Multitasking Biomolecules in ME/CFS Pathogenesis

Known Players on Their Unexpected Journey

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Fourth Annual Working Group Meeting on the Molecular Basis of ME/CFS
September 8-11, 2020

IRSC CIHR Instituts de recherche en santé du Canada Canadian Institutes of Health Research

CIHR IRSC Institute of Musculoskeletal Health and Arthritis Institut de l'appareil locomoteur et de l'arthrite

ICanCME

FONDATION Sibylla Hesse

HOPE OpenMedicine Foundation CANADA

Research Centre CHU Sainte-Justine Mother and Child University Hospital Centre

Université de Montréal

2 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

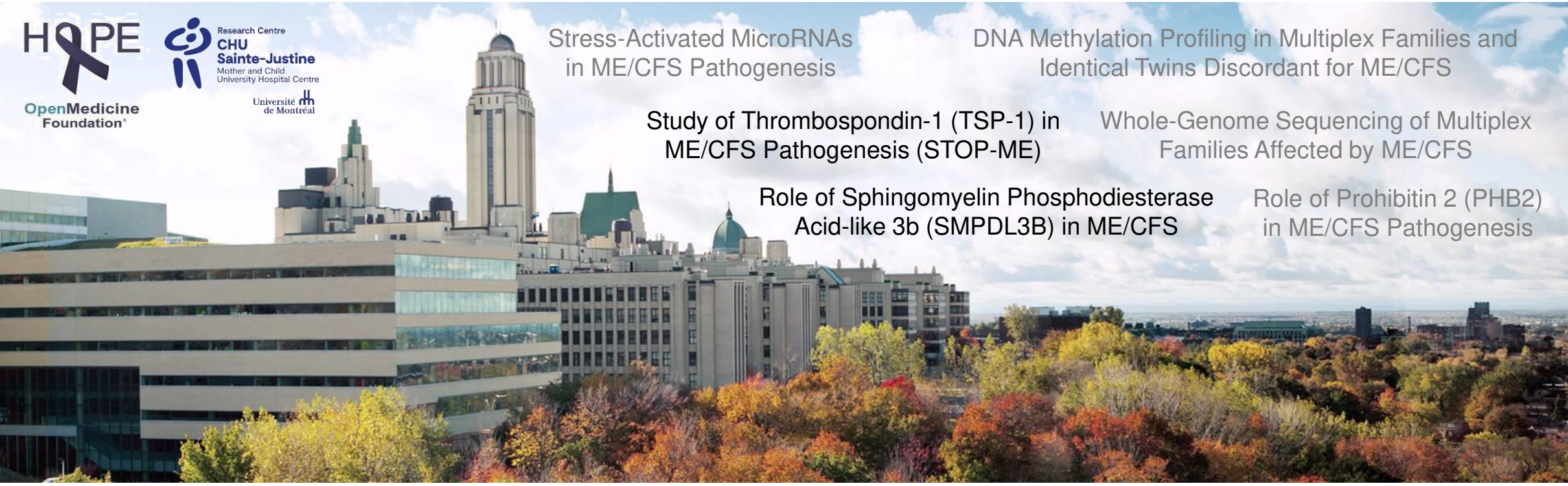
Pr. Moreau osteoarthritis research program has been approved by Sainte-Justine University Hospital Ethic Review Board (protocol #2018-1630)

- 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/



Alain Moreau PhD, Scientific Director
Anita Franco, MSc, Lab coordinator
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Corinne Leveau BSc, PhD student
Evguenia Nepotchatykh BSc, PhD student
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Bita Rostmani, undergraduate student
Marie-Yvonne Akoume PhD, Professor (Université de Libraville)
Dawei Li, PhD, Associate Professor (University of Vermont)

Stanford University
HARVARD UNIVERSITY
UPPSALA UNIVERSITET
MASSACHUSETTS GENERAL HOSPITAL

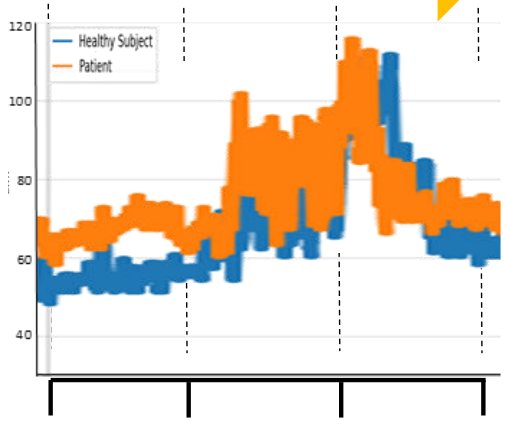
4 PROVOCATION STUDY: A NEW APPROACH

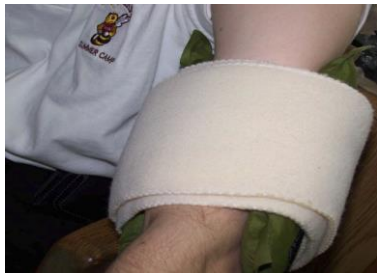
Development of a stress challenge inducing post-exertional malaise (PEM)

