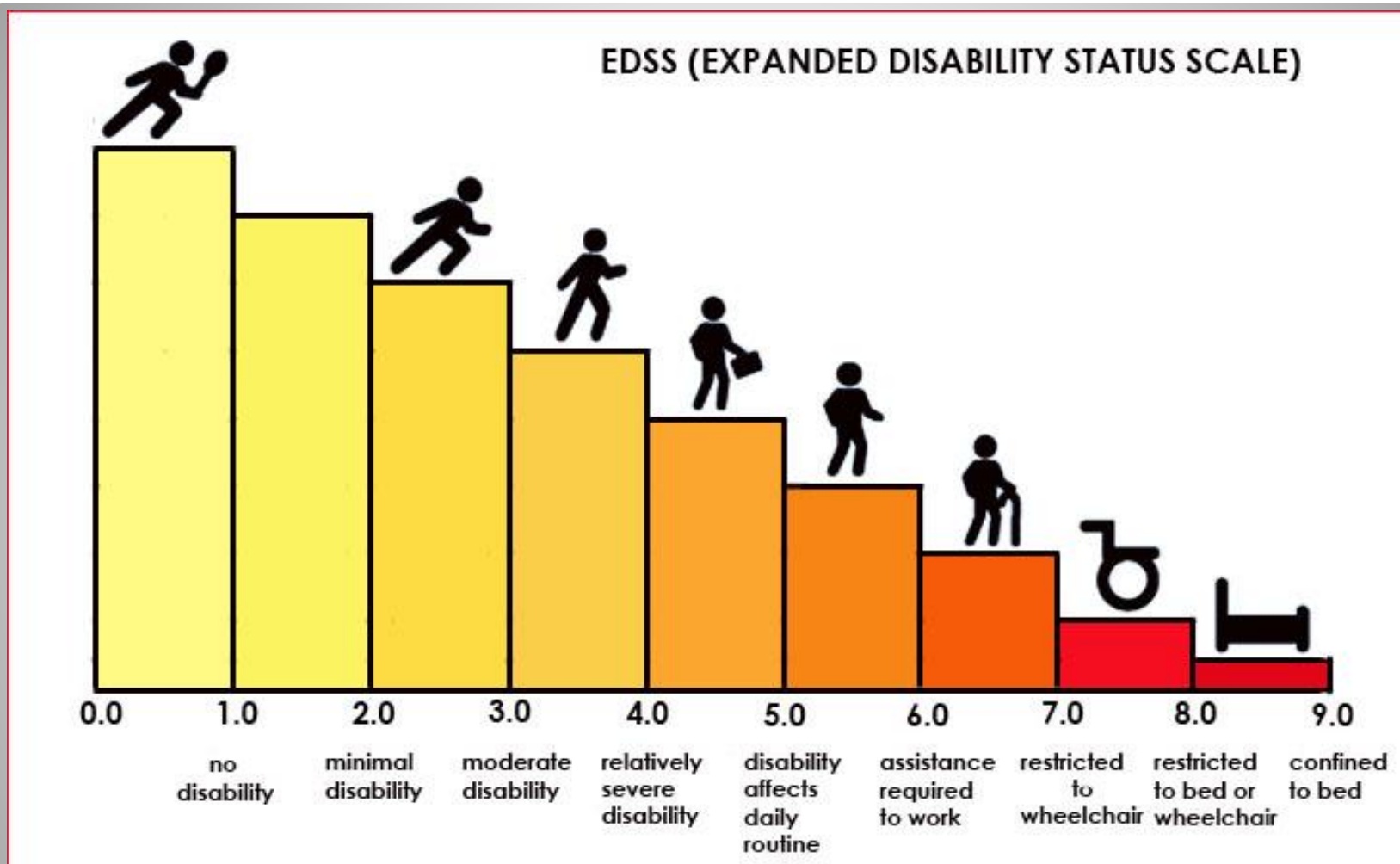
Multiple Sclerosis

- **Multiple sclerosis (MS)** is the most common immune-mediated inflammatory demyelinating disease of the central nervous system
- Diagnosis is based on McDonald's criteria (2010) – imaging (MRI) and clinical findings
- The disease onset typically occurs at ages 20-40
- 2%-5% of MS onsets occur under the age of 18. These cases are defined as **pediatric MS**.


EDSS (EXPANDED DISABILITY STATUS SCALE)
0.0
1.0
no disability
2.0
minimal disability
3.0
moderate disability
4.0
relatively severe disability
5.0
disability affects daily routine
6.0
assistance required to work
7.0
restricted to wheelchair
8.0
restricted to bed or wheelchair
9.0
confined to bed

Motivation

- Pediatric and adult MS patients differ in clinical onset and outcome features.
- Pediatric patients have slower disease progression and better clinical recovery.
- Although there are growing numbers of clinical studies published on pediatric MS, the molecular mechanism behind the disease remains relatively unclear.

Objectives

To assess common and specific blood transcriptional profile of pediatric and adult MS patients and evaluate the underlying molecular mechanism responsible for age-related clinical differences

methods

- Inclusion criteria:
  - Diagnosis of RRMS according to the 2010 McDonald criteria
  - Age 12-17 for pediatric patients, age 20-50 for adult patients
  - At least 1 month from last steroid treatment
  - No previous immuno-modulatory drugs (IMD) treatment

- Peripheral blood mononuclear cell (PBMC) samples of pediatric and adult MS patients were identified in the Sheba Medical Center blood sample database.

Study stages

- Clinical assessment of pediatric and adult MS patients
- Microarray chip production using Affymetrix Inc. technology (HG-U133A2 arrays)
- Data processing and statistical analysis
- Identifying the biological pathways related to the differently expressed genes that were found

