

Multitasking Biomolecules in ME/CFS Pathogenesis

Known Players on Their Unexpected Journey

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DISCLOSURES

Pr. Moreau's current research programs are funded by:

Research program on ME/CFS

Pr. Moreau ME/CFS research program has been approved by Sainte-Justine University Hospital Ethic Review Board (protocol #2015-829)



Research program on idiopathic scoliosis

Pr. Moreau scoliosis research program has been approved by Sainte-Justine University Hospital Ethic Review Board (protocol #2018-1935)



Research program on osteoarthritis

Pr. Moreau osteoarthritis research program has been approved by Sainte-Justine University Hospital Ethic Review Board (protocol #2018-1630)



- Member of the Institute of Musculoskeletal Health & Arthritis (CIHR), Institute Advisory Board
- Member of Open Medicine Foundation Scientific Advisory Board (USA)
- Senior Editorial Board Member, Scientific Reports, Nature Co (UK)
- Chief Scientific Officer and Co-Founder, Inception Therapeutics Inc., (Montreal, Canada)

3 OPEN MEDICINE FOUNDATION

ME/CFS Collaborative Research Center at *CHU Sainte-Justine/Université de Montréal*

<https://www.omf.ngo/collaborative-research-center-montreal/>



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Stress-Activated MicroRNAs
in ME/CFS Pathogenesis

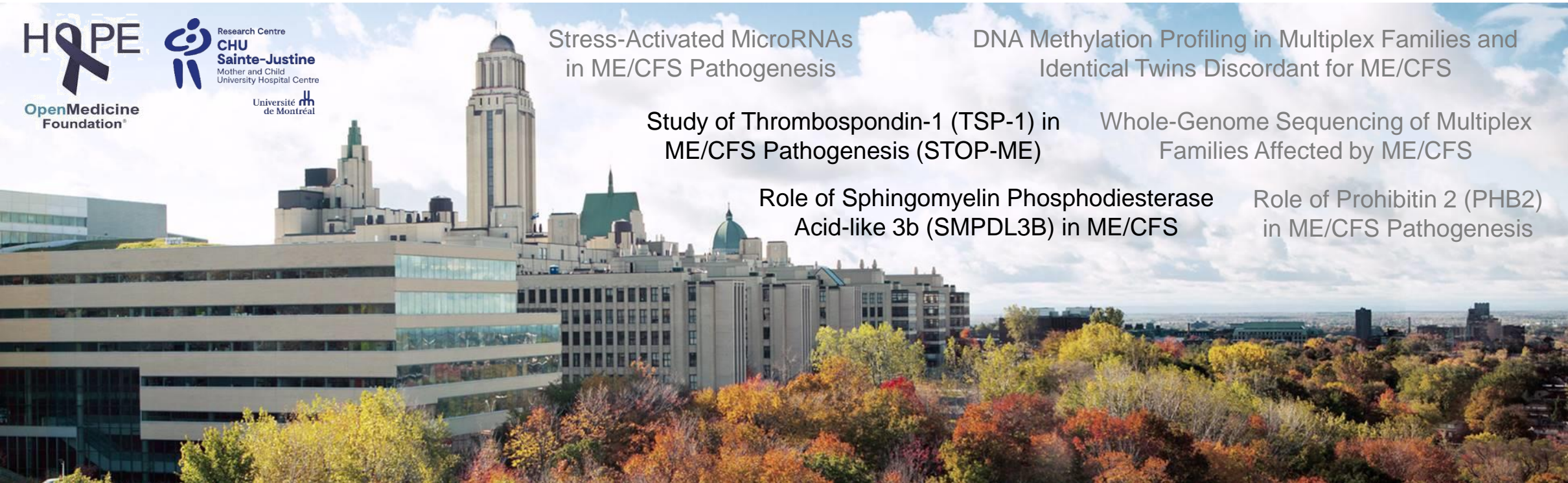
DNA Methylation Profiling in Multiplex Families and
Identical Twins Discordant for ME/CFS

Study of Thrombospondin-1 (TSP-1) in
ME/CFS Pathogenesis (STOP-ME)

Whole-Genome Sequencing of Multiplex
Families Affected by ME/CFS

Role of Sphingomyelin Phosphodiesterase
Acid-like 3b (SMPDL3B) in ME/CFS

Role of Prohibitin 2 (PHB2)
in ME/CFS Pathogenesis



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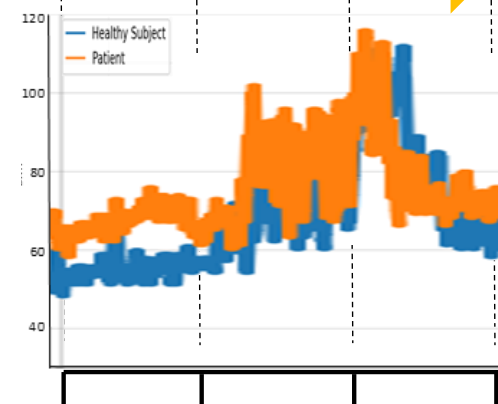
PROVOCATION STUDY: A NEW APPROACH

Development of a stress challenge inducing post-exertional malaise (PEM)



Compression at 0,006 Hz,
varying from 0-4 psi

stimulation



Blood sampling

T_0

$T_{90 \text{ min}}$



Brain oxymetry

Hexoskin
smart vest

during the stress-test



Valérie



Sophie



Anita



Wesam



Viorica

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NEW EXPERIMENTAL APPROACH

Stress-test version 2.0



Valérie



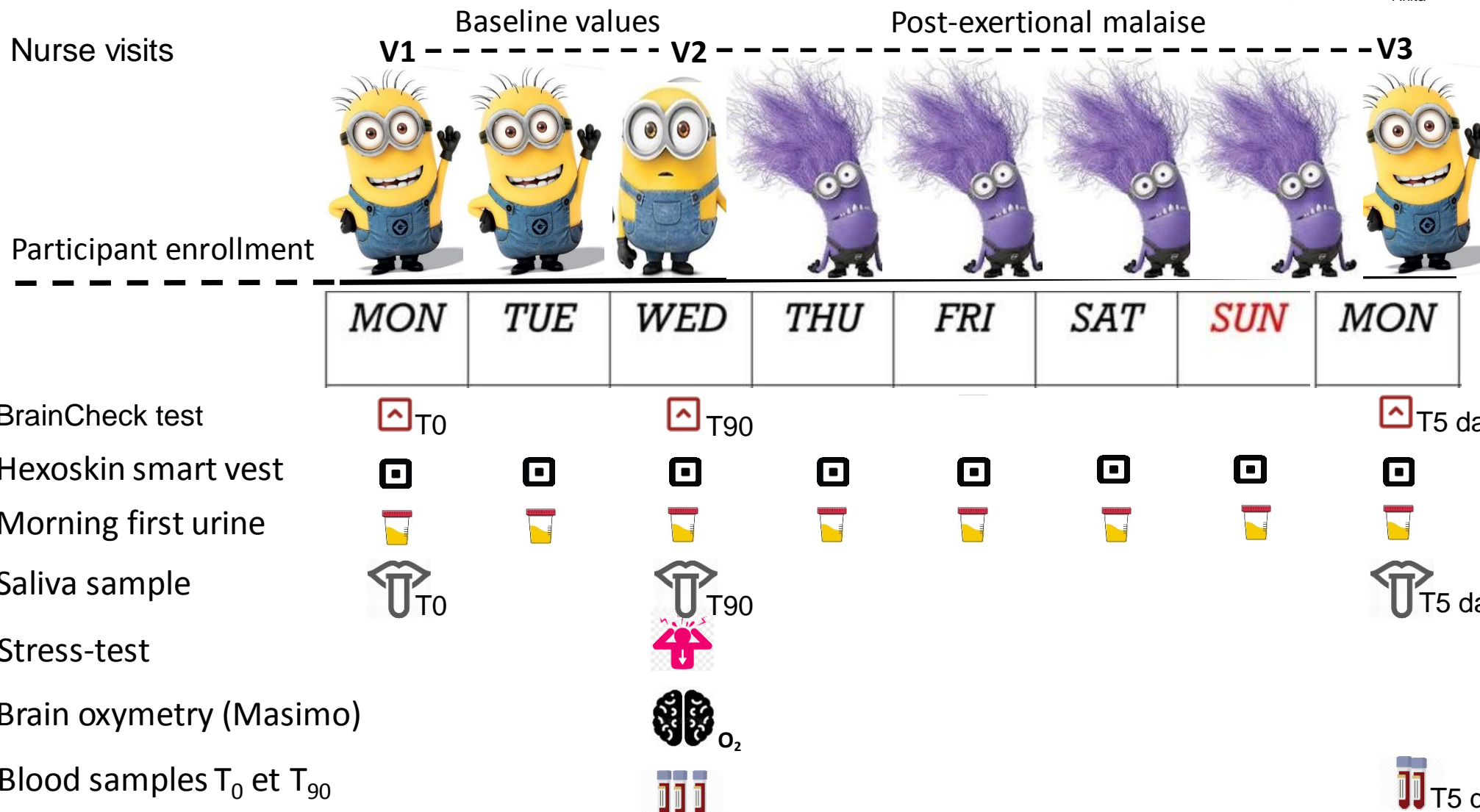
Sophie



Anita



Wesam





SLEEP DISTURBANCES IN ME/CFS

Longitudinal sleep assessment with Hexoskin smart biometric vest

Sleep Data – EM-169 (F, 42 y)	Pre-Stress Test (night 2)	Post-Stress Test (night 3)	Post-Stress Test (night 7)	Post-Stress Test Worse Night (#3)
Sleep Position Changes (#)	47	105	46	105
Total Sleep Time (hh:mm:ss)	07:08:40	07:13:20	08:28:20	07:13:20
REM Sleep Time (hh:mm:ss)	02:04:20	01:52:20	03:33:20	01:52:20
Non-REM Sleep Time (hh:mm:ss)	05:04:20	05:21:00	05:55:00	5:21:00
Time Awake (hh:mm:ss)	00:22:40	00:47:00	00:47:20	00:47:00