HEXOSKIN
HEALTH SENSORS & AI

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

stimulation





Blood sampling T_0 $T_{90\ min}$

Brain oxymetry

Hexoskin smart vest

during the stress-test

Valérie

Sophie

Anita

Wesam

Viorica

5 NEW EXPERIMENTAL APPROACH

Stress-test version 2.0


Valérie


Sophie

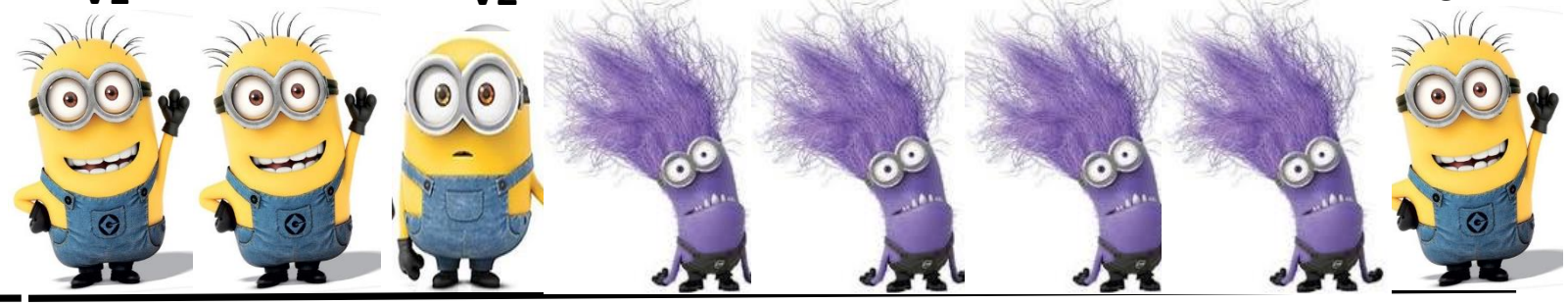

Anita


Wesam

Baseline values

Post-exertional malaise

Nurse visits V1 V2 V3



Participant enrollment

MON	TUE	WED	THU	FRI	SAT	SUN	MON

BrainCheck test T0 T90 T5 day

Hexoskin smart vest

Morning first urine

Saliva sample T0 T90 T5 day

Stress-test

Brain oxymetry (Masimo) O_2

Blood samples T_0 et T_{90} T5 day

6 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

7 MULTITASKING BIOMOLECULES (1)

Study of ThrombOsPondin-1 in ME/CFS – STOP-ME


Corinne Leveau


Dr. M-Y Akoume

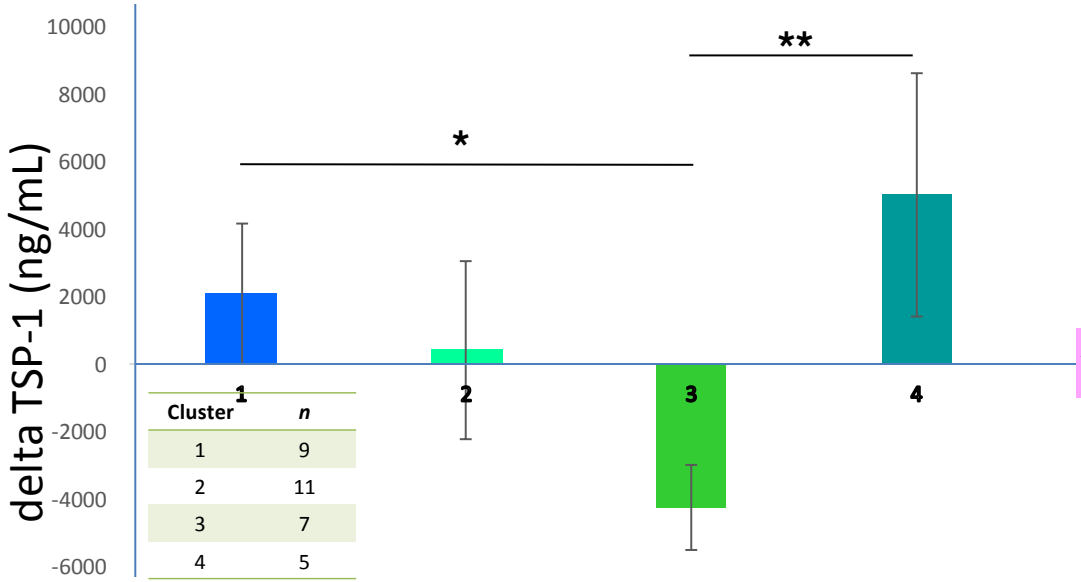

Dr. Wesam Elremaly

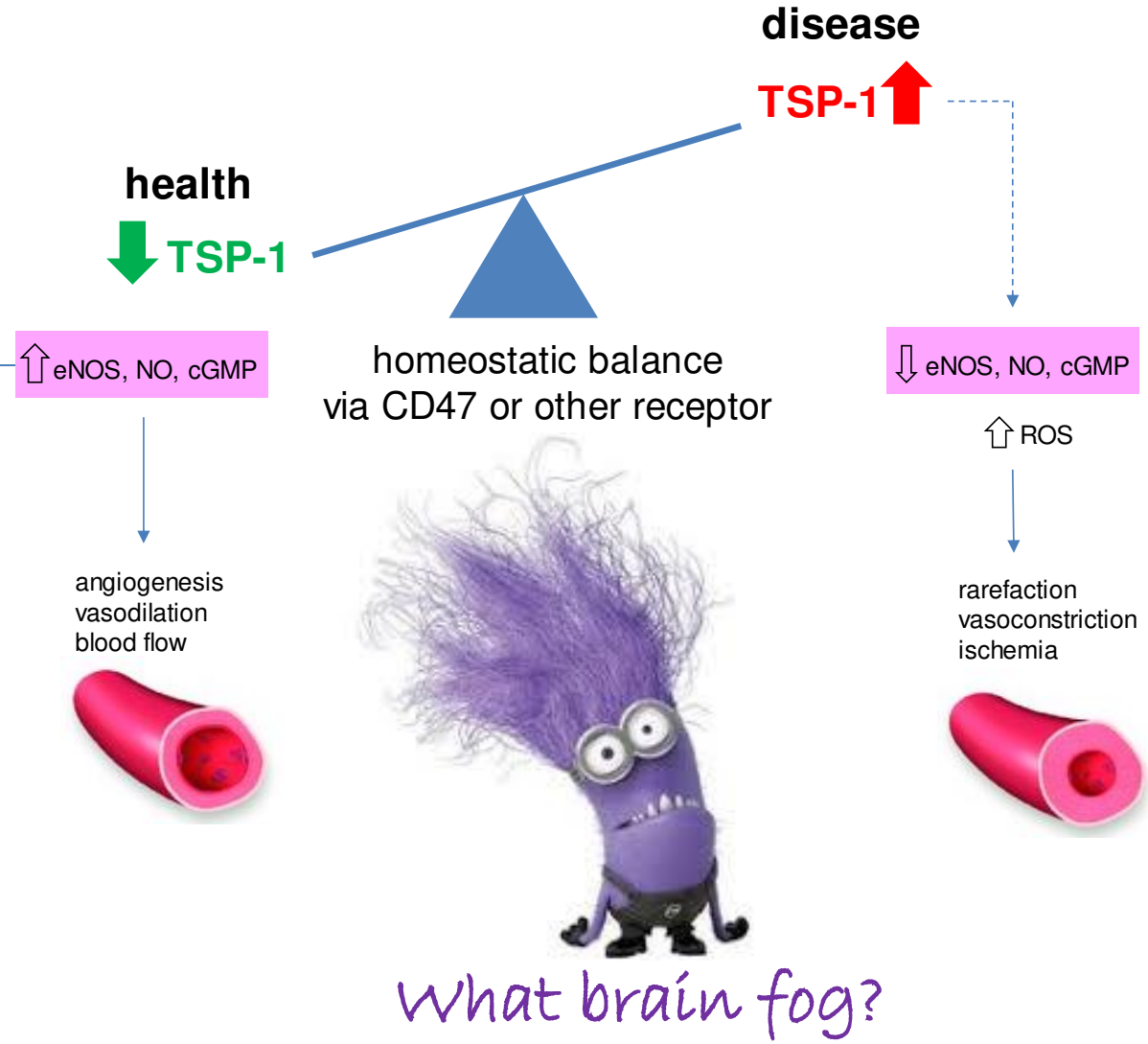
THE PROBLEM: Little is known about the mechanisms causing brain fog, orthostatic intolerance as well as postural orthostatic tachycardia (POTS) in ME/CFS.

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.

8 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.