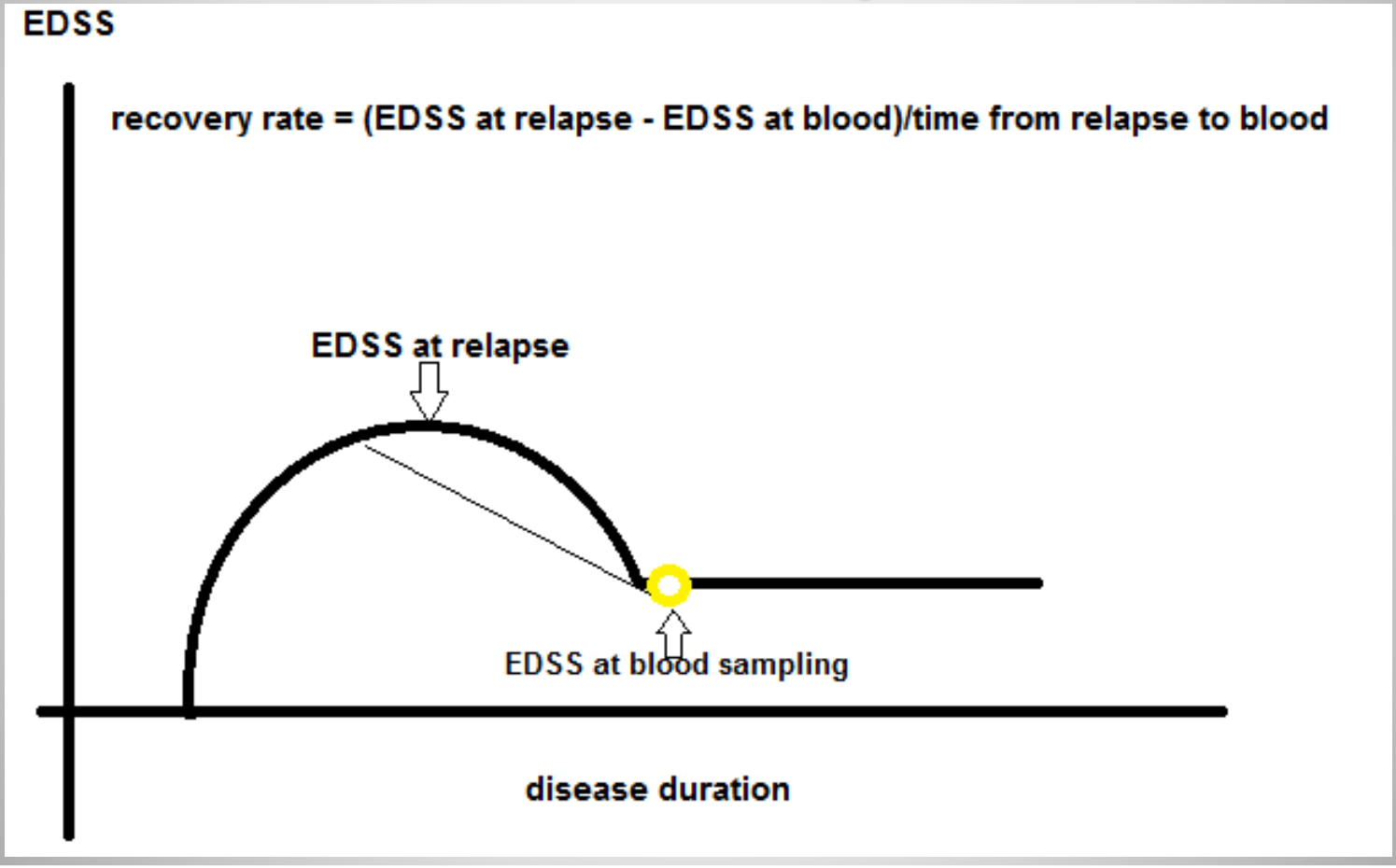
- **Clinical assessment of pediatric and adult MS patients**

Clinical analysis

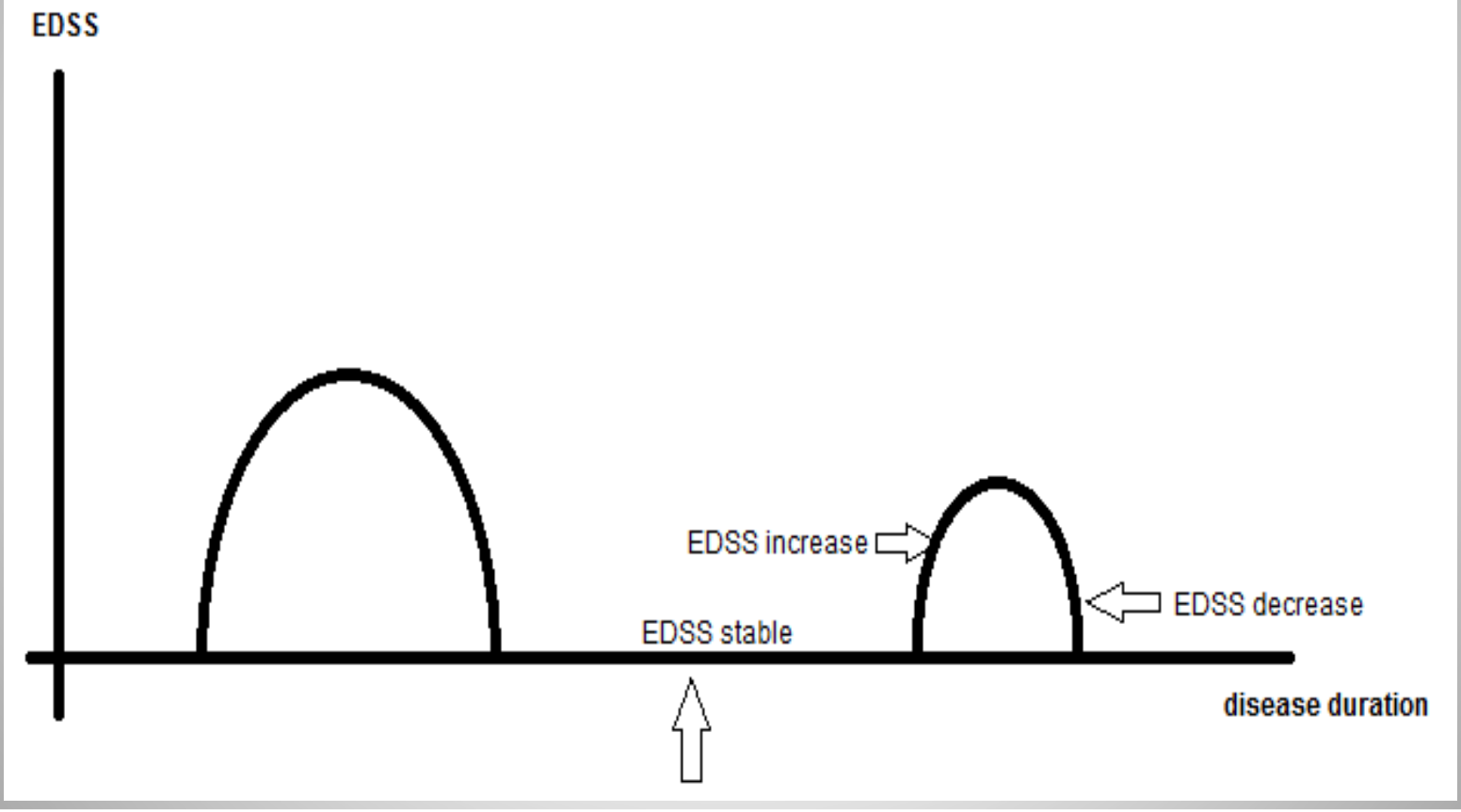
Clinical parameters of pediatric and adult MS patients were assessed, including:

- EDSS at blood sampling
- changes in EDSS ± one year from blood sampling
- Recovery rate from relapse previous to blood sampling

Clinical analysis



Clinical analysis



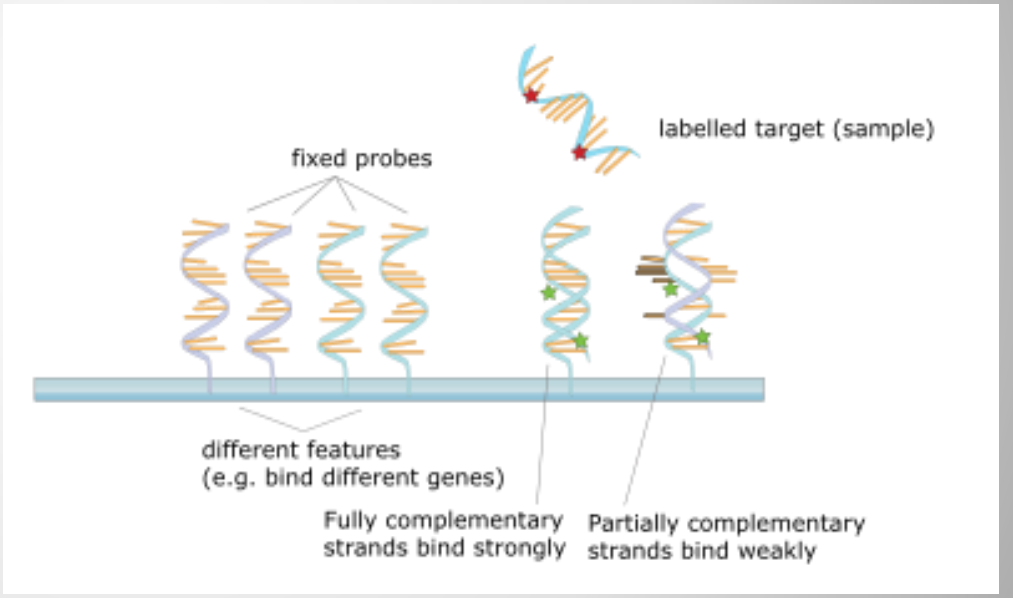
- **Microarray gene expression analysis**

Microarray gene expression analysis

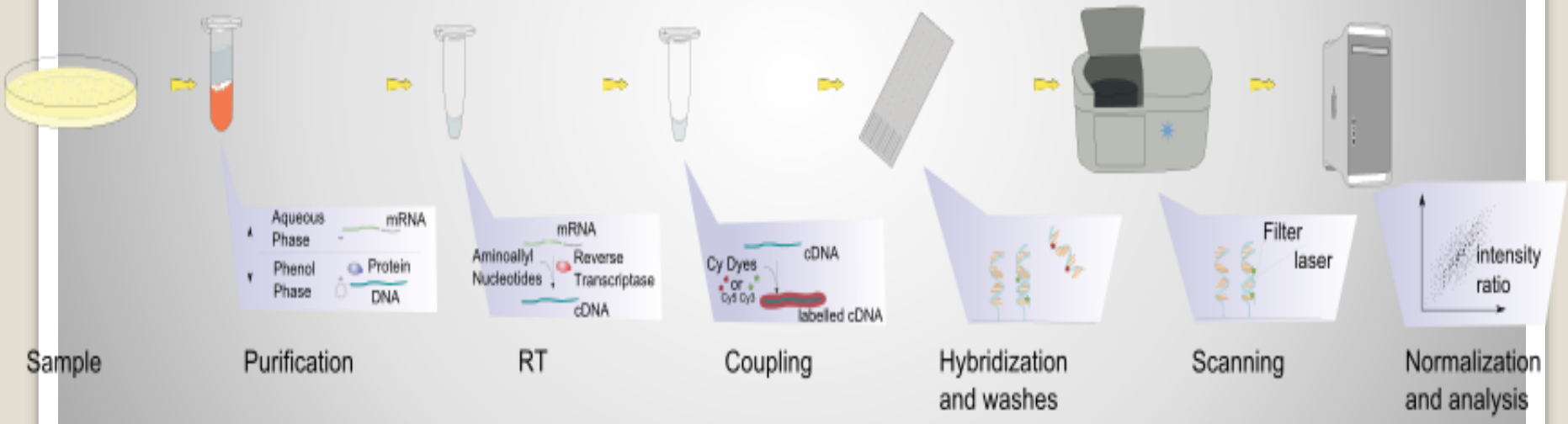
- A **DNA microarray** is a collection of microscopic DNA spots attached to a solid surface, used to measure the expression levels of large numbers of genes simultaneously. Each DNA spot contains large numbers of **probes**: a specific DNA sequence that is used to hybridize a cDNA or cRNA sample