Sleep Data – EM-170 (F, 50 y)	Pre-Stress Test (night 2)	Post-Stress Test (night 3)	Post-Stress Test (night 7)	Post-Stress Test Worse Night (#5)
Sleep Position Changes (#)	61	65	64	59
Total Sleep Time (hh:mm:ss)	08:47:40	10:53:00	07:15:20	6:13:40
REM Sleep Time (hh:mm:ss)	1:51:00	3:23:20	01:08:00	00:55:00
Non-REM Sleep Time (hh:mm:ss)	6:56:40	7:29:40	6:07:20	5:18:00
Time Awake (hh:mm:ss)	00:42:00	1:02:20	00:33:00	1:23:20

Sleep Data – EM-171 (M, 40 y)	Pre-Stress Test (night 2)	Post-Stress Test (night 3)	Post-Stress Test (night 7)	Post-Stress Test Worse Night (#6)
Sleep Position Changes (#)	16	22	21	76
Total Sleep Time (hh:mm:ss)	07:54:40	08:27:40	06:18:00	05:57:00
REM Sleep Time (hh:mm:ss)	02:09:00	02:30:00	01:41:00	01:18:00
Non-REM Sleep Time (hh:mm:ss)	05:45:40	05:57:40	04:36:00	4:39:00
Time Awake (hh:mm:ss)	00:08:00	00:21:00	00:24:20	01:10:20



Corinne Leveau



Dr. M-Y Akoume



Dr. Wesam Elremaly



THE PROBLEM: Little is known about the mechanisms causing brain fog, orthostatic intolerance as well as postural orthostatic tachycardia (POTS) in ME/CFS.

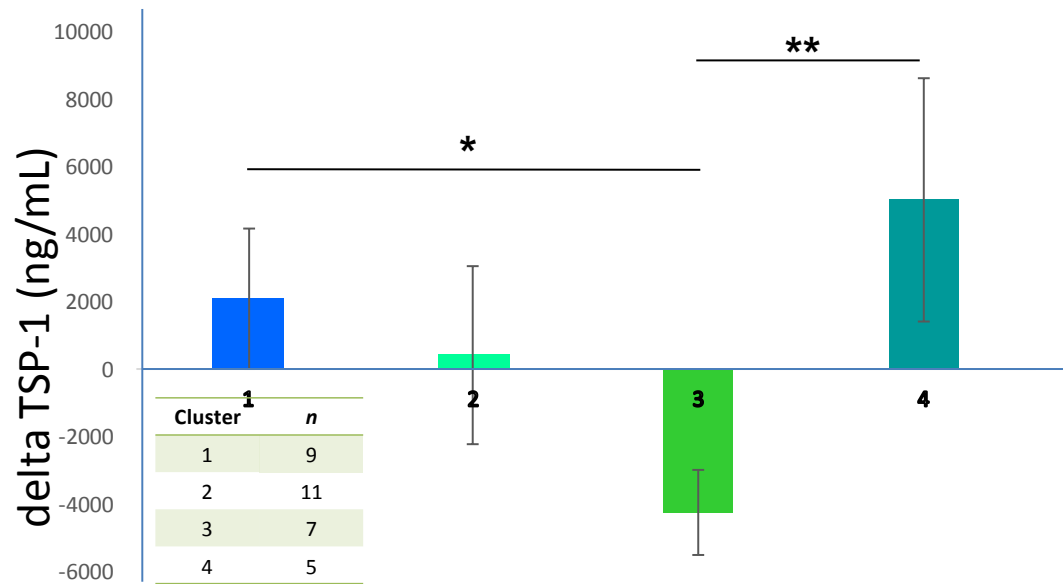


OUR HYPOTHESIS: We propose that elevation of circulating thrombospondin-1 (TSP-1) levels could induce a brain fog and PEM in ME/CFS by reducing brain-blood flow. Conversely, a rapid decrease in blood TSP-1 levels could induce an orthostatic intolerance or even POTS.

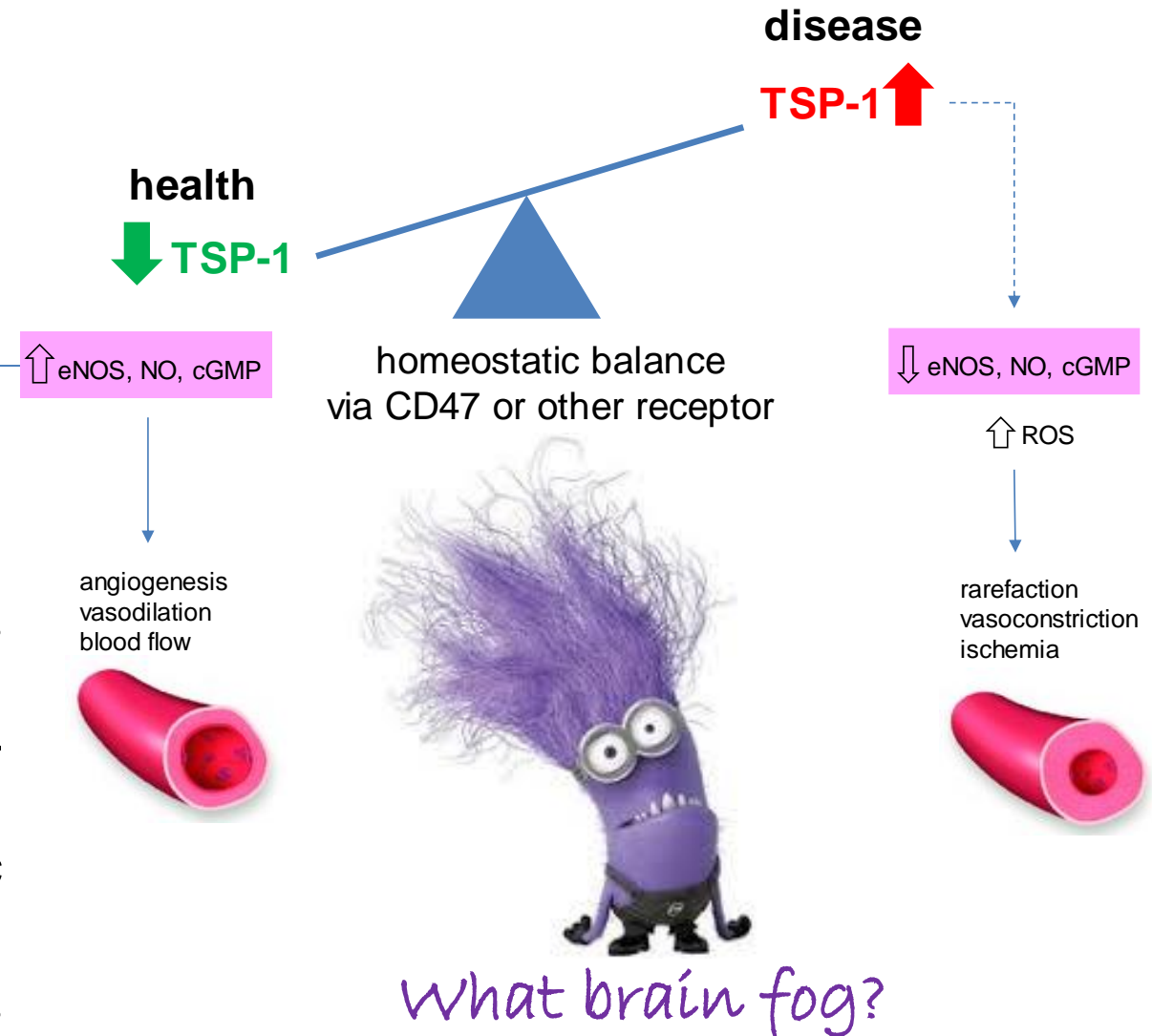
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ROLE OF THROMBOSPONDIN-1 IN ME/CFS?

Changes in plasma TSP-1 levels could be involved in ME/CFS pathogenesis



- **Cluster 3** encompasses ME/CFS patients showing a strong elevation of TSP-1 blood levels after the application of the stress-test. This subgroup including all ME/CFS patients exhibiting an orthostatic intolerance.
- **Cluster 4** encompasses ME/CFS patients at-risk of developing brain fog.