Adapted from Rogers N.M.et al., Cardiovascular Research (2017) 113, 858–868

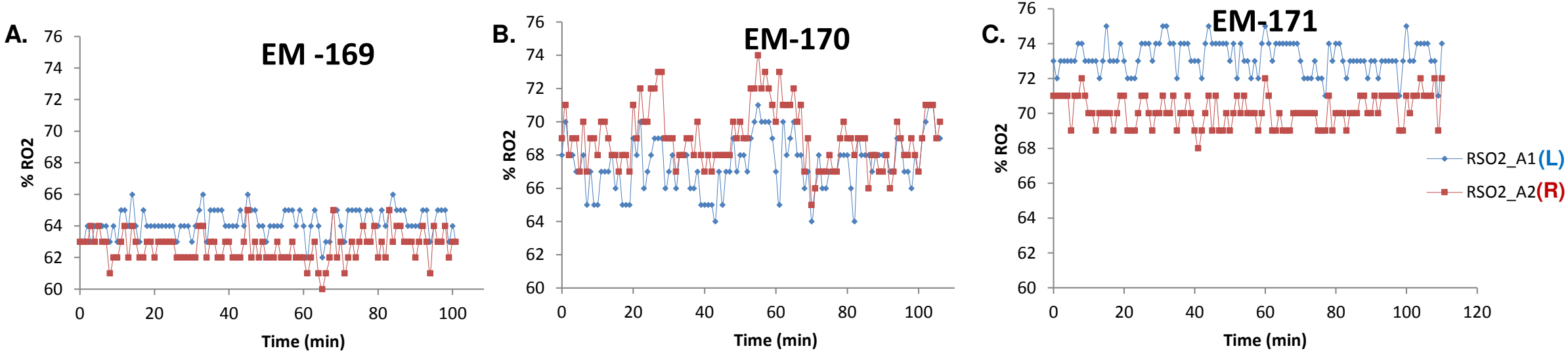
9 PRELIMINARY DATA

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

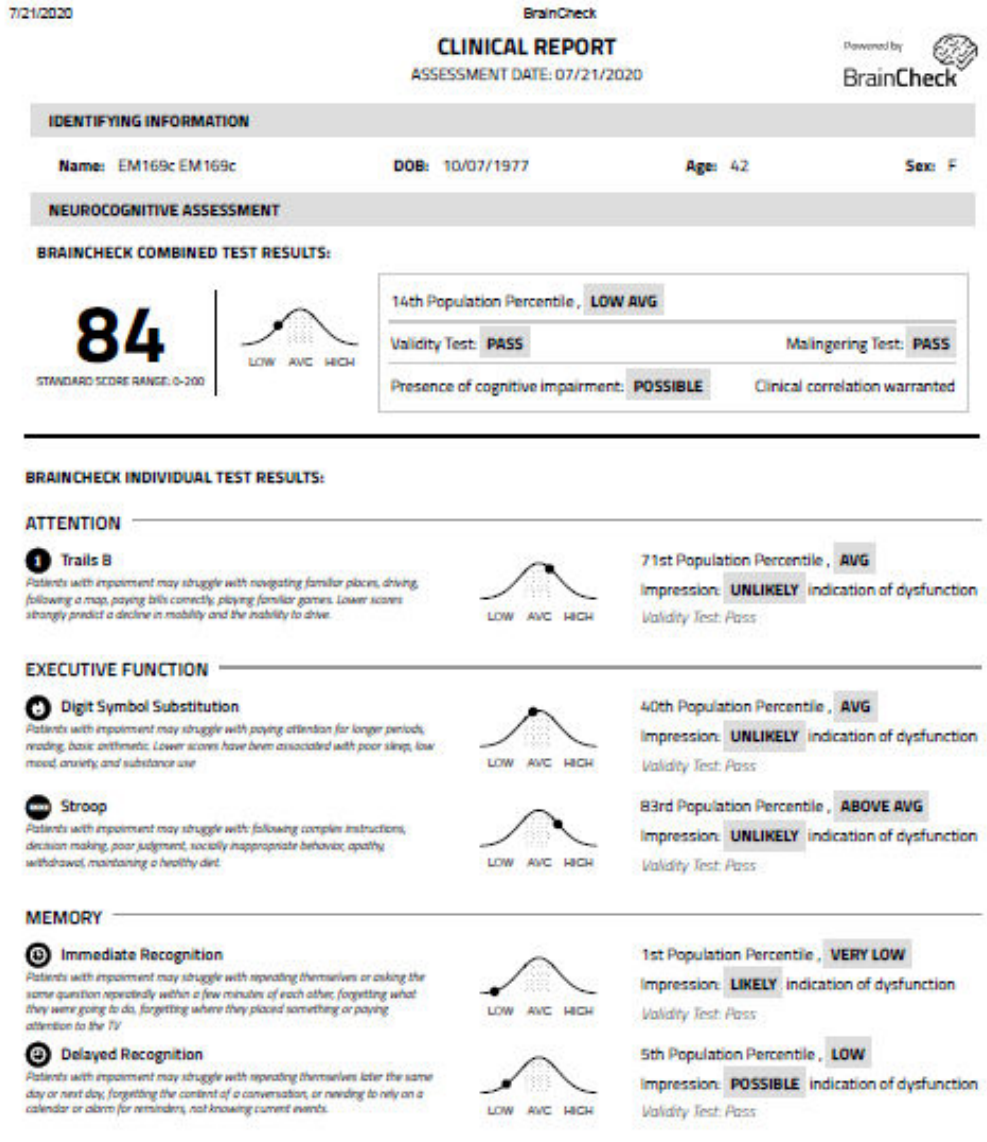
Changes in brain oxygen levels during the stress-test



10 LONGITUDINAL NEUROCOGNITIVE ASSESSMENT

Effects of plasma TSP-1 levels on neurocognitive functions

Dr. Wesam Elremaly



7/21/2020 BrainCheck

CLINICAL REPORT

ASSESSMENT DATE: 07/21/2020

Powered by BrainCheck

IDENTIFYING INFORMATION

Name: EM169c EM169c DOB: 10/07/1977 Age: 42 Sex: F

NEUROCOGNITIVE ASSESSMENT

BRAINCHECK COMBINED TEST RESULTS:

84

STANDARD SCORE RANGE: 0-200

LOW AVG HIGH

14th Population Percentile, LOW AVG

Validity Test: PASS Malingering Test: PASS

Presence of cognitive impairment: POSSIBLE Clinical correlation warranted

BRAINCHECK INDIVIDUAL TEST RESULTS:

ATTENTION

Trails B

Patients with impairment may struggle with navigating familiar places, driving, following a map, paying bills correctly, playing familiar games. Lower scores strongly predict a decline in mobility and the inability to drive.

LOW AVG HIGH

71st Population Percentile, AVG

Impression: UNLIKELY indication of dysfunction

Validity Test: Pass

EXECUTIVE FUNCTION

Digit Symbol Substitution

Patients with impairment may struggle with paying attention for longer periods, reading, basic arithmetic. Lower scores have been associated with poor sleep, low mood, anxiety, and substance use

LOW AVG HIGH

40th Population Percentile, AVG

Impression: UNLIKELY indication of dysfunction

Validity Test: Pass

Stroop

Patients with impairment may struggle with following complex instructions, decision making, poor judgment, socially inappropriate behavior, apathy, withdrawal, maintaining a healthy diet.

LOW AVG HIGH

83rd Population Percentile, ABOVE AVG

Impression: UNLIKELY indication of dysfunction

Validity Test: Pass

MEMORY

Immediate Recognition

Patients with impairment may struggle with repeating themselves or asking the same question repeatedly within a few minutes of each other, forgetting what they were going to do, forgetting where they placed something or paying attention to the TV

LOW AVG HIGH

1st Population Percentile, VERY LOW

Impression: LIKELY indication of dysfunction

Validity Test: Pass

Delayed Recognition

Patients with impairment may struggle with repeating themselves later the same day or next day, forgetting the content of a conversation, or needing to rely on a calendar or alarm for reminders, not knowing current events.