- Probe-target hybridization is detected and quantified using a scanner

Microarray gene expression analysis



Microarray gene expression analysis

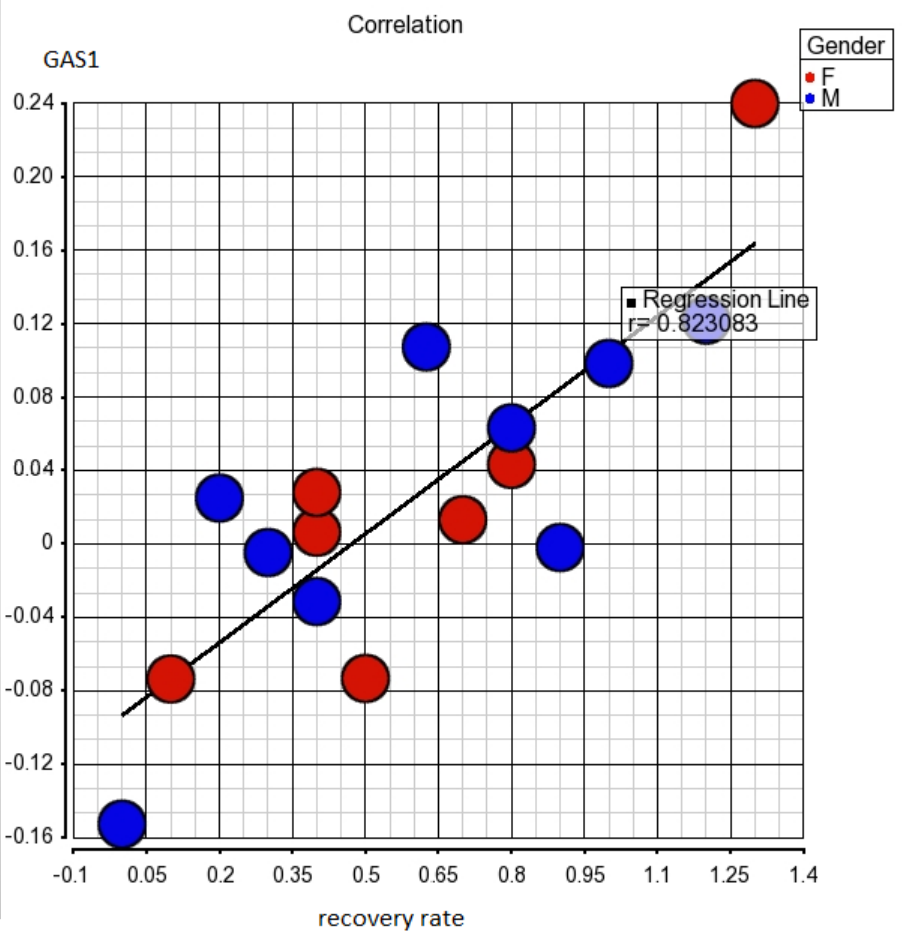


- **Data processing and statistical analysis**

Data processing and statistical analysis

- Out of 22,000 genes, we selected only genes that correlate with clinical parameters: recovery rate from previous relapse, EDSS at blood sample sampling and changes in EDSS around time of blood sampling

Data processing and statistical analysis



- Identifying the related biological pathways

Results

- We have enrolled 16 pediatric MS patients and 15 adult MS patients

- Microarrays were hybridized in batches of 12 samples, assembled according to the principles of controlling batch effect:

Pediatric MS	Adult MS	Healthy pediatric	Healthy adults
2F, 2M	2F, 2M	1F, 1M	1F, 1M

Results: clinical analysis

Pediatric MS	gender	Age at onset	Age at blood sampling	Disease duration	Recovery rate	EDSS at blood sampling
average	8F, 8M	14.1	15.5	1.3	0.6	1.1
S.E		0.4	0.4	0.3	0.3	0.1

adult MS	gender	Age at onset	Age at blood sampling	Disease duration	Recovery rate	EDSS at blood sampling
average	8F, 7M	30.1	31.9	1.6	0.3	1.6
S.E		1.6	1.6	0.5	0.1	0.3

Results: clinical analysis

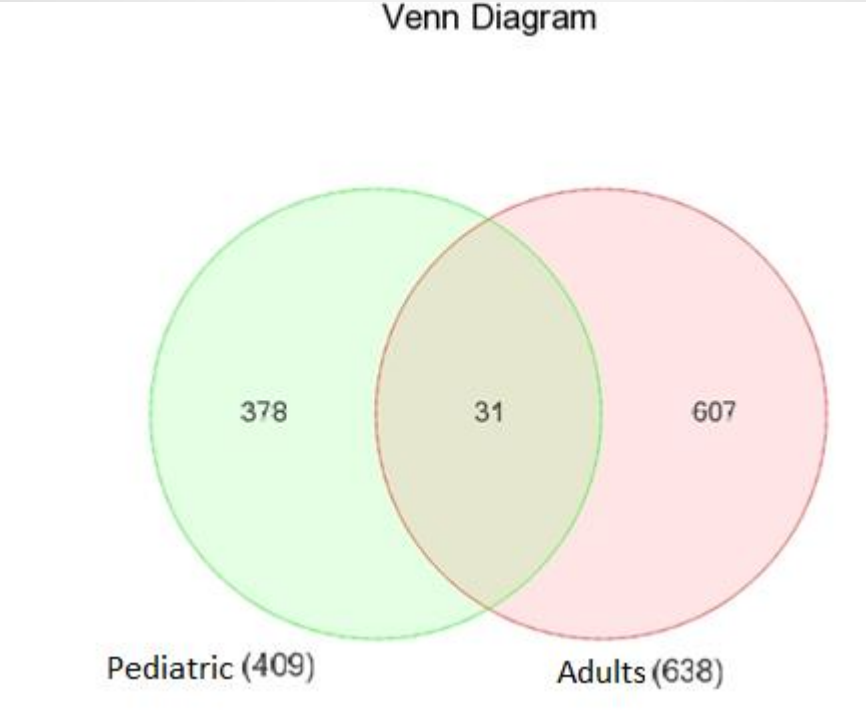
- As expected, the pediatric MS patients had significantly better clinical recovery than adult MS patients (0.6 ± 0.3 vs 0.3 ± 0.1, p-value = 0.02)

Results: statistical analysis

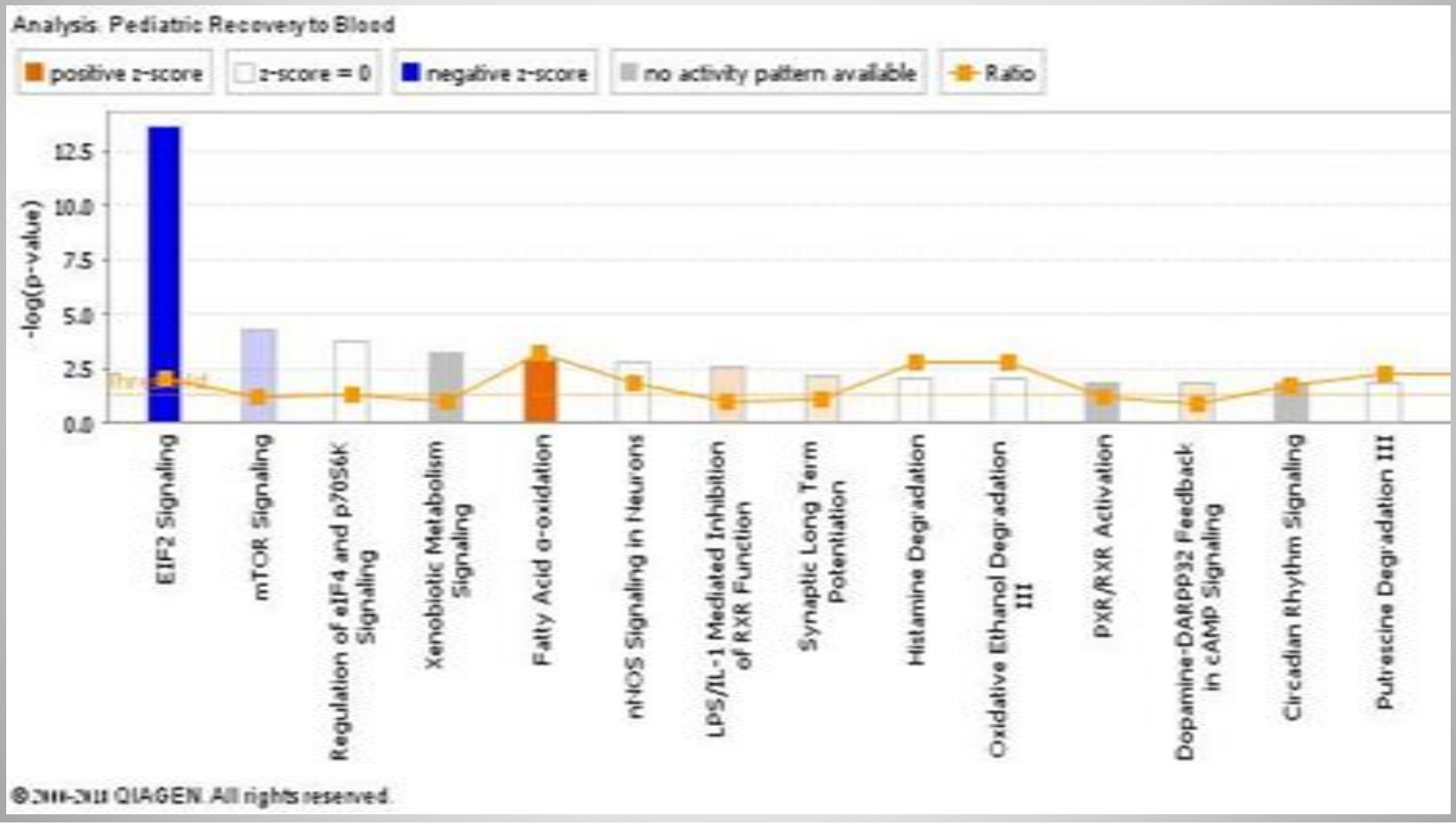
- In pediatric MS patients, 409 genes were found correlated with recovery rate, with coefficients of correlation ranging from -0.71 to -0.9 and from 0.71 to 0.94, p-value < 0.01
- In adult MS patients, 638 genes were found correlated with recovery rate, with coefficients of correlation ranging from -0.93 to -0.74 and from 0.74 to 0.94, p-value < 0.01