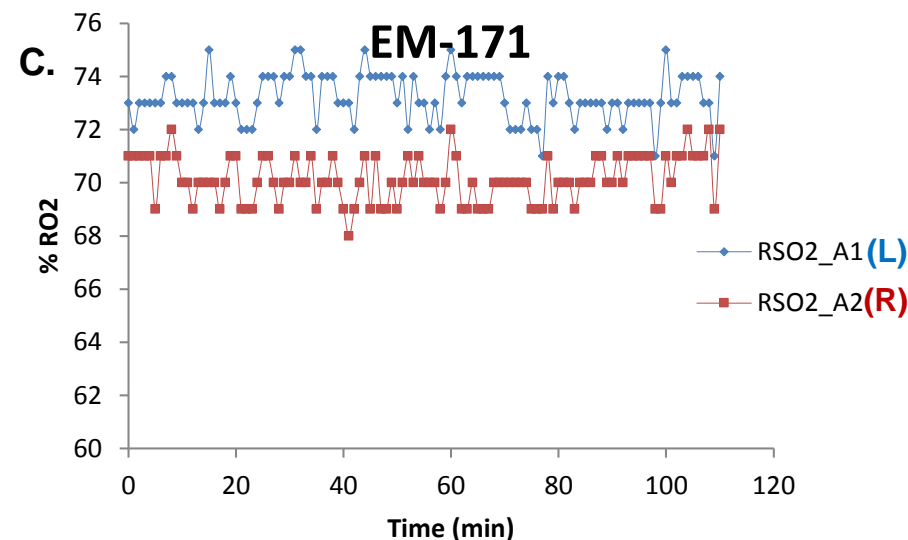
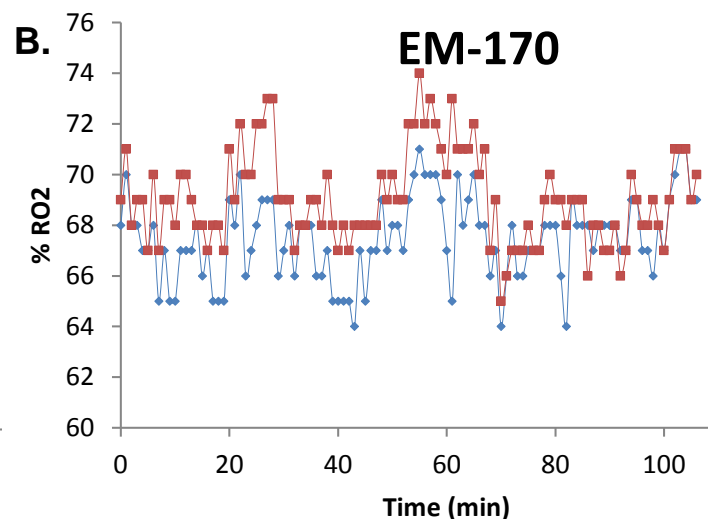
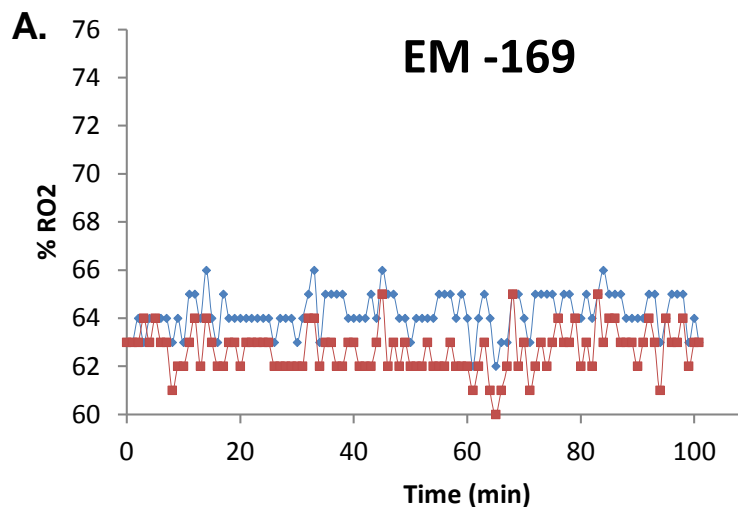
PRELIMINARY DATA

Changes in plasma TSP-1 levels and brain oxygen levels during the stress-test

Table 3. Changes in plasma TSP-1 levels at different time points

Patient ID	Sex	Age (year)	TSP-1 at baseline (T0 min)	TSP-1 post-stress test (T90 min)	TSP-1 post-stress test (T+ 5 days)	PEM score (DSQ)	Medication
EM 169	F	42	25 665 ng/mL	16 956 ng/mL	13 993 ng/mL	92	
EM 170	F	50	18 926 ng/mL	18 602 ng/mL	10 038 ng/mL	65	Pregabalin
EM 171	M	40	8 054 ng/mL	17 325 ng/mL	7 718 ng/mL	86	Pregabalin (25mg+125mg) + Vit D3

Changes in brain oxygen levels during the stress-test



10 LONGITUDINAL NEUROCOGNITIVE ASSESSMENT

Effects of plasma TSP-1 levels on neurocognitive functions



Dr. Wesam Elremaly

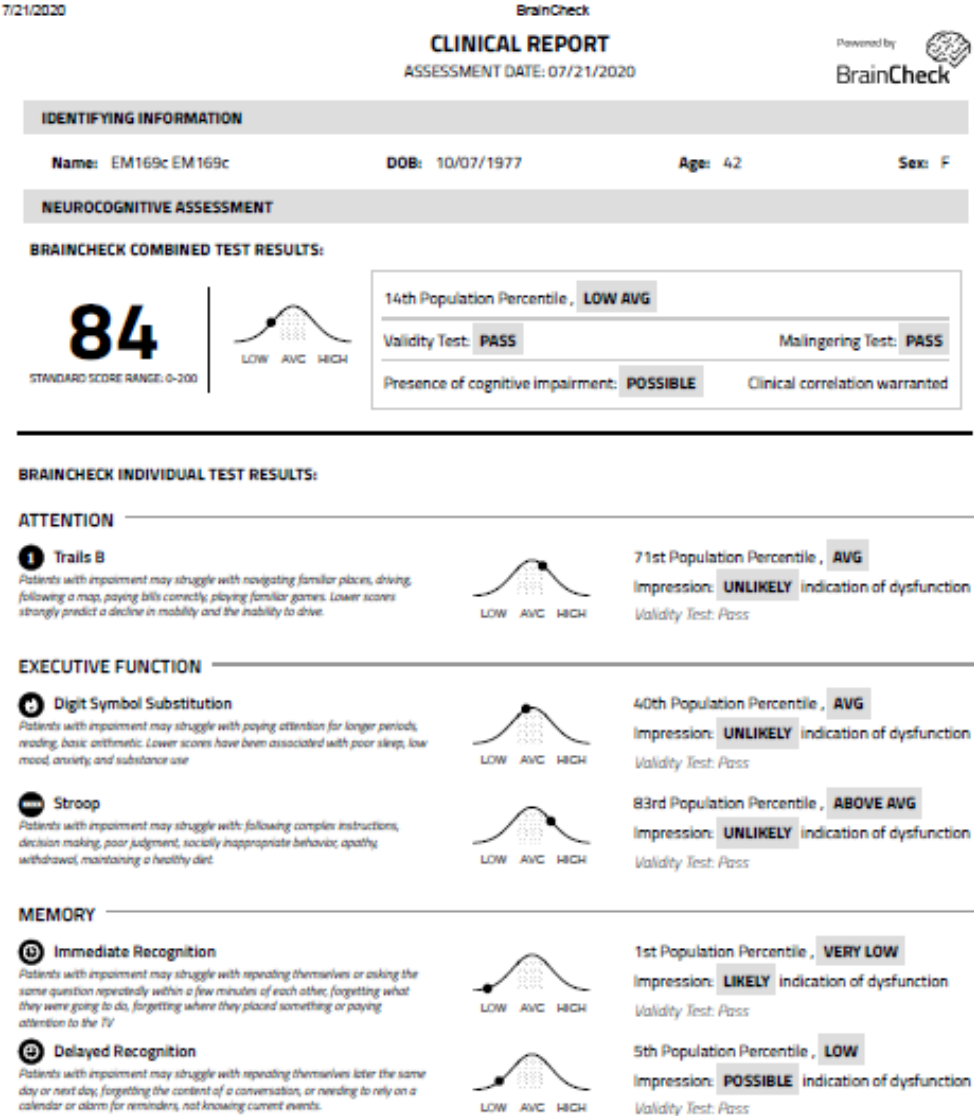


Table 1. Clinical and demographic data of participants

Patient ID	Sex	Age (year)	Illness duration (years)	Sleep score	Cognitive score	PEM score	ANI score
EM 169	F	42	6	28	61	92	33
EM 170	F	50	5	38	69	65	34
EM 171	M	40	3	53	60	86	41