LOW AVG HIGH

5th Population Percentile, LOW

Impression: POSSIBLE indication of dysfunction

Validity Test: Pass

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

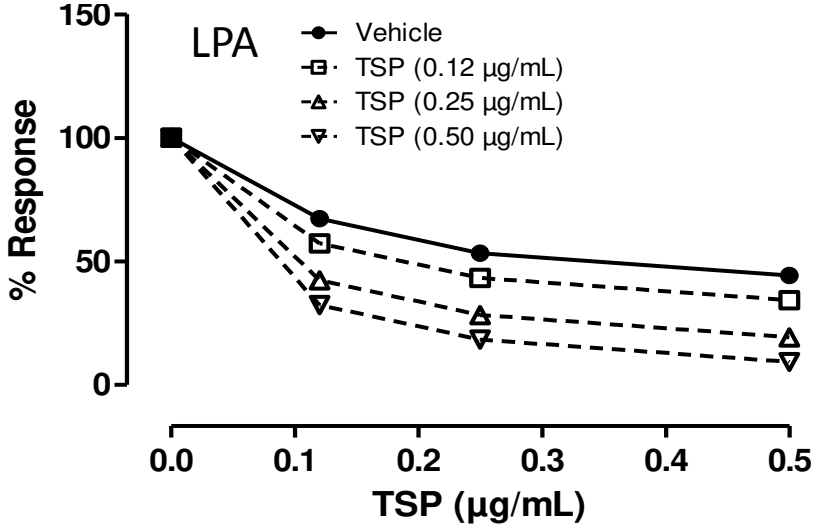
Table 2. Clinical results with BrainCheck

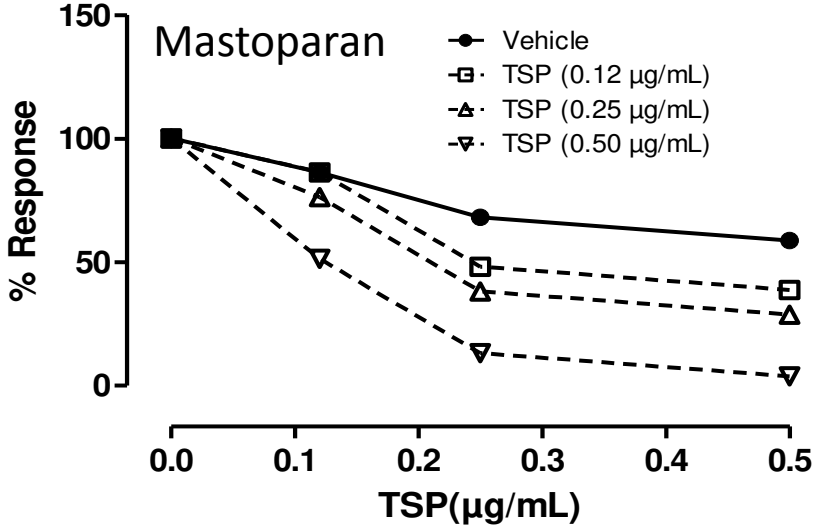
Patient ID	At baseline (T0 min)	Post-stress test (T90 min)	Post-stress test (T+5 days)	Neurocognitive effects
EM 169	98	94	**84**	Likely a memory dysfunction
EM 170	**83**	96	101	Likely a memory dysfunction
EM 171	101	**85**	111	Possible executive function dysfunction

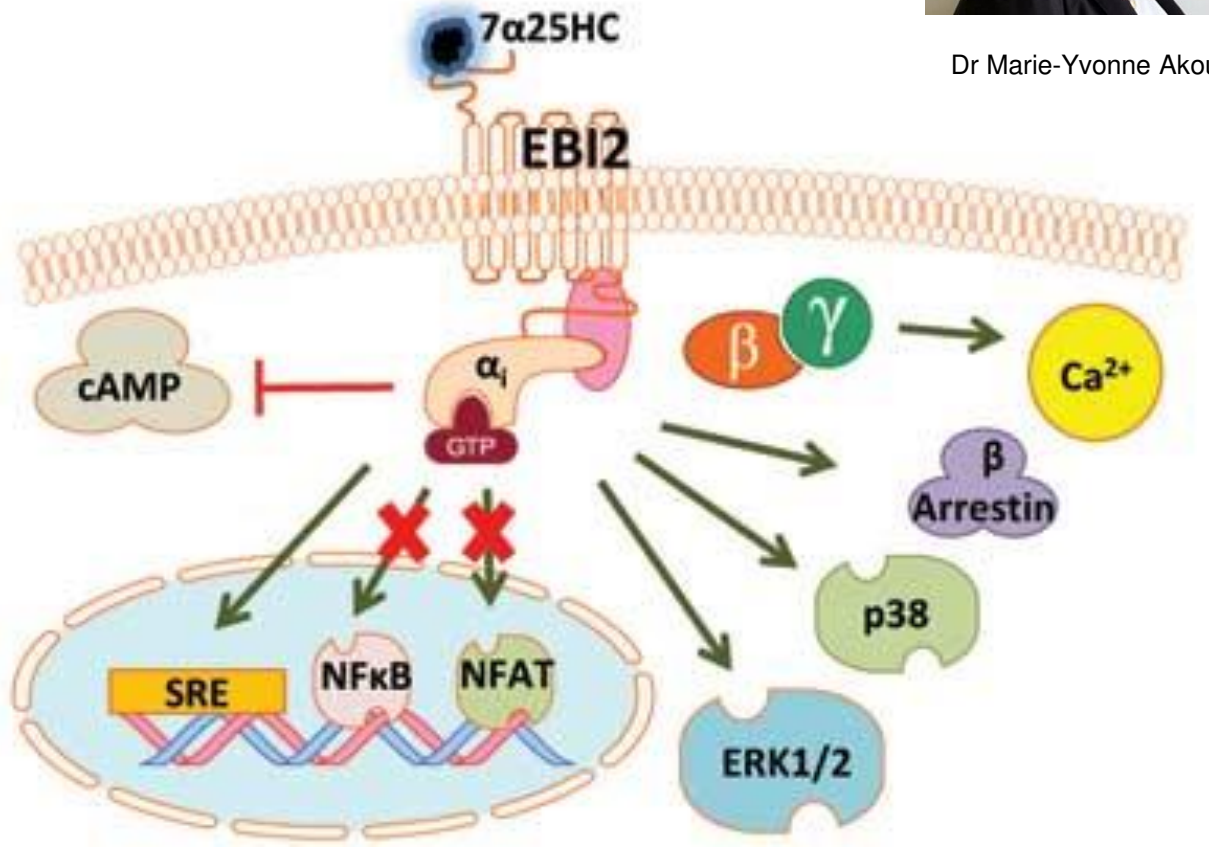
11 ROLE OF THROMBOSPONDIN-1 IN ME/CFS?