Data processing and statistical analysis

31 of these genes were common for pediatric and adult MS:



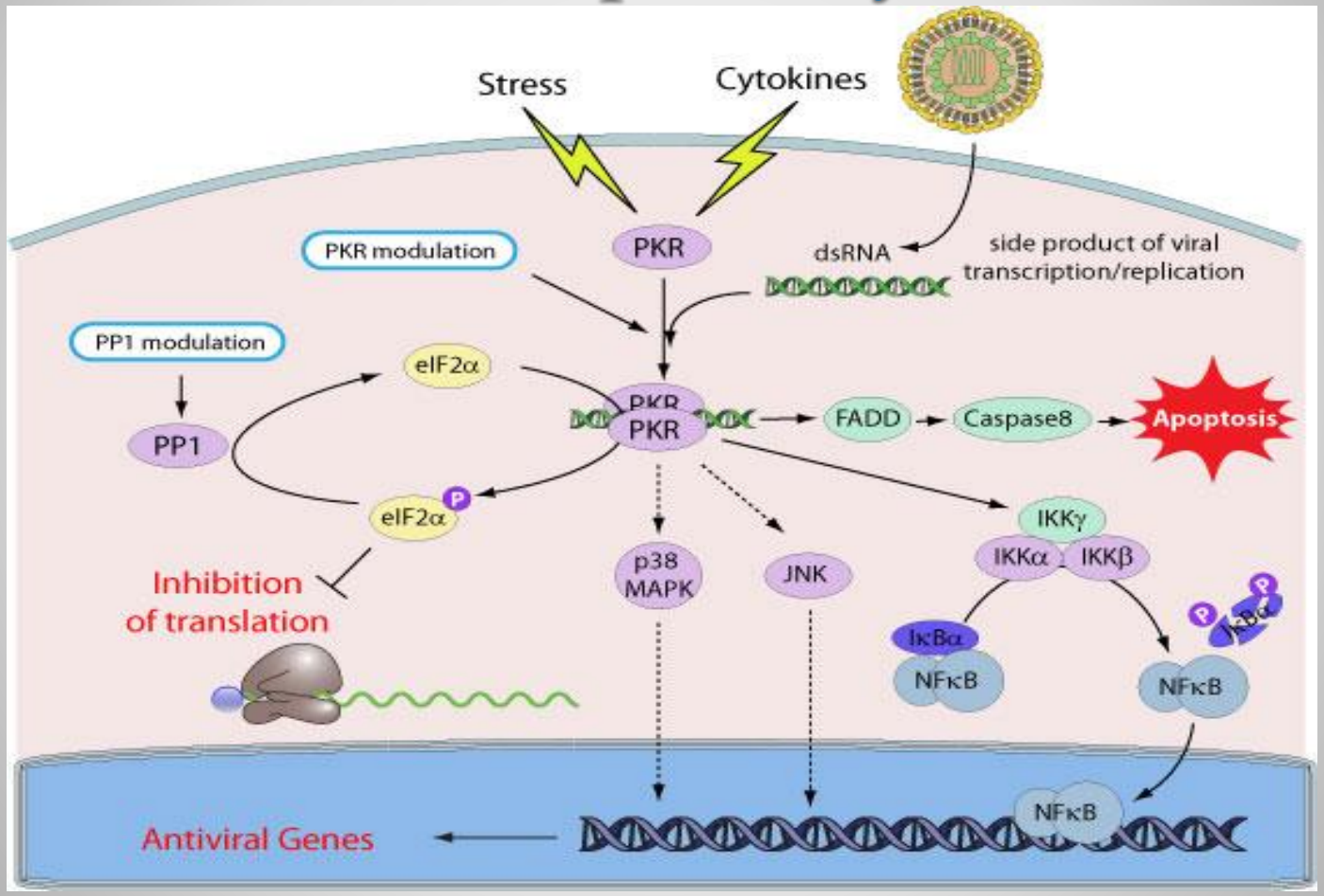
Results: pediatric MS



Results: pediatric MS

Ingenuity Canonical Pathways	P-value
EIF2 Signaling	**2.51E-14**
mTOR Signaling	**4.57E-05**
Regulation of eIF4 and p70S6K Signaling	0.000158
Xenobiotic Metabolism Signaling	0.000646
Fatty Acid α-oxidation	0.001318
nNOS Signaling in Neurons	0.001698
LPS/IL-1 Mediated Inhibition of RXR Function	0.002344
Synaptic Long Term Potentiation	0.006761
Histamine Degradation	0.00871
Oxidative Ethanol Degradation III	0.00871

