Biomarkers and Genetic Variations Related to Glutamate Concentration in the MS Brain

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Outline of the presentation

1) Brief Review of Glutamate Biology in MS

2) Brain Glutamate ($^1$H-MRS) as a Biomarker?
   - $^1$H-MR Spectroscopy

3) Genetic Variations associated with Brain Glutamate
   - Individual susceptibility to MS severity/progression?

4) Summary and Future Directions
Why being interested in glutamate biology in MS?

Glutamate-Glutamine Neuro-Glial Cycle
Potential Excitotoxic Cascades

![Diagram of excitotoxic cascades](image)


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Glutamate in MS

- …
Glutamate excitotoxicity:

Dysregulation of glutamate signaling, including sustained activation of ionotropic glutamate receptors or reduced glutamate uptake, impairs cellular calcium homeostasis and activates nitric oxide synthesis, leading to free radical generation and cell death.

Targeting Glutamate in Clinical Trials

No clear-cut answers from MS drug trials:

- Plaut 1987 (RRMS) - Amantadine weak NMDAR antagonist
- Killestein 2005 (PPMS) - Riluzole kainate and NMDAR antagonist
- Lovera 2010 (all MS) - Memantine NMDAR blocker
- Waubant 2015 (early MS) - Riluzole kainate and NMDAR antagonist

Agents, dose, route, trial design, PEP, etc…

- Lisak 2014 (cell culture) – Dextromethorphan NMDAR antagonist protects oligodendrocyte and OPC against glutamate excitotoxicity
In vivo Brain Glutamate in MS

Hurd et al. MRM 2004
Unobstructed glutamate

Srinivasan et al. Brain 2005
~30% in gad+ lesion

Srinivasan et al. Neuroimage 2006
glutamate NAWM, NAGM maps

In vivo Brain Glutamate in MS

Srinivasan et al. Neuroimage 2006
Glutamate as predictor of poor outcomes in MS

Table 1. Baseline Demographics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients (N=343)</th>
<th>Controls (N=42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>234</td>
<td>26</td>
</tr>
<tr>
<td>Male</td>
<td>109</td>
<td>16</td>
</tr>
<tr>
<td>Age (years)</td>
<td>43.2 ± 9.6</td>
<td>40.5 ± 10.0</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>9.1 ± 8.0</td>
<td></td>
</tr>
<tr>
<td>Clinical Subtype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIS</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>RRMS</td>
<td>263</td>
<td></td>
</tr>
<tr>
<td>SPMS</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>PPMS</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>EDSS (score)</td>
<td>2 (0-7.5)</td>
<td></td>
</tr>
<tr>
<td>MSFC (z score)</td>
<td>0.20 ± 0.59</td>
<td></td>
</tr>
<tr>
<td>Subjects on DMT</td>
<td>226</td>
<td></td>
</tr>
</tbody>
</table>

Statistics:
- mixed-effects models for repeated measures
- slope modifiers
- interaction of time taken into account

Azevedo et al. Ann Neurol 2014;76:269-278
Glutamate as predictor of poor outcomes in MS

NAA decline over 2 years

Table 3. Glutamate and Sustained Elevation of Glutamate as Predictors of NAA Decline (N=343 and 211, respectively)*#  

<table>
<thead>
<tr>
<th>Predictor</th>
<th>NAA in NAWM</th>
<th>NAA in GM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glu [mM]</td>
<td>-0.03 (-0.07, 0.01) p=0.12</td>
<td>-0.06 (-0.11, -0.02) p=0.004</td>
</tr>
<tr>
<td>Glu [mM]</td>
<td>-0.03 (-0.07, -0.002) p=0.038</td>
<td>-0.08 (-0.08, -0.02) p=0.003</td>
</tr>
<tr>
<td>Sustained Elevation Glu [mM]</td>
<td>-0.28 (-0.41, -0.15) p=0.001</td>
<td>-0.15 (-0.30, 0.004) p=0.056</td>
</tr>
<tr>
<td>Sustained Elevation Glu [mM]</td>
<td>0.21 (-0.14, 0.56) p=0.24</td>
<td>-0.07 (-0.46, 0.33) p=0.75</td>
</tr>
</tbody>
</table>

NAA = N-acetyl aspartate, NAWM = Normal Appearing White Matter, GM = Grey Matter  
*Parameter estimate for the predictor by time interaction and 95% confidence intervals are given for each model; models in which the predictor by time interaction was statistically significant at alpha=0.05 are bolded.  
#Mean follow up time was 2.2 years for NAA.
Table 4. Glu/NAA as a Predictor of Clinical Outcomes (N=211)*