Thrombospondin-1 inhibits Gi-coupled receptor signaling

Dr Marie-Yvonne Akoume



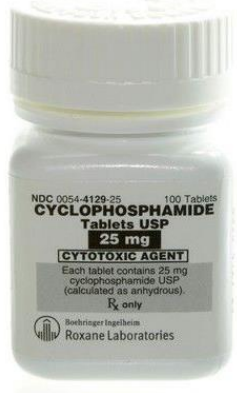




Kerr JR. Epstein-Barr Virus Induced Gene-2 Upregulation Identifies a Particular Subtype of Chronic Fatigue Syndrome/Myalgic Encephalomyelitis. Front Pediatr. 2019;7:59.

12 THERAPEUTIC OPTIONS FOR ME/CFS PATIENTS

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

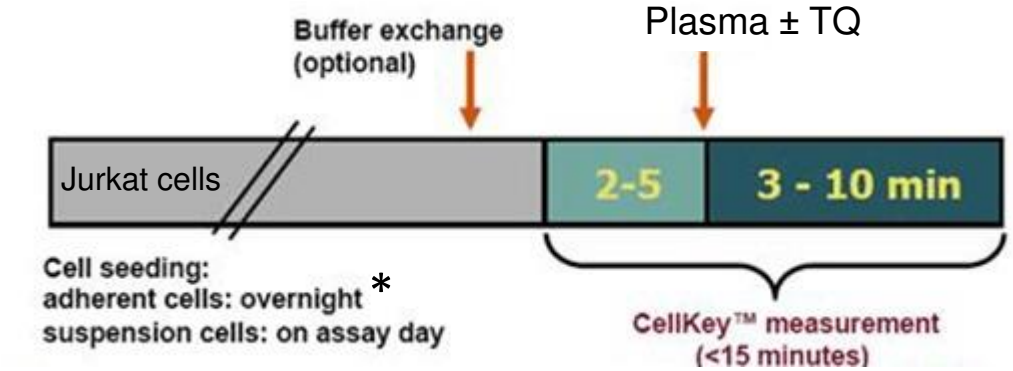


- Interestingly, α2δ-1 is the high affinity receptor for TSP-1 in the brain.
- Two commonly prescribed anti-epileptic, anti-neuropathic pain medications, **gabapentin** (Neurontin™) and **pregabalin** (Lyrica™) are targeting α2δ-1 receptor. Both drugs are being used off-label for ME/CFS and fibromyalgia 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]

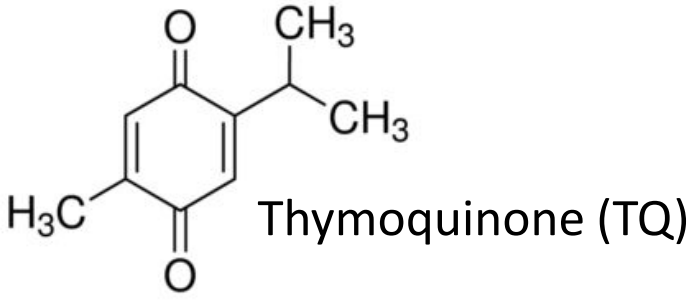
[1] Amarasekera AT, et al. Vitamin D supplementation lowers thrombospondin-1 levels and blood pressure in healthy adults. *PLoS One*. 2017;12(5):e0174435.
[2] Lansiaux, A. et al. Circulating thrombospondin 1 level as a surrogate marker in patients receiving cyclophosphamide-based metronomic chemotherapy. 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

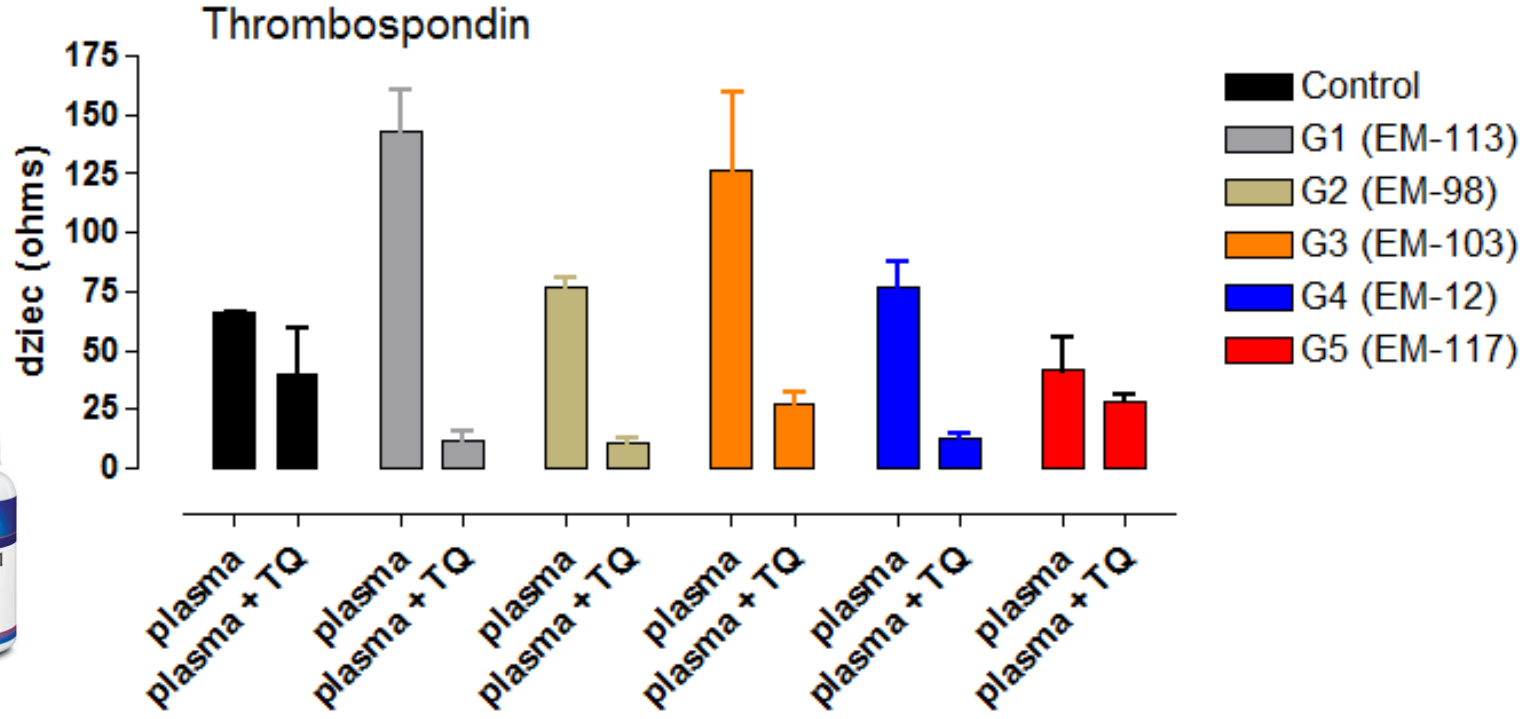
How to block TSP-1 signaling action?



*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 α2δ-1 and CD47 receptors but not CD36 receptor.