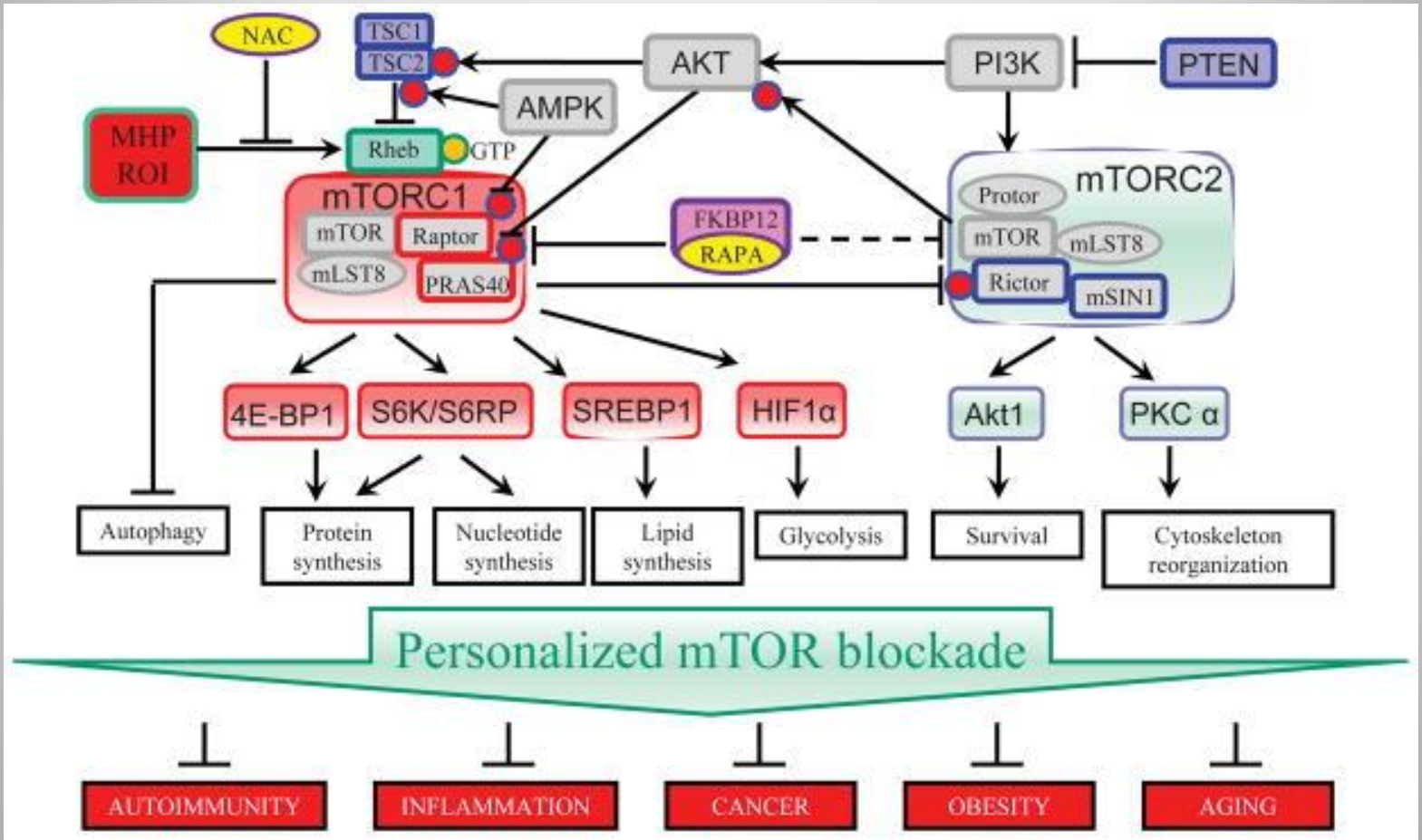
EIF2 pathway



EIF2 and MS

- EIF2 is one of the dysregulated pathways in MS patients (Srinivasan S, 2017)
- EIF2 is upregulated in RRMS INF-beta non-responders (Bustamante MF, et al, 2013)