Table 2. Clinical results with BrainCheck

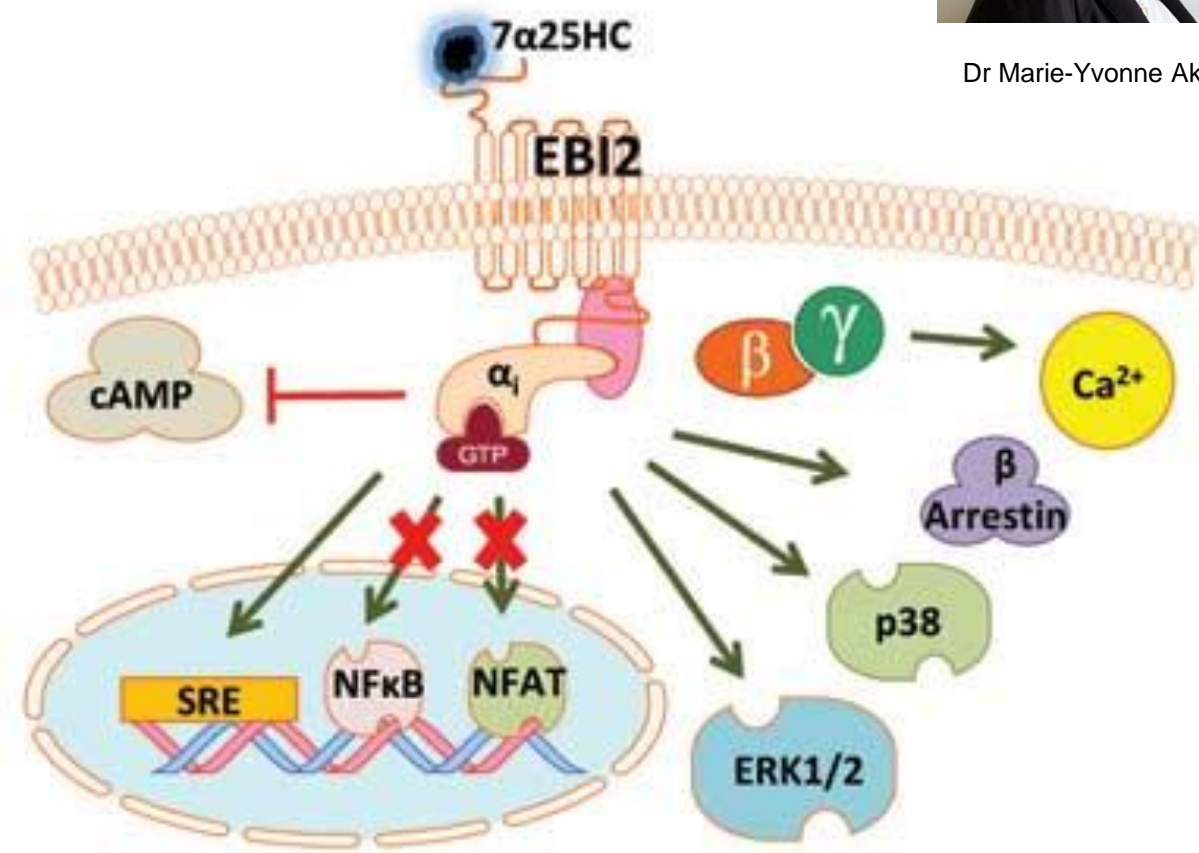
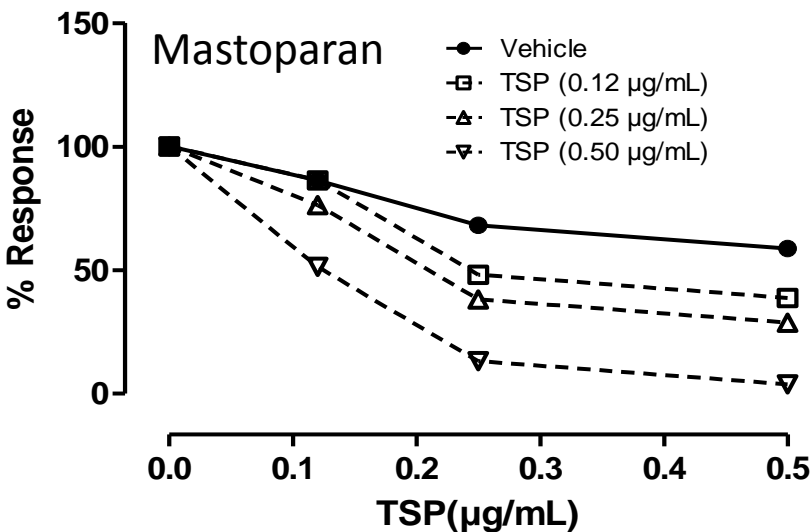
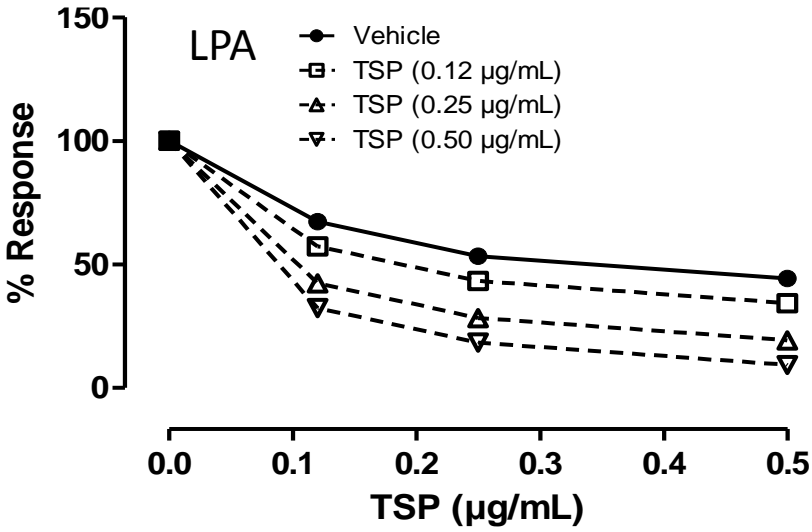
Patient ID	At baseline (T0 min)	Post-stress test (T90 min)	Post-stress test (T+5 days)	Neurocognitive effects
EM 169	98	94	84	Likely a memory dysfunction
EM 170	83	96	101	Likely a memory dysfunction
EM 171	101	85	111	Possible executive function dysfunction

ROLE OF THROMBOSPONDIN-1 IN ME/CFS?

Thrombospondin-1 inhibits Gi-coupled receptor signaling



Dr Marie-Yvonne Akoume



Kerr JR. Epstein-Barr Virus Induced Gene-2 Upregulation Identifies a Particular Subtype of Chronic Fatigue Syndrome/Myalgic Encephalomyelitis. Front Pediatr. 2019;7:59.

12 THERAPEUTIC OPTIONS FOR ME/CFS PATIENTS

How to decrease plasma TSP-1 levels or block its signaling action?



- Interestingly, $\alpha 2\delta$ -1 is the high affinity receptor for TSP-1 in the brain.
- Two commonly prescribed anti-epileptic, anti-neuropathic pain medications, **gabapentin** (Neurontin™) and **pregabalin** (Lyrica™) are targeting $\alpha 2\delta$ -1 receptor. Both drugs are being used off-label for ME/CFS and fibromyalgia patients.
- **Vitamin D3 supplementation** for 12 weeks markedly reduced TSP-1 levels by almost 2.5 fold (522.7 ± 379.8 ng/mL vs 206.7 ± 204.5 ng/mL, $p < 0.001$).¹
- Low-dose of **cyclophosphamide**.²
- **Hyperbaric oxygenation therapy** could be effective to decrease blood TSP-1 levels but it remains to be tested by a clinical trial. A direct link between TSP-1 activity and hyperoxic condition has not been made yet.³

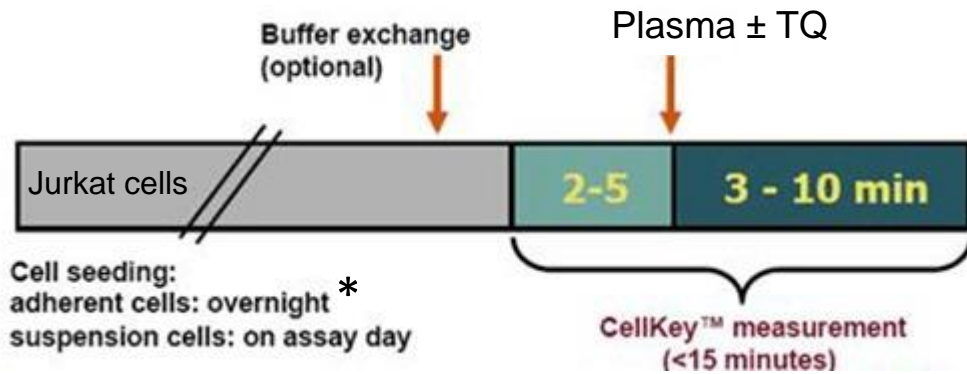
¹ Amarasekera AT, et al. [Vitamin D supplementation lowers thrombospondin-1 levels and blood pressure in healthy adults](#). *PLoS One*. 2017;12(5):e0174435.

² Lansiaux, A. et al. [Circulating thrombospondin 1 level as a surrogate marker in patients receiving cyclophosphamide-based metronomic chemotherapy](#). *Invest New Drugs* 30, 403–404 (2012).

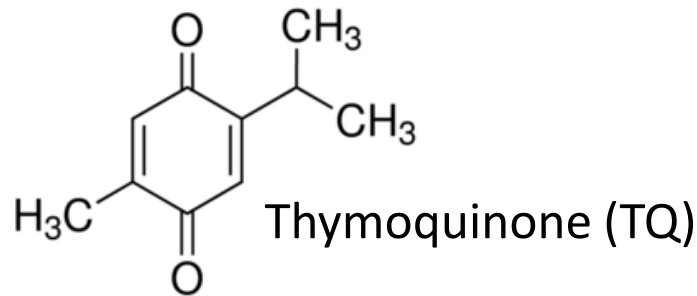
³ Asadamongkol B, Zhang JH. [The development of hyperbaric oxygen therapy for skin rejuvenation and treatment of photoaging](#). *Med Gas Res*. 2014;4(1):7

13 THERAPEUTIC OPTIONS FOR ME/CFS PATIENTS

How to block TSP-1 signaling action?