Thymoquinone (TQ)



14 MULTITASKING BIOMOLECULES (2)

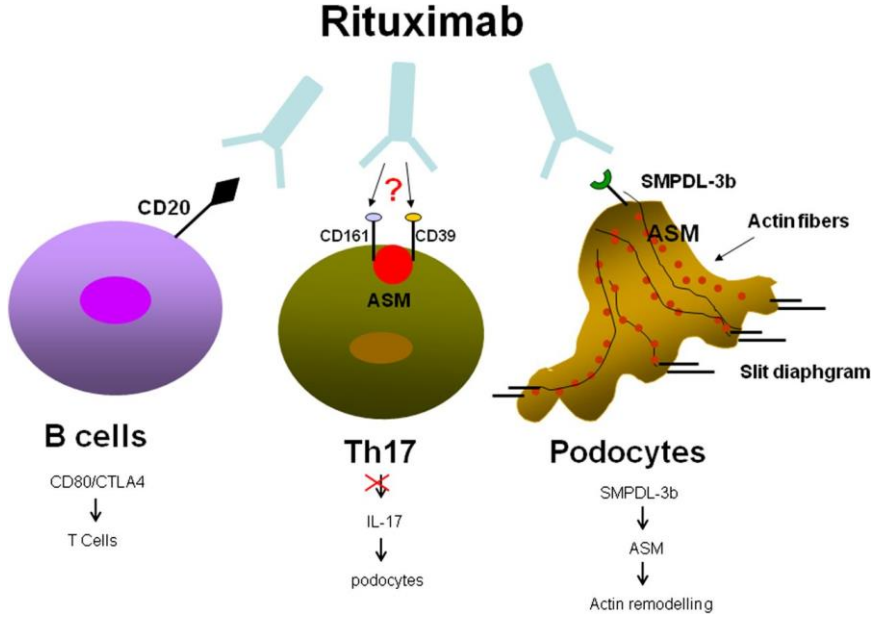
Role of SMPDL3B in ME/CFS pathophysiology

Bita Rostami

Dr. Wesam Elremaly

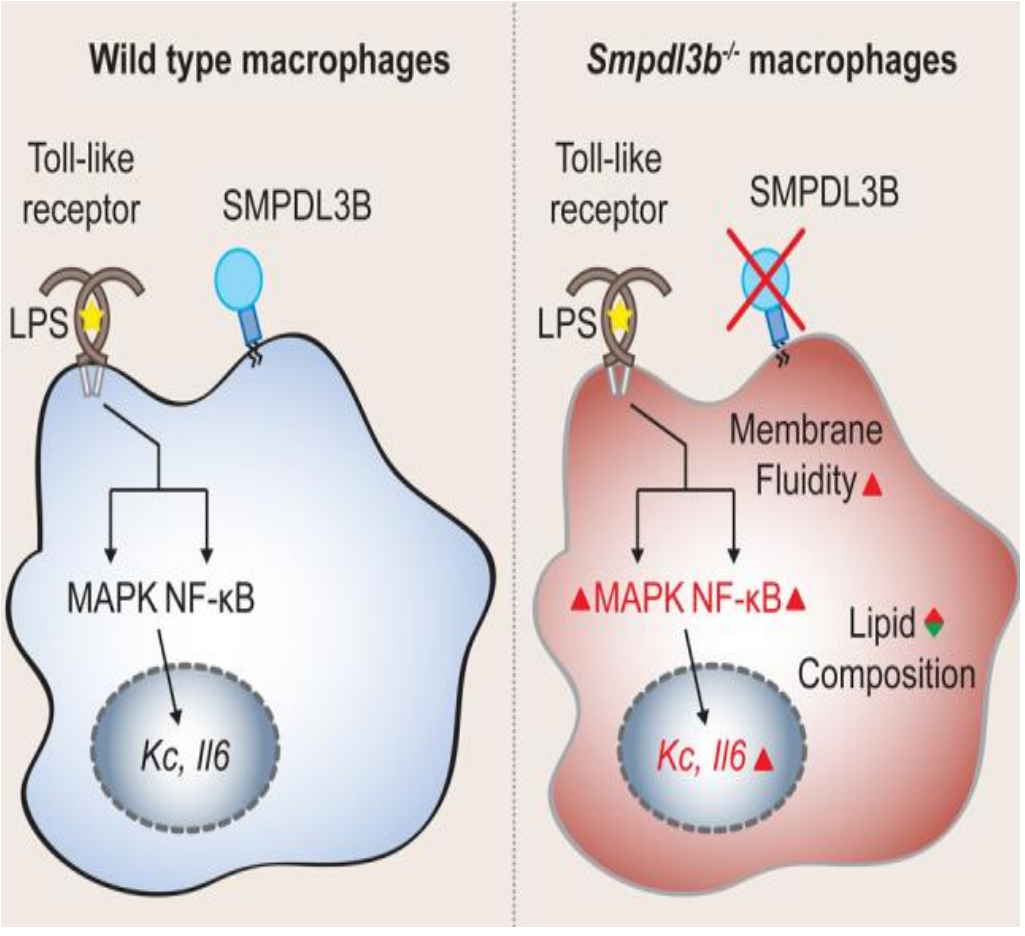
THE PROBLEM: Little is known about the mechanism underlying lipid metabolism alteration occurring in ME/CFS.

