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

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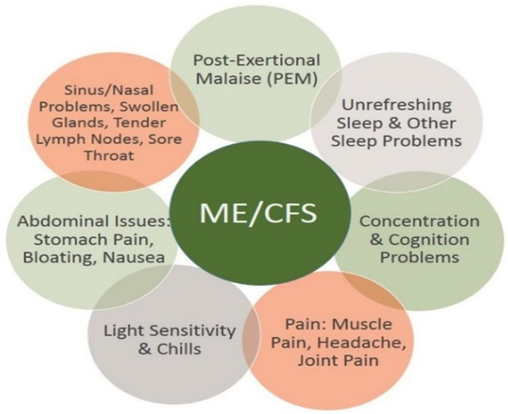
Introduction

- Myalgic Encecphalomyelitis, often refered to as Chronic Fatigue Syndrome, is a complexe 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

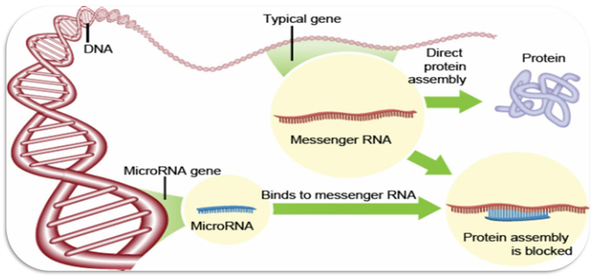


Figure 1: Small but powerful. MiRNAs regulate the expression of several genes *Ref: Van Rooij.E «The Art of microRNA Research» Review Circulation Research, 2011*

Could miRNA orchestrate the development and progression of Myalgic Encephalomyelitis?

Objectives

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

Methods

Recrutement of patients
- French-Canadian patients (n=111, 87F + 24M)
- Diagnosed with ME/CFS (according to Canadian Consensus Criteria)
- Analysed and compared to age and gender-matched healthy controls (n=58, 24F + 34M)

Immunodosage of biochimical markers
- Evaluation of plasma levels of different biochimical 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-Human miRNA 8 x 60k

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

Results

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

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

- 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 (using a 16µmol/L cut-off), when compared to the matched healthy controls (10±8 µmol/L)

Folic acid and methionine metabolic pathways

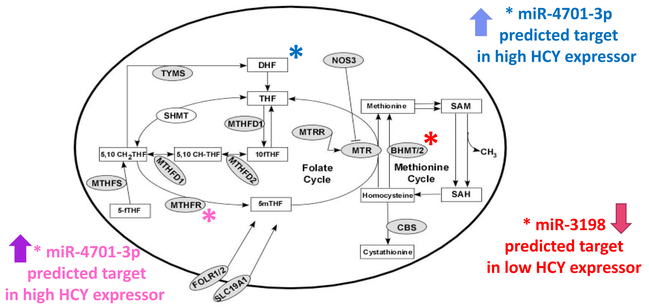


Figure 2. MicroRNAs deregulated in ME/CFS patients and modulating key components of folic acid and methionine metabolic pathways

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

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AQEM Association Québécoise de l'Encéphalomyélite Myalgique

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