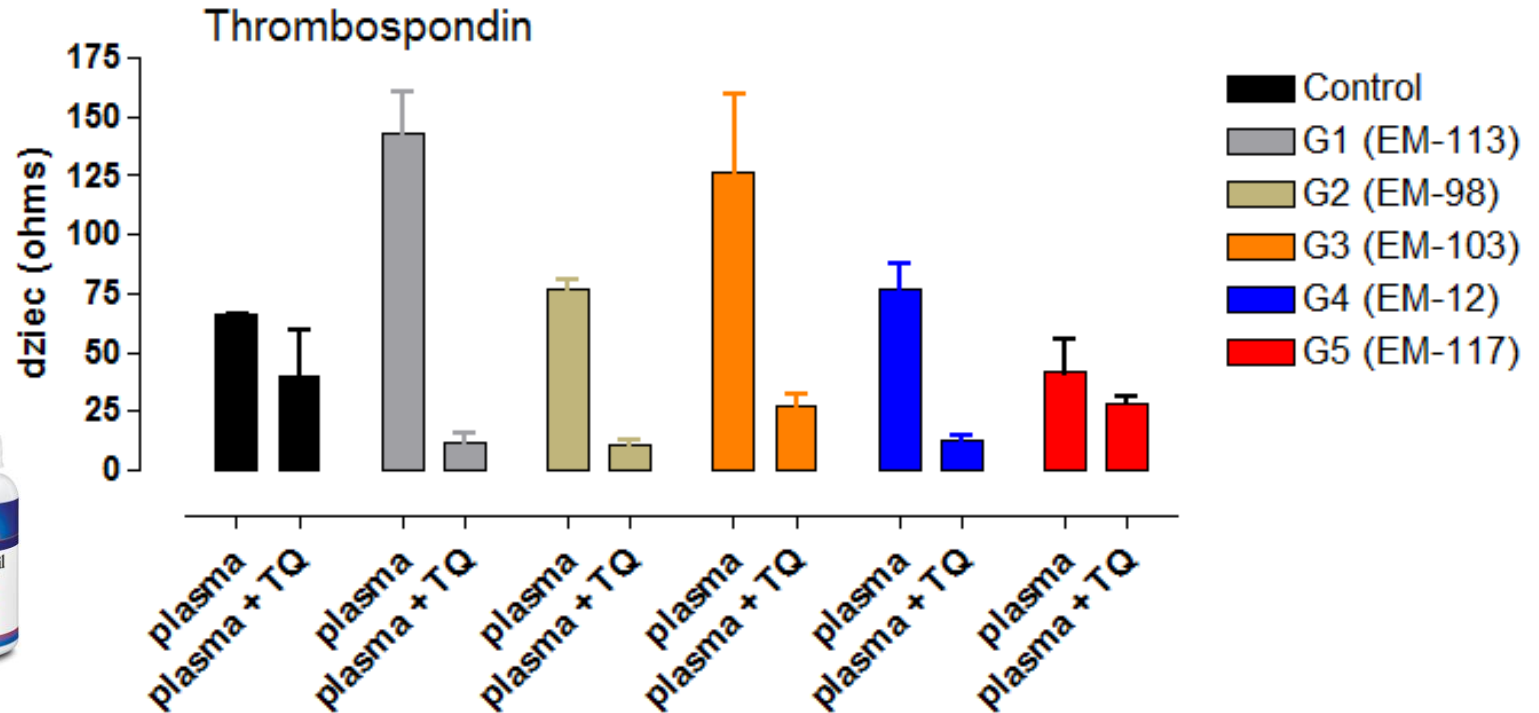
* Jurkat cells (immortalized human lymphocytes T) were pretreated for 2 hours with plasma with or without 50μM of thymoquinone (TQ). Then stimulated with 10μM of recombinant thrombospondin-1 proteins. Of note, Jurkat cells express $\alpha 2\delta$ -1 and CD47 receptors but not CD36 receptor.



Nigella sativa



Black seed



14 MULTITASKING BIOMOLECULES (2)

Role of SMPDL3B in ME/CFS pathophysiology



Bitu Rostami



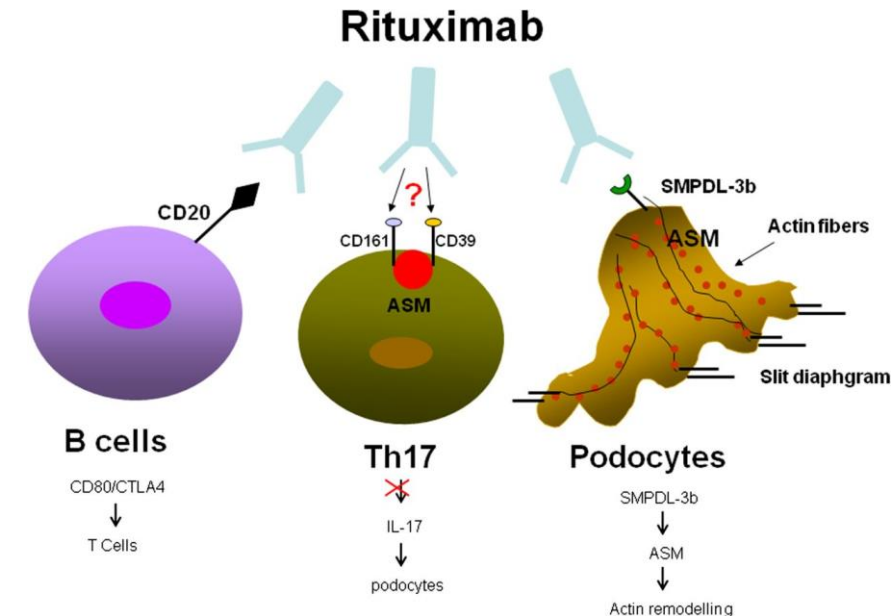
Dr. Wesam Elremaly



THE PROBLEM: Little is known about the mechanism underlying lipid metabolism alteration occurring in ME/CFS.

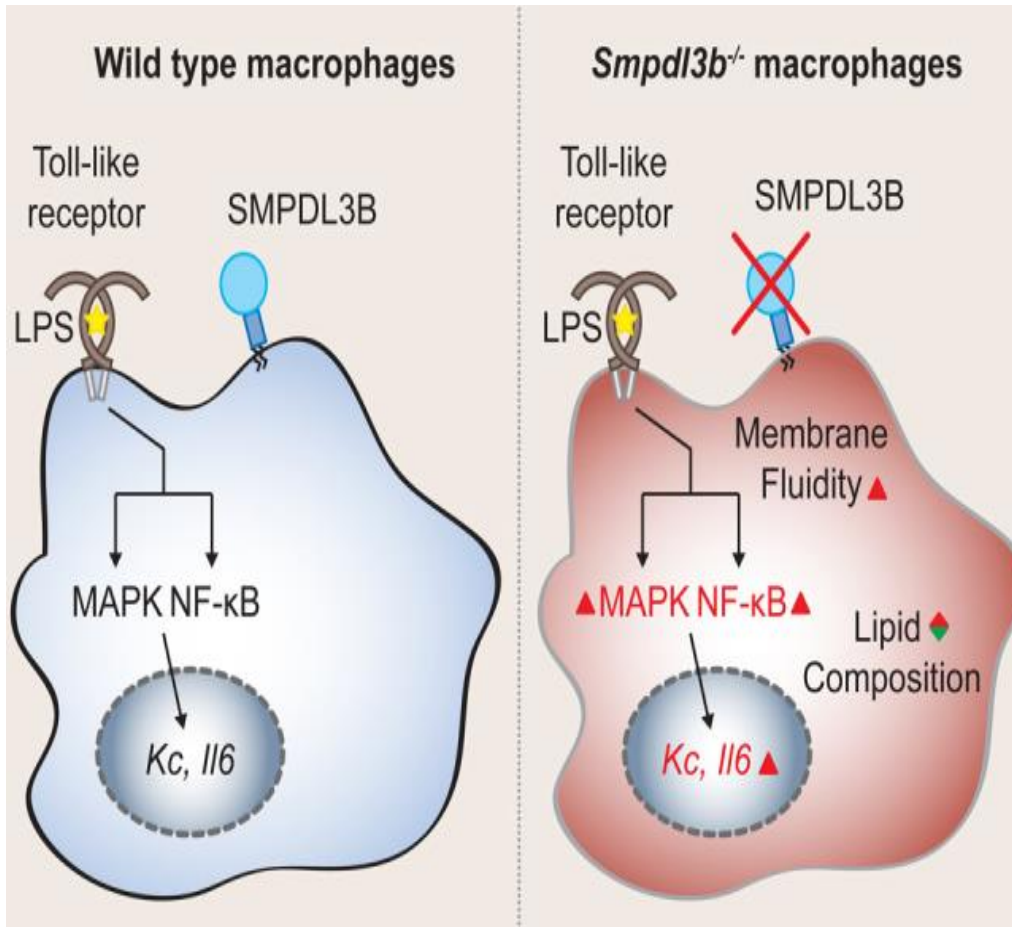


OUR HYPOTHESIS: We propose that sphingomyelin phosphodiesterase acid-like 3b (SMPDL3B) is involved in ME/CFS pathogenesis by modulating innate immunity and lipid metabolism. We have identified SMPDL3B as a possible alternative target of Rituximab in ME/CFS pathogenesis.