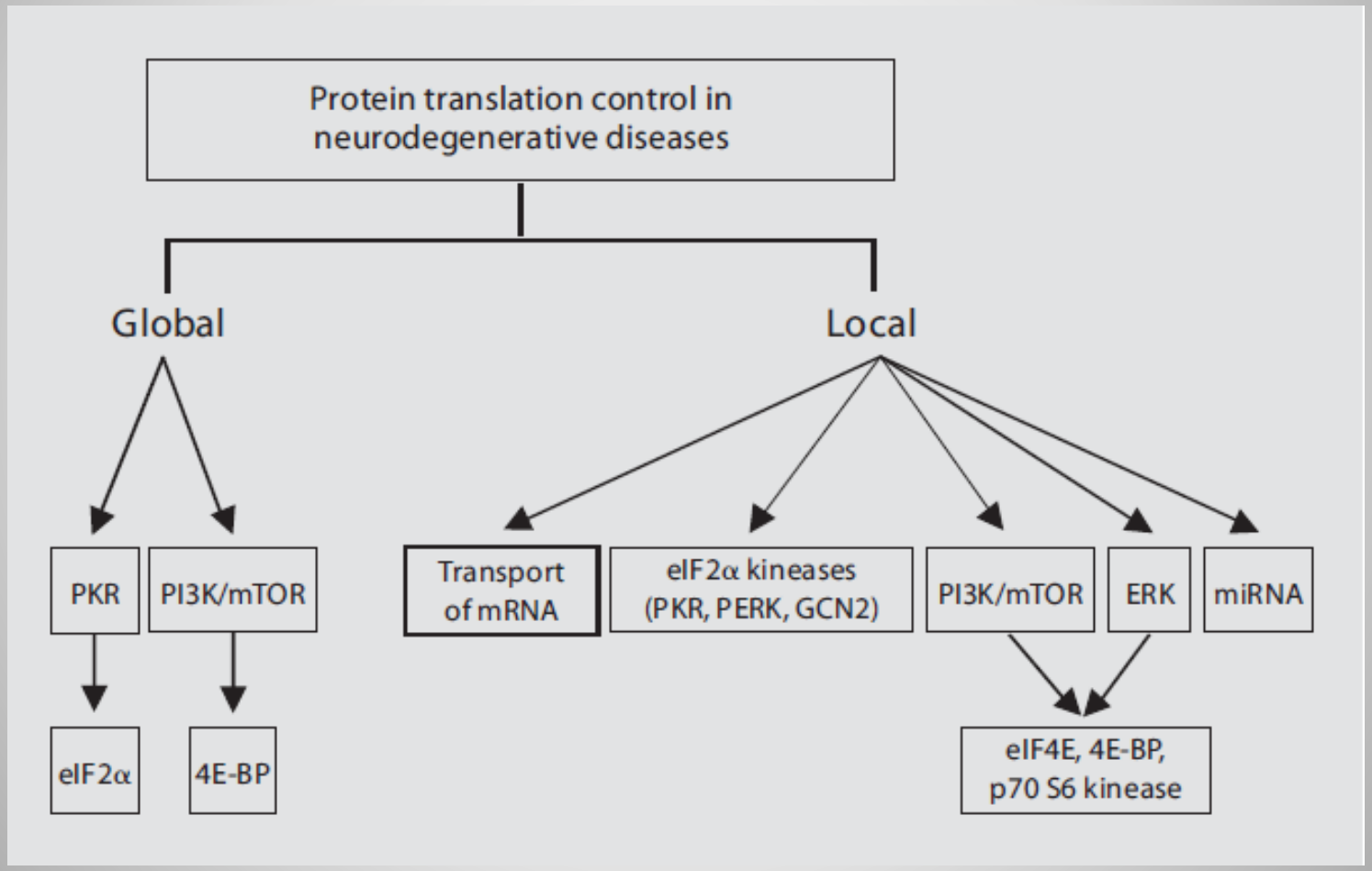
mTOR pathway



mTOR and MS

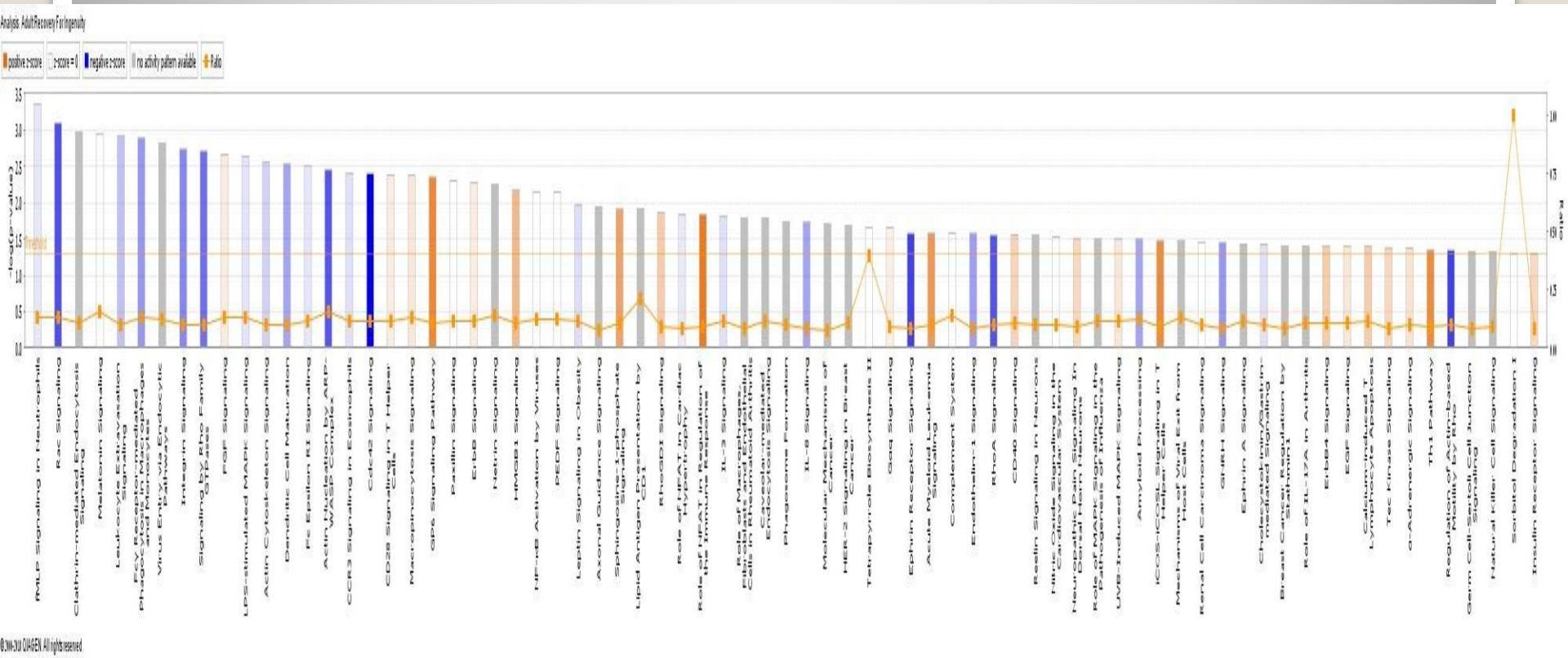
- mTOR inhibitors have beneficial effects in different experimental models of MS, due to their ability to reduce T-cell activation and increase T-regulatory cell function (Dello Russo et al, 2016)