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

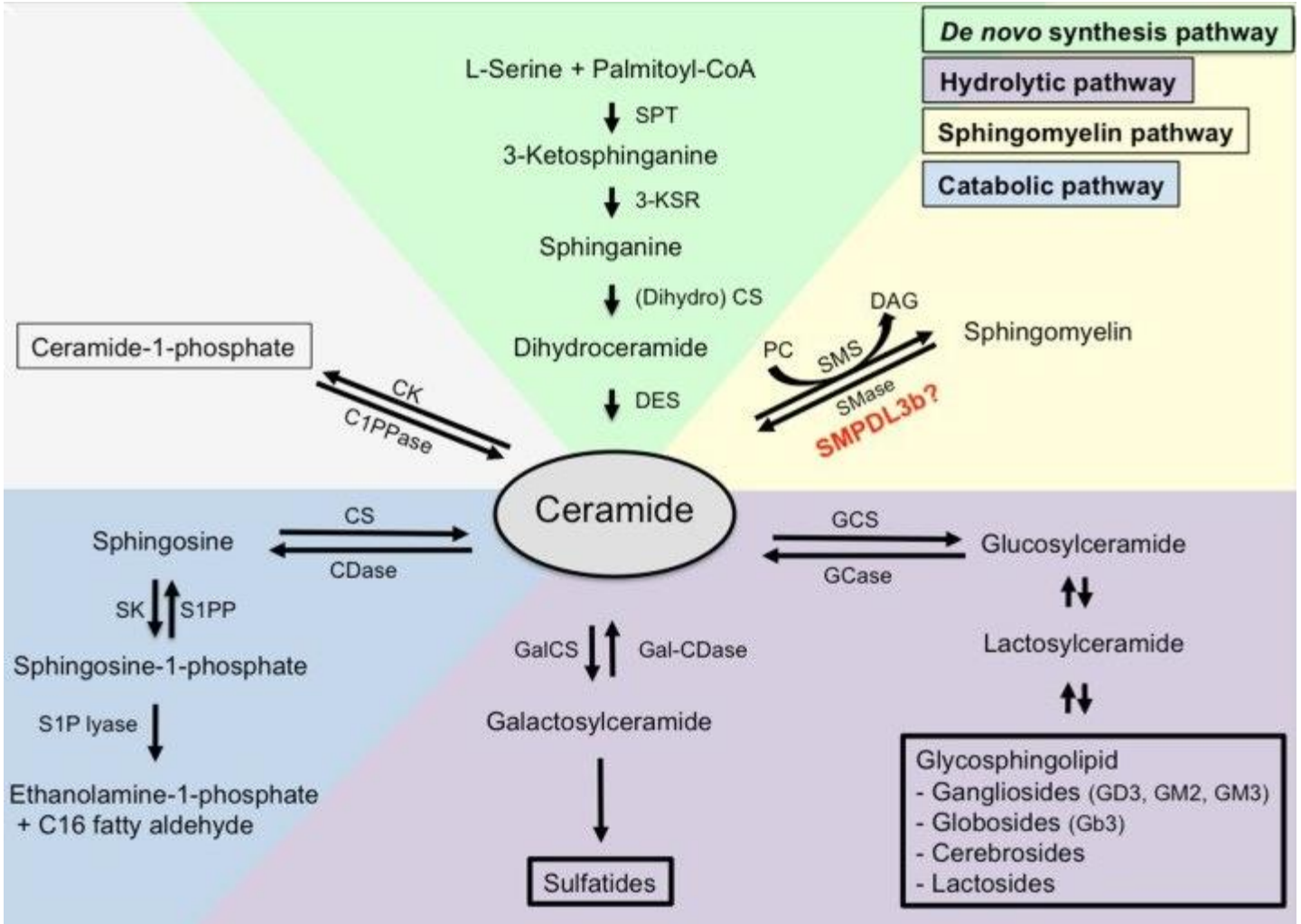


- SMPDL3B expression is prominently observed in macrophages and DCs.
- Consistent with a possible role for this enzyme in the course of inflammatory processes.
- Smpdl3b transcription in bone marrow-derived macrophages (BMDMs) and DCs (BMDCs) is robustly induced upon TLR stimulation

Heinz LX, Baumann CL, Köberlin MS, et al. The Lipid-Modifying Enzyme SMPDL3B Negatively Regulates Innate Immunity. Cell Rep. 2015;11(12):1919-1928.

16 LIPID-MODIFYING ENZYME SMPDL3B

SMPDL3B is a relevant molecule if ME/CFS pathogenesis



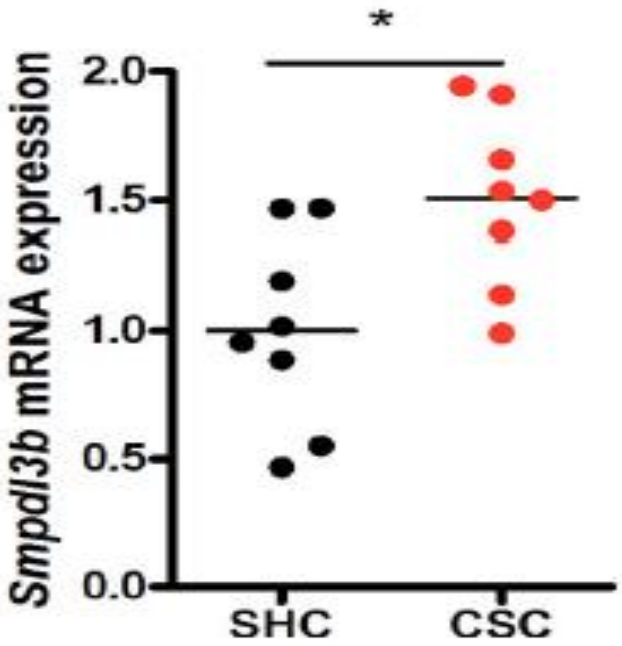
Merscher S, Fornoni A. Podocyte pathology and nephropathy - sphingolipids in glomerular diseases. Front Endocrinol (Lausanne). 2014;5:127.

- 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 s**phingomyelins** in females.

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

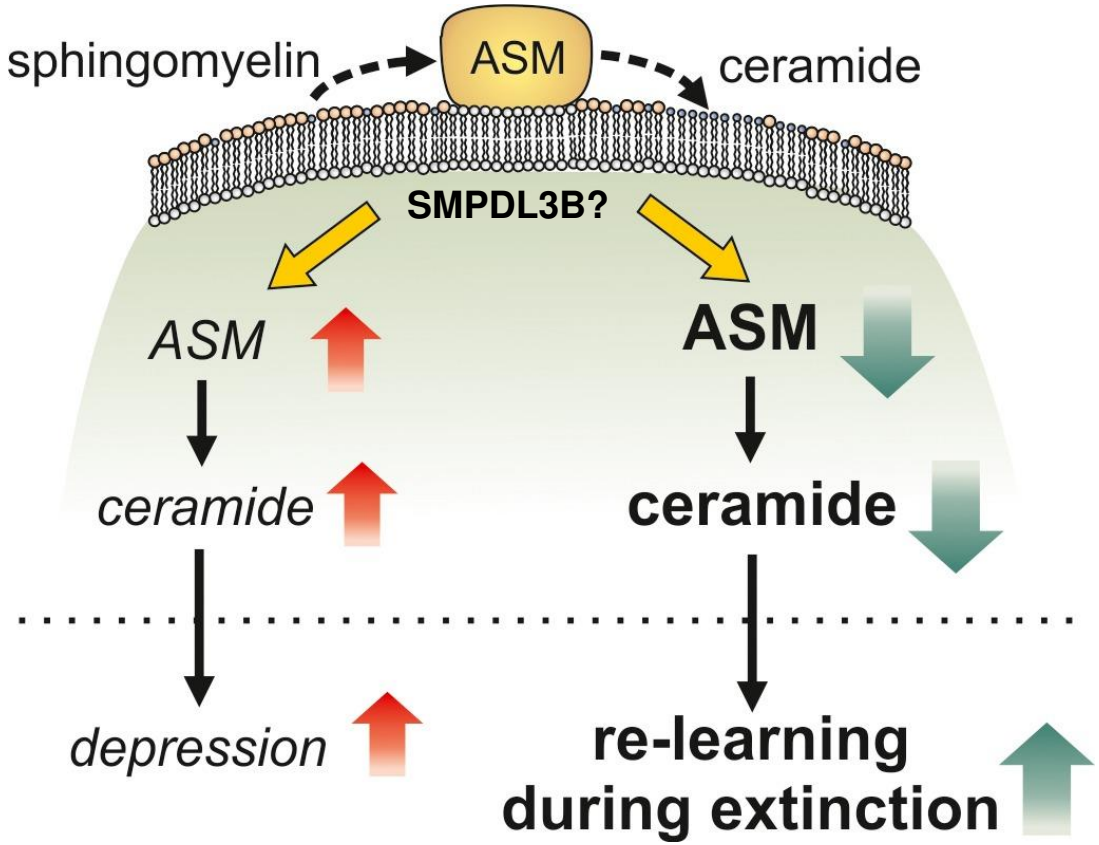
17 LIPID-MODIFYING ENZYME SMPDL3B

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.

