Plasma homocysteine levels and circulating microRNA profiles in patients with ME/CFS

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Introduction
- Myalgic Encephalomyelitis, often referred to as Chronic Fatigue Syndrome, is a complex chronic disease with biochemical, metabolic, and genetic components.
- Given the clinical heterogeneity and gender differences, its etiology is not well understood. Progression varies among individuals and little is known regarding validated biomarkers for ME/CFS.
- There is a need to find biomarkers for ME/CFS, and we have identified biochemical factors and circulating miRNA that may be relevant.

Objectives
- We investigated whether alterations exist in the plasmatic levels of specific biochemical factors and circulating microRNAs in ME/CFS patients.

Methods
- Recruitment of patients:
  - French-Canadian patients (n=111, 87F + 24M) & low age & gender-matched healthy controls (n=58, 24F + 34M)

- Immunodosage of biochemical markers:
  - Evaluation of plasma levels of different biochemical markers, by ELISA method

- miRNA extraction from plasma:
  - Platelet-poor plasma from a discovery panel (ME/CFS patients n=11, 9F + 2M) & healthy controls without familial antecedent of ME/CFS (n=7, 5F + 2M)
  - miRNA extraction using the miRNeasy kit (Qiagen)

- Analysis of circulating miRNA:
  - Analysis of circulating miRNA expression profile, by hybridization array, using Agilent expression array

- Identification of miRNA:
  - Agilent GeneSpring software
  - Clustering analyses
  - miRNAs differentially expressed between ME/CFS and controls
  - Significant expression difference: ±2 fold-change, false-discovery rate ≤0.005

Results
- Among the several biomarkers tested, the mean plasma homocysteine (HCY) levels were significantly increased in a subset of ME/CFS patients when compared to controls (p < 0.05; Student’s t-test two-tailed, equal variance)
- The average values were 30±18 µmol/L and 7±3 µmol/L for high HCY ME/CFS and low HCY ME/CFS subgroup respectively. When compared to the matched healthy controls (10±8 µmol/L)

Table 1. Plasma HCY values in healthy subjects and ME patients

<table>
<thead>
<tr>
<th>Subjects</th>
<th>N</th>
<th>Age (years)</th>
<th>Sub-group</th>
<th>HCY Level (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Controls</td>
<td>50</td>
<td>47 ± 10 (28-65)</td>
<td>Normal</td>
<td>10 ± 8 (2-37)</td>
</tr>
<tr>
<td>All ME Patients</td>
<td>98</td>
<td>51 ± 10 (28-78)</td>
<td>Low HCY</td>
<td>7 ± 3 (2-15)</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>49 ± 12 (13-64)</td>
<td>High HCY</td>
<td>30 ± 18 (16-79)</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Female Controls</td>
<td>20</td>
<td>46 ± 11 (28-65)</td>
<td>Normal</td>
<td>9 ± 8 (2-28)</td>
</tr>
<tr>
<td>Female ME Patients</td>
<td>62</td>
<td>51 ± 10 (28-78)</td>
<td>Low HCY</td>
<td>8 ± 4 (2-15)</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>51 ± 9 (26-64)</td>
<td>High HCY</td>
<td>32 ± 20 (16-79)</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Male Controls</td>
<td>30</td>
<td>49 ± 10 (30-65)</td>
<td>Normal</td>
<td>10 ± 9 (2-37)</td>
</tr>
<tr>
<td>Male ME Patients</td>
<td>14</td>
<td>50 ± 11 (32-67)</td>
<td>Low HCY</td>
<td>6 ± 2 (3-10)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>43 ± 20 (13-62)</td>
<td>High HCY</td>
<td>22 ± 8 (16-35)</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Conclusions
- Elevated levels of HCY have previously been reported in the cerebrospinal fluid of patients with fibromyalgia and ME/CFS (Regland et al. Scand J Rheumatol. 1997; 26 (4):301-7), and correlated with fatigability
- Our preliminary data strongly suggests that microRNAs could play an important role in the elevation of circulating HCY levels in a subset of ME/CFS patients

Acknowledgments
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Figure 1: Small but powerful. MiRNAs regulate the expression of several genes Ref: Van Rooij, E. “The Art of microRNA Research” Review Circulation Research, 2011
Could miRNA orchestrate the development and progression of Myalgic Encephalomyelitis?

Figure 2. MicroRNAs deregulated in ME/CFS patients and modulating key components of folic acid and methionine metabolic pathways

- * miR-4701-3p predicted target in high HCY expressor
- * miR-4701-3p predicted target in low HCY expressor
- * miR-3198 predicted target in high HCY expressor
- * miR-4701-3p predicted target in low HCY expressor

Figure 3. Biochemical pathway of homocysteine metabolism

Folic acid and methionine metabolic pathways