EIF2 & mTOR pathways



Raymod C et al, 2007

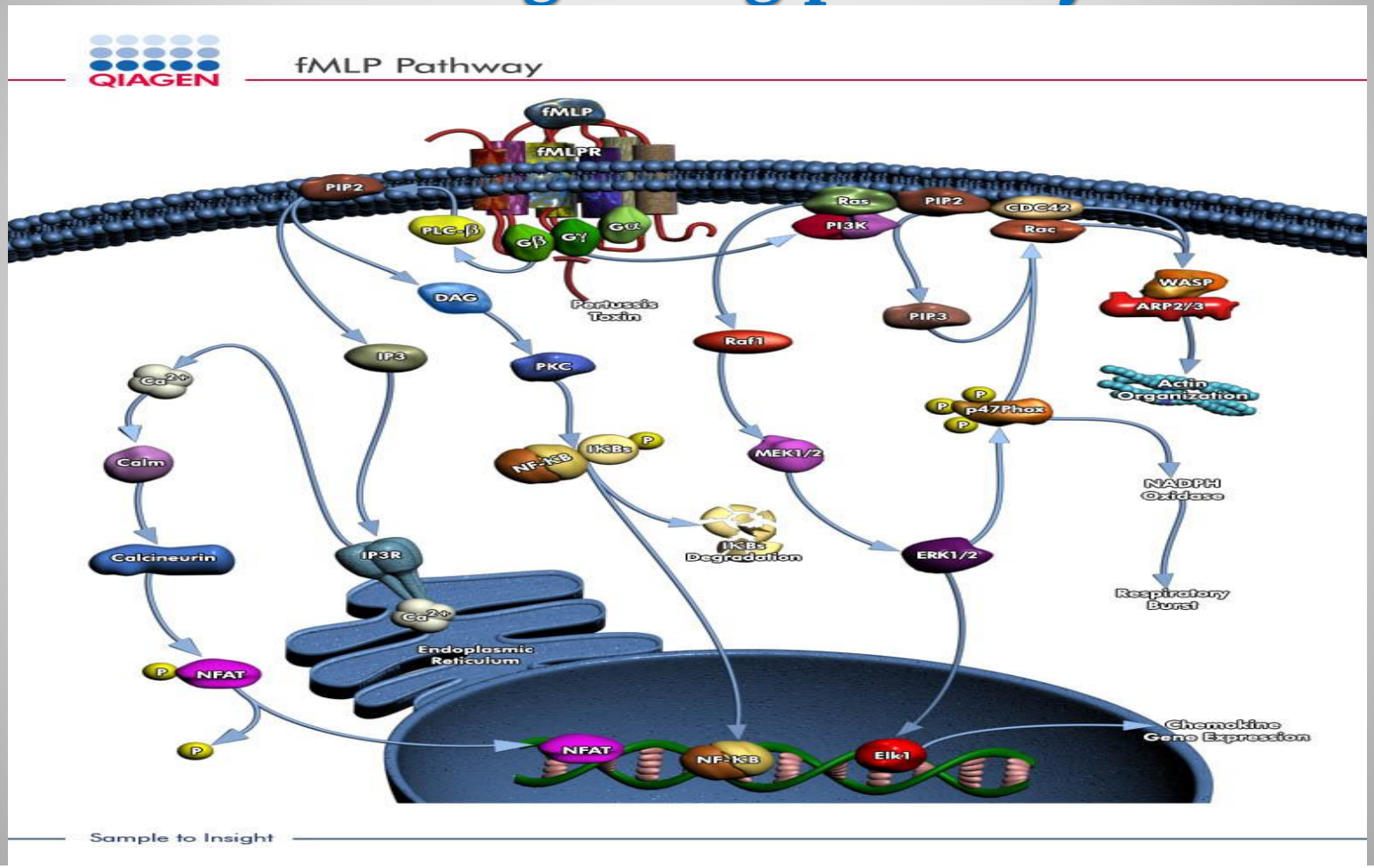
Results: adult MS



Results: adult MS

Ingenuity Canonical Pathways	P-value
fMLP Signaling in Neutrophils	0.000447
Rac Signaling	0.000794
Leukocyte Extravasation Signaling	0.001202
Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes	0.001318
Integrin Signaling	0.001862
Signaling by Rho Family GTPases	0.001905
FGF Signaling	0.002138
CD28 Signaling in T Helper Cells	0.004169
IL-8 Signaling	0.018197

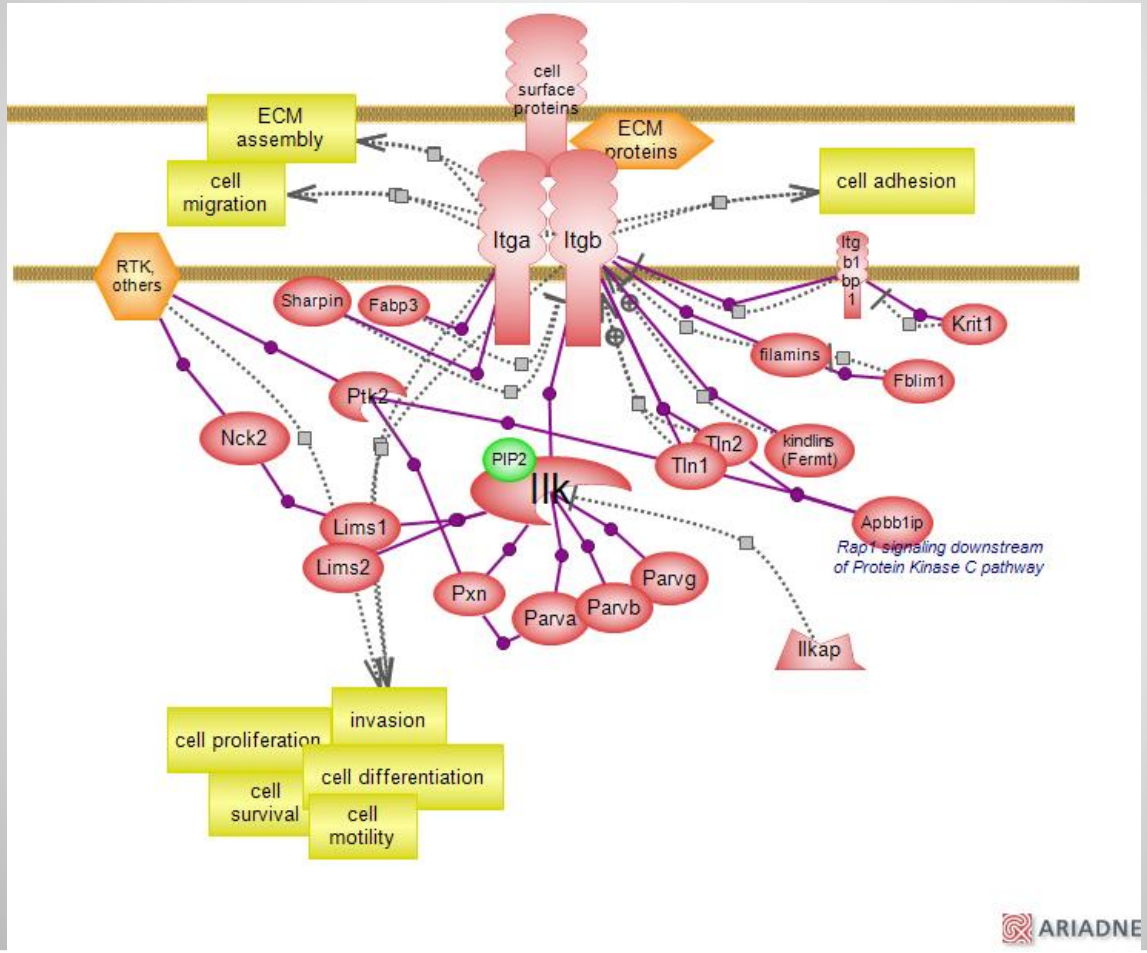
fMLP signaling pathway



fMLP and MS

- neutrophils in multiple sclerosis patients are more numerous and exhibit higher expression of fMLP receptor, probably due to the chronic inflammatory environment in multiple sclerosis which underlies this inappropriate neutrophil priming (Naegele M. et al, 2012)

integrin signaling pathway



Integrins and MS

Natalizumab (Tysabry), an efficient immunomodulatory treatment for MS, is targeted against the protein ITGA4/VLA4 alpha 4 subunit from the integrin pathway

Conclusion

- Pediatric MS patients are characterized by better clinical recovery compared to adults
- The biological mechanism associated with clinical recovery in pediatric and adult population is different: pediatric recovery is associated with EIF2 and mTOR pathways, and adults recovery with multiple mechanisms including fMLP and integrin pathways.

Thank you