15 **LIPID-MODIFYING ENZYME SMPDL3B**

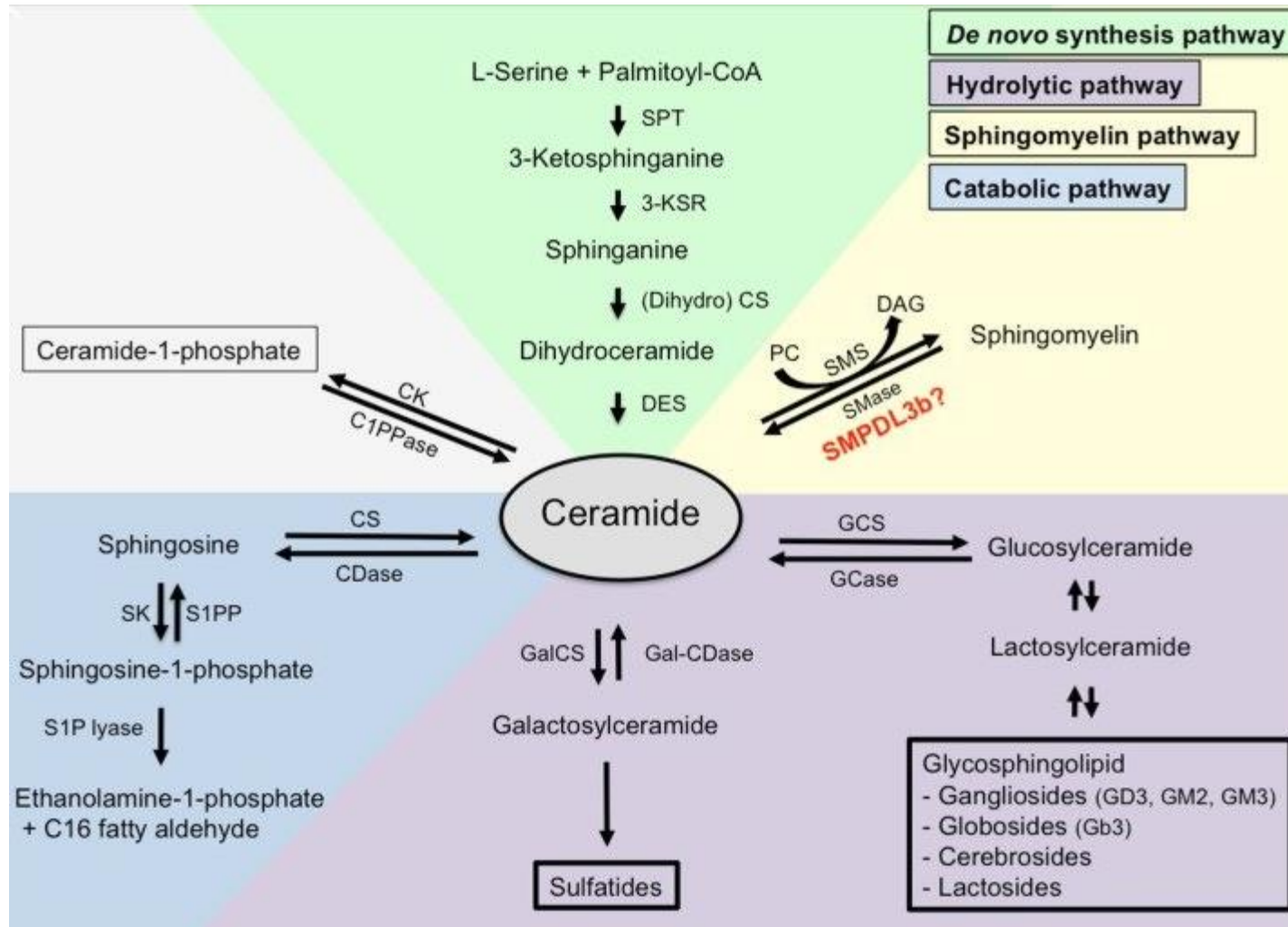
Possible role of in the regulation of innate immunity in ME/CFS



- SMPDL3B expression is prominently observed in macrophages and DCs.
- Consistent with a possible role for this enzyme in the course of inflammatory processes.
- *Smpdl3b* transcription in bone marrow-derived macrophages (BMDMs) and DCs (BMDCs) is robustly induced upon TLR stimulation

16 LIPID-MODIFYING ENZYME SMPDL3B

SMPDL3B is a relevant molecule if ME/CFS pathogenesis



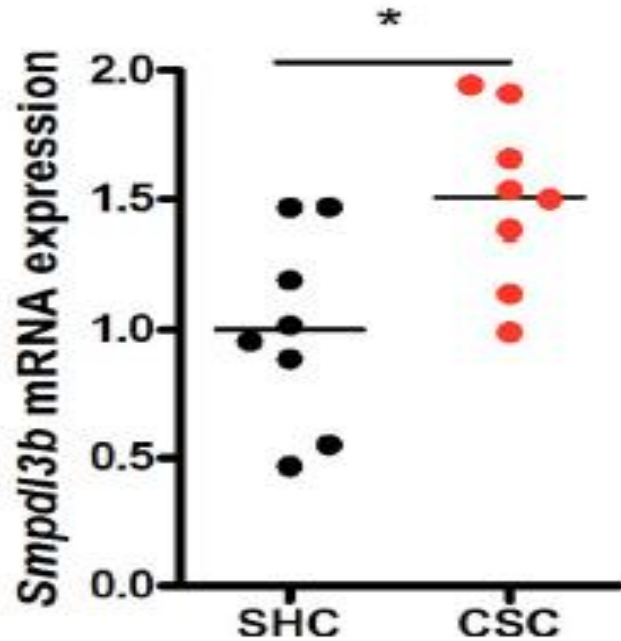
- In males, over 50% (16/30) of the sphingolipids that were decreased were **ceramides**, and 47% (14/30) were **sphingomyelin** species.
- In females, 86% (18/21) were **ceramides** and 14% (3/21) were **sphingomyelins** in females.

Naviaux R.K. et al. "Metabolic features of chronic fatigue syndrome." PNAS 2016; 113(37): E5472–E5480

Merscher S, Fornoni A. Podocyte pathology and nephropathy - sphingolipids in glomerular diseases. Front Endocrinol (Lausanne). 2014;5:127.

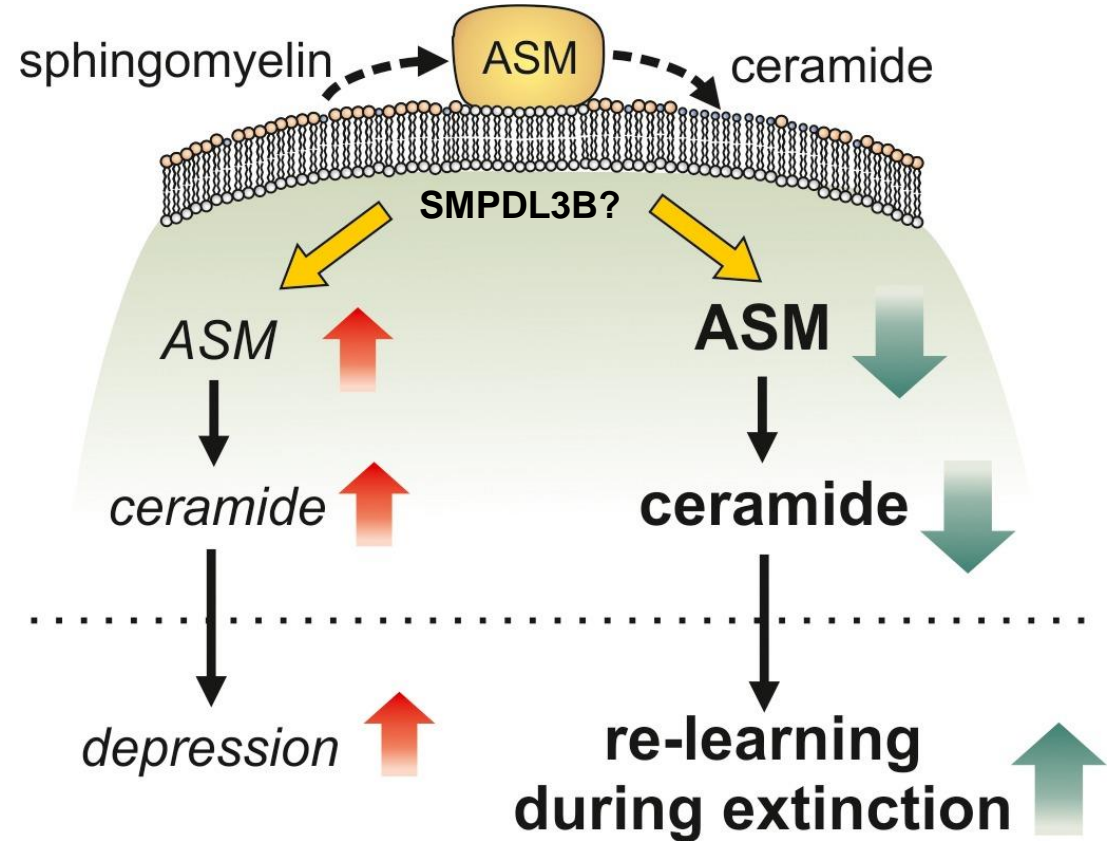
17 LIPID-MODIFYING ENZYME SMPDL3B

Possible role of SMPDL3B in ME/CFS pathogenesis



Chronic psychosocial stress is associated with altered gene expression in the liver of enzymes regulating ceramide production.