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Annualized PBVC</th>
<th>MSFC</th>
<th>PASAT</th>
<th>EDSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glu/NAA_{[U,ml]}</td>
<td>0.33 (0.13, 0.52) p=0.002</td>
<td>0.009 (0.004, 0.014) p&lt;0.001</td>
<td>0.17 (0.07, 0.27) p&lt;0.001</td>
<td>0.001 (-0.02, 0.02) p=0.92</td>
</tr>
<tr>
<td>Glu/NAA_{[U,ml]}</td>
<td>0.06 (-0.06, 0.20) p=0.42</td>
<td>-0.003 (-0.007, 0.001) p=0.11</td>
<td>0.004 (-0.07, 0.08) p=0.92</td>
<td>-0.007 (-0.02, 0.01) p=0.29</td>
</tr>
</tbody>
</table>

*Parameter estimate for the predictor by time interaction and 95% confidence intervals are given for each model; models in which the predictor by time interaction was statistically significant at alpha=0.05 are bolded.

*The value given in the table reflects the effect of a 10% change in Glu/NAA.

Azevedo et al. Ann Neurol 2014;76:269-278
Genome Wide Association Study and *in vivo* glutamate

**Figure 2. Strategy**

Baranzini et al. Brain 2010
Genome Wide Association Study and *in vivo* glutamate

**Green:** glutamate receptor and transporter organization; **Red:** TGBb signaling; **Pink:** regulators of glutamatergic synaptic activity; **Yellow:** glutamate receptors; **Blue:** axon guidance

Highest Scoring - Module 14
70 genes

**Individual Genetic Scores**

- Pilot study: Toward individual tx approaches for susceptible patients?

- Genetic Score = For a module of N genes, each patient was assigned a genetic score (from 0 to 2*N) corresponding to the total number of risk alleles carried at the N loci.

  \[(N=70 \text{ genes})\]
Individual Glutamate Genetic Scores and Correlation with Markers of disease severity

[Glutamate] vs. Genetic Score

Baranzini et al. Brain 2010

Individual Glutamate Genetic Scores and Correlation with Markers of disease severity

[NAA] decline vs. Genetic Score

Baranzini et al. Brain 2010
Individual Glutamate Genetic Scores and Correlation with Markers of disease severity

Whole Brain Atrophy vs. Genetic Score

Better phenotyping leads to better genotyping!
Some future directions

- Learning: Sample size of ~500 MRI datasets provides pilot data
- Replication is necessary
- Deep genotyping/phenotyping. Larger observational, prospective, longitudinal MS cohorts are on-going.

**Individual Level**

↑Genetic score vs. ↓Genetic score

Glutamate pathway (GWAS)

• Long term whole brain volume loss
• Thalamic atrophy/Cortical thinning
• Cognitive impairment
• Time to reach EDSS 3 and 6, MSFC

We also want to pursue our findings and investigation further this glutamate biological pathway *in vivo* with advanced metabolite imaging experiments ("indirect measures of extracellular glutamate").
Some future directions

$^{13}$C-MRS in vivo to measure:
- glutamate/glutamine neuro-glial cycle ($V_{cycle}$) 
  (Rothman et al. 2011)
- mitochondrial energetic function (TCA cycles)

Hypothesis: excess glutamate mediated by activated microglia coupled with deficient glial re-uptake

Expected Outcome: abnormal $V_{cycle}$ as an indirect indicator of an excess of extracellular glutamate

Glutathione: an oxygen radicals scavenger


Towards an *in vivo* metabolite profile for neurodegeneration and neuroprotection

Indication for oxidative stress in Multiple Sclerosis measured using proton spectroscopic imaging at 7T

Radhika Srinivasan, Sarah Nelson, Kate Hammond, Duan Xu, Douglas Kelley, Daniel Vigneron, and Daniel Pelletier

- NAA,
- Glutamate,
- Glutathione (GSH),
- GABA,
- (Vitamin C),
- macromolecules

Are genetically susceptible patients with
- ↑ Glutamate,
- ↓ GSH
more at risk of loss of NAA and brain atrophy?


Summary

1) Brain Glutamate, measured by $^1$H-MRSI, is elevated in MS white matter.
2) Brain Glutamate predicts NAA decline over 2 years.
3) Glu/NAA$^{[NAWM]}$ predicts brain atrophy and MSFC over 4 years.
4) Through GWAS study we have observed genetic variations that could increase susceptibility to worse MS outcomes at the individual level.
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Thank you for your attention!

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USC MS Center in 2017!