Reichel M, Rhein C, Hofmann LM, et al. [Chronic Psychosocial Stress in Mice Is Associated With Increased Acid Sphingomyelinase Activity in Liver and Serum and With Hepatic C16:0-Ceramide Accumulation](#). Front Psychiatry. 2018;9:496.



Huston JP, Kornhuber J, Mühle C, et al. [A sphingolipid mechanism for behavioral extinction](#). J Neurochem. 2016;137(4):589-603.

18 THERAPEUTIC OPTION FOR ME/CFS PATIENTS

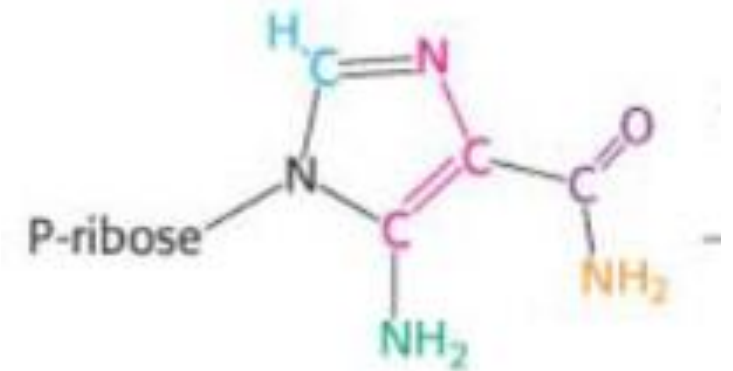
How to increase Smpdl3b gene expression?

List of top 5 up-regulated genes in Lateral Entorhinal Cortex after 7 days of AICAR administration (ACR7) and exercise (RUN7)

GENE	FOLD	
	ACR7	RUN7
Igsf1	3.21	2.29
Nr2f2	2.40	2.37
Smpdl3b	2.35	2.10
Tmie	2.19	1.93
Gm4983	1.90	1.96



AMPK agonist



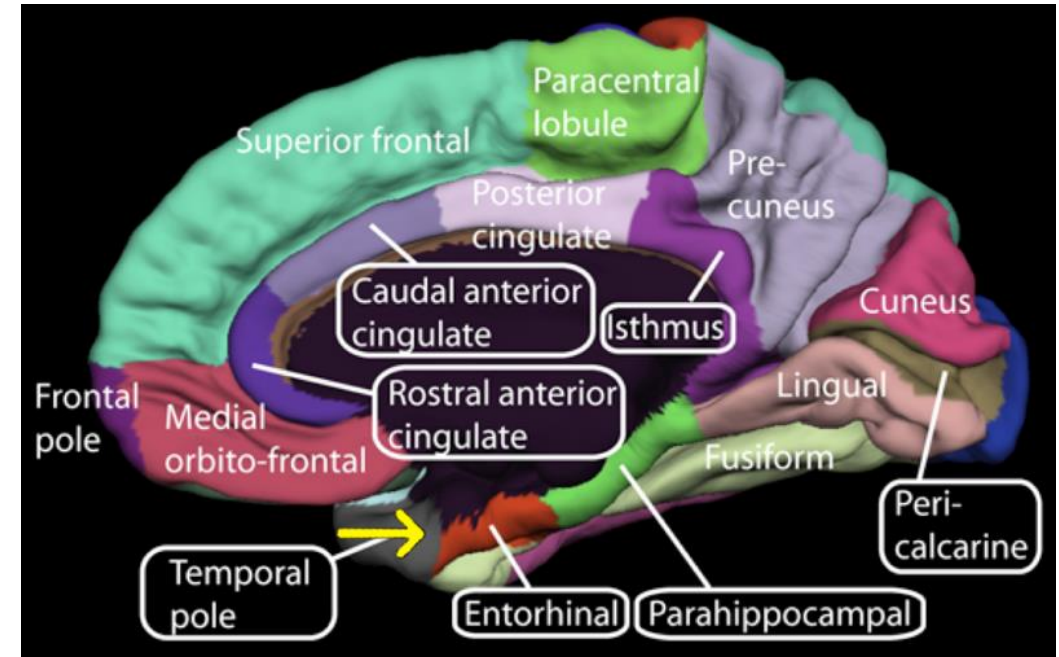
5-Aminoimidazole-4-carboxamide ribonucleotide

The **entorhinal cortex** (EC) is an area of the brain located in the medial temporal lobe and functions as a hub in a widespread network for memory, navigation and the perception of time. The EC is the main interface between the hippocampus and neocortex.

19 IMPORTANCE OF ENTORHINAL CORTEX

Possible link between memory, bodily sensation and fatigue rating in ME/CFS

- The parahippocampal gyrus (PaHcG), which includes the **entorhinal cortex**, is involved in aspects of limbic function as well as memory retrieval and storage.
- Reduced connectivity in ME/CFS participants between PaHcG and regions that encompassed left postcentral gyrus (i.e., primary sensory cortex) and supramarginal gyrus suggests abnormality in the link between memory and bodily sensation.¹
- Such lower connectivity was strongly correlated to higher fatigue ratings of ME/CFS participants.¹

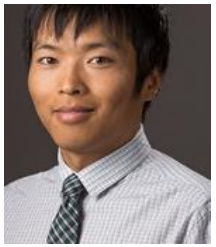


¹ Boissoneault J, Letzen J, Lai S, et al. [Abnormal resting state functional connectivity in patients with chronic fatigue syndrome: an arterial spin-labeling fMRI study](#). *Magn Reson Imaging*. 2016;34(4):603-608.

Hypermethylation of SMPDL3B gene occurs in ME/CFS

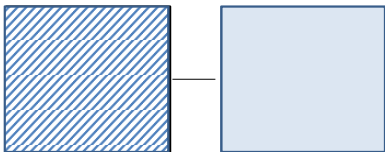


Lynda Chalder



Dr. Dawei Li

Family #14

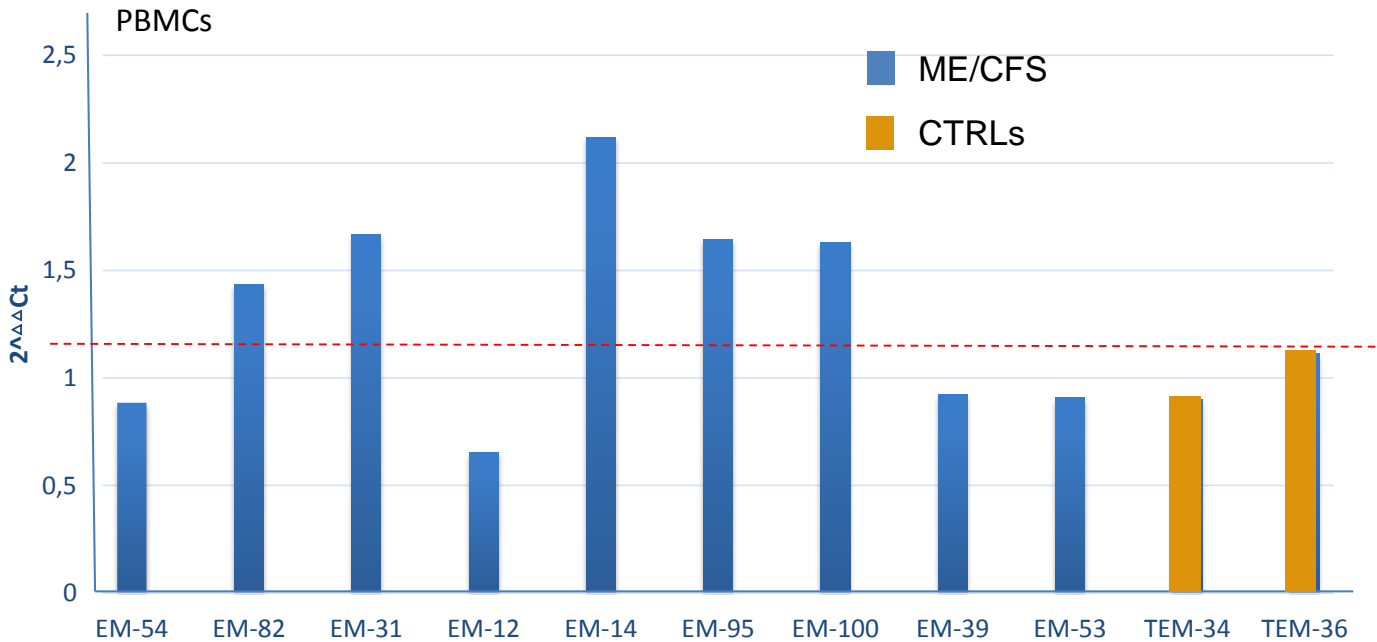


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F14	bValDiff	absbValDiff	F14-01 ME	F14-02 Healthy	gene
cg05320933	0,3630	0,3630	0,6202	0,2572	SMPDL3B
cg23448720	0,3148	0,3148	0,4982	0,1834	SMPDL3B

SMPDL3B expression



Bitra Rostami

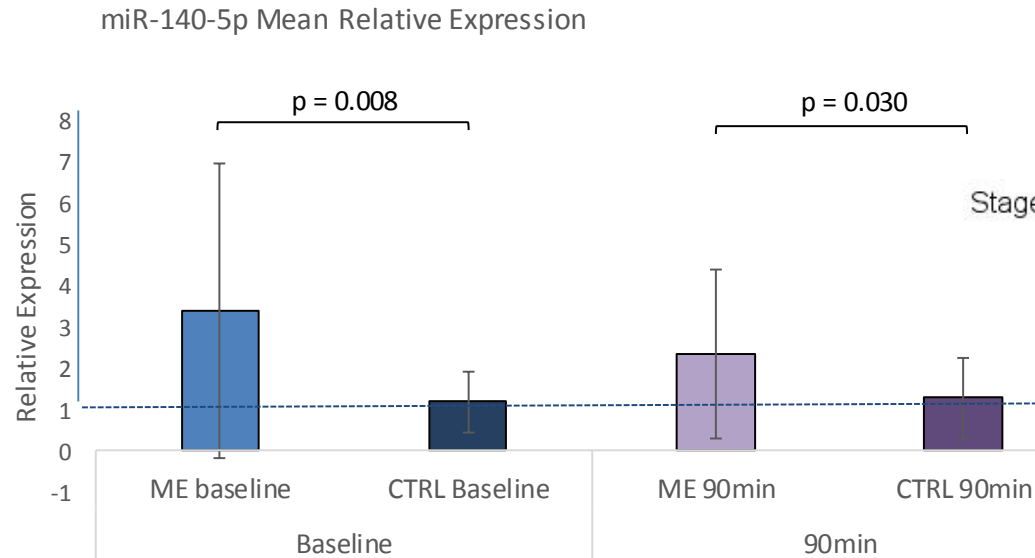
RITUXIMAB EFFECTS IN ME/CFS?

MiR-140-5p expression could influence Rituximab bioavailability

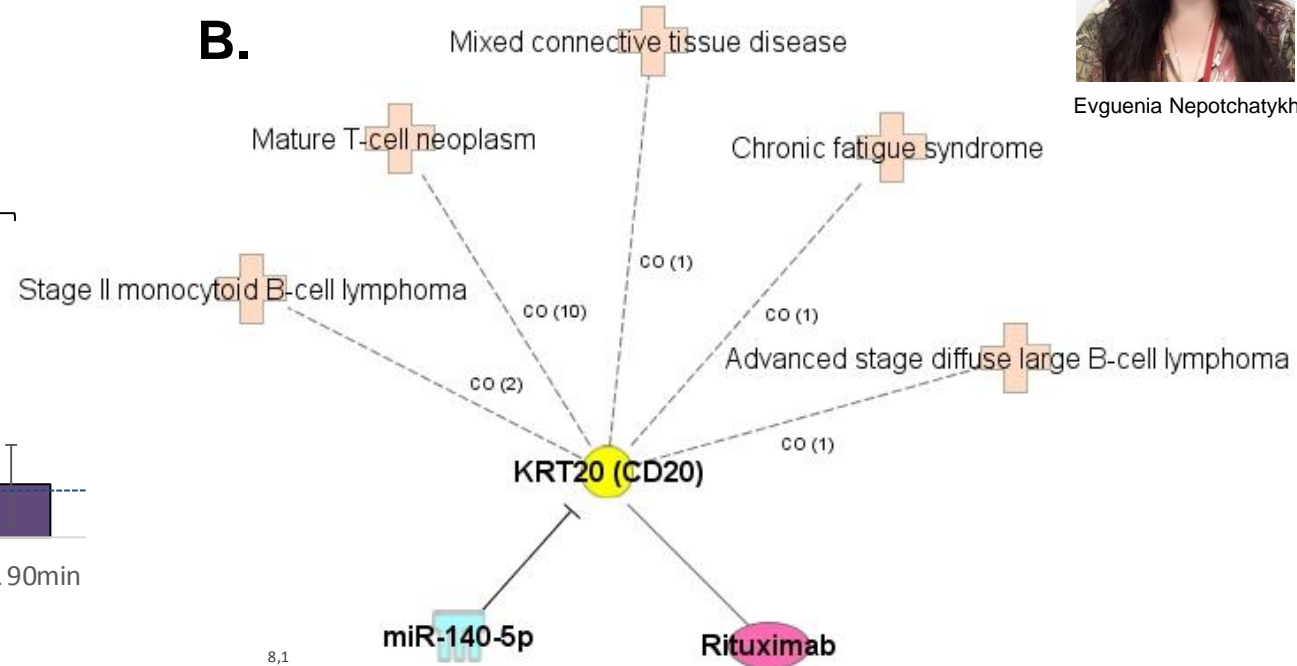


Evguenia Nepotchatykh

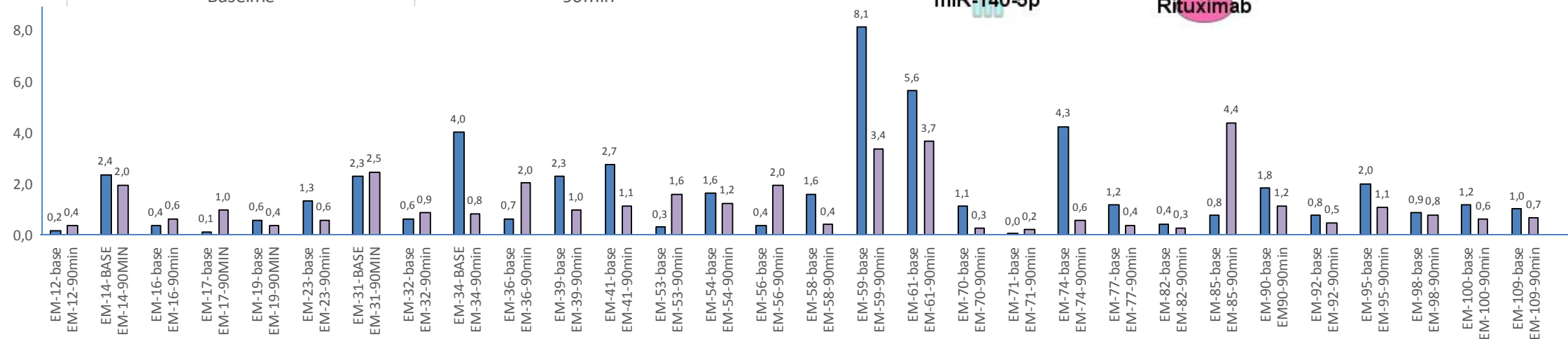
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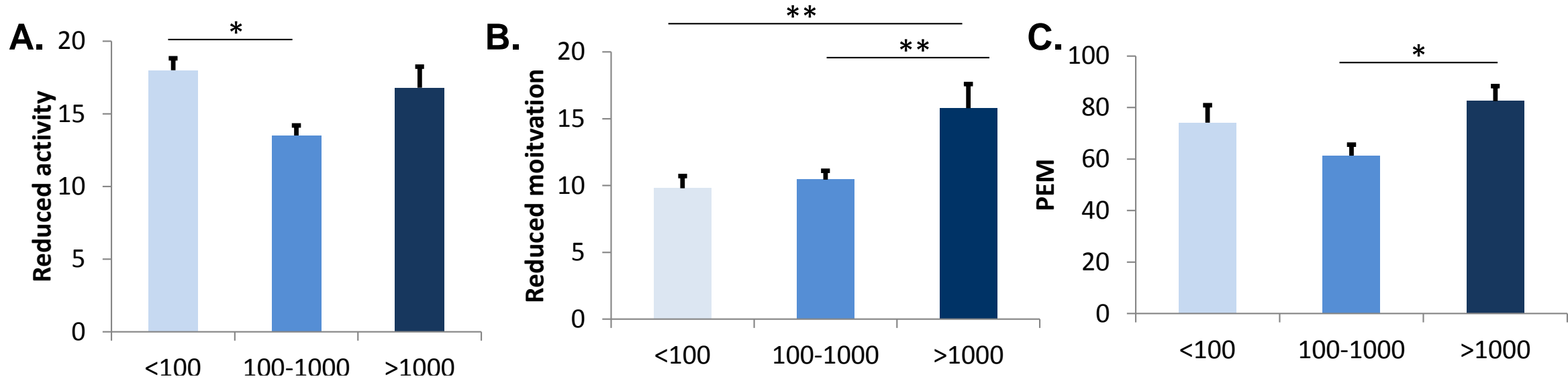


22 CHANGES IN URINARY SMPDL3B LEVELS

Association of urinary SMPDL3b levels and ME/CFS symptoms



Bitra Rostami



Classification of urinary SMPDL3B into <100, 100-1000 and > 1000 ng/ml/mg creatinine and DSQ questionnaires

23 ACKNOWLEDGEMENTS

Collaborative research is the key!



A special thanks to all the participants and families for their contribution to this study as well as to AQEM, National ME/FM and Action CIND for their kind assistance.

ME/CFS Collaborative Research Center at CHU Sainte-Justine/Université de Montréal



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Valérie Tremblay



Corinne Leveau



Dr. Marie-Yvonne Akoume



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