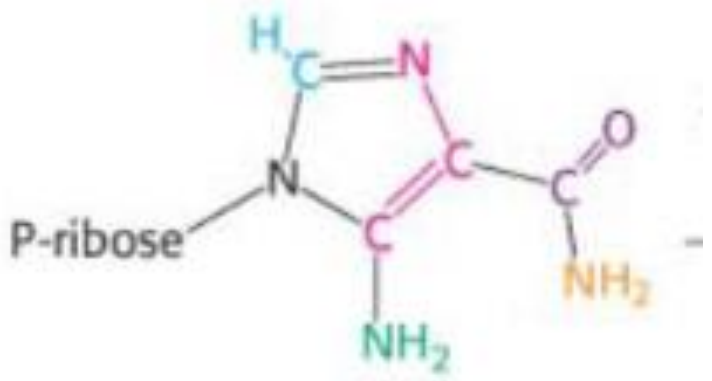
18 THERAPEUTIC OPTION FOR ME/CFS PATIENTS

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



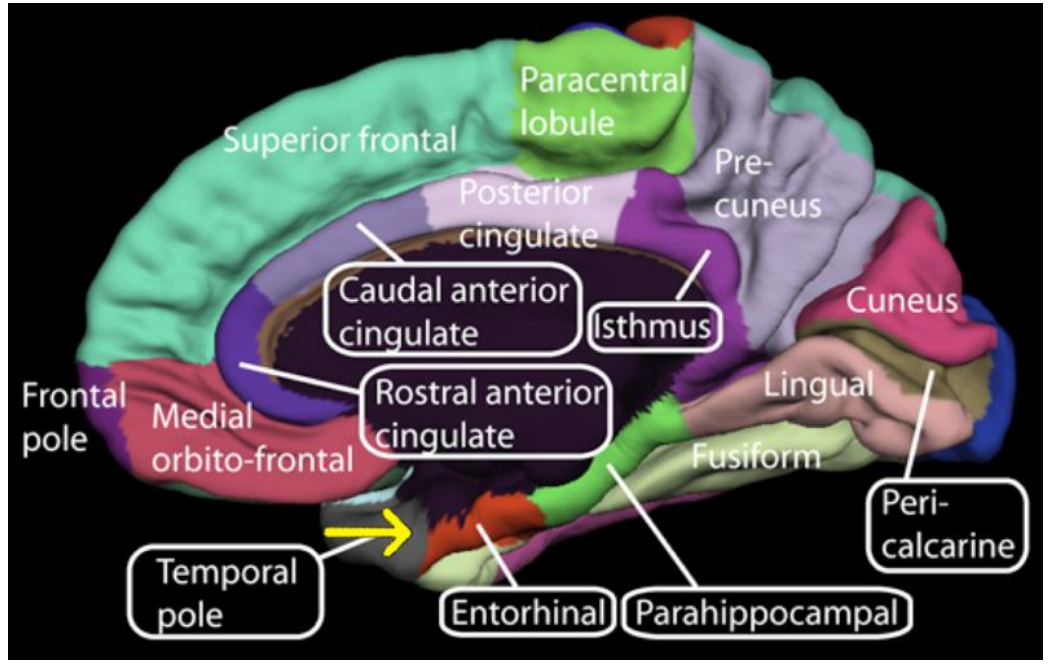
5-Aminoimidazole-4-carboxamide ribonucleotide

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]



By Hagmann P, Cammoun L, Gigandet X, Meuli R, Honey CJ, et al. - https://commons.wikimedia.org/w/index.php?curid=8636113

[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.

PRELIMINARY DATA

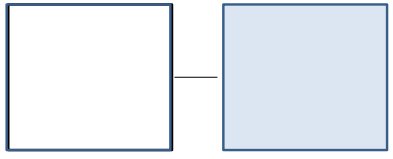
Possible role of SMPDL3B in ME/CFS pathogenesis

Lynda Chalder

Dr. Dawei Li

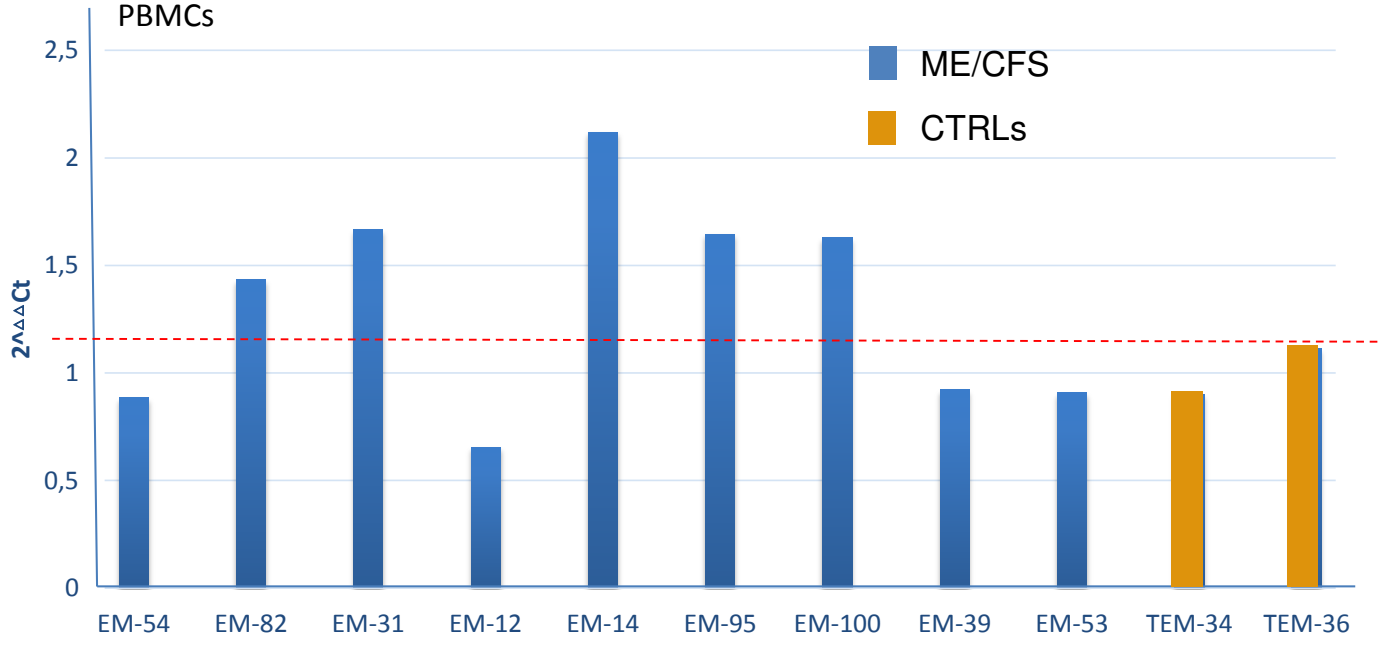
Hypermethylation of SMPDL3B gene occurs in ME/CFS

Family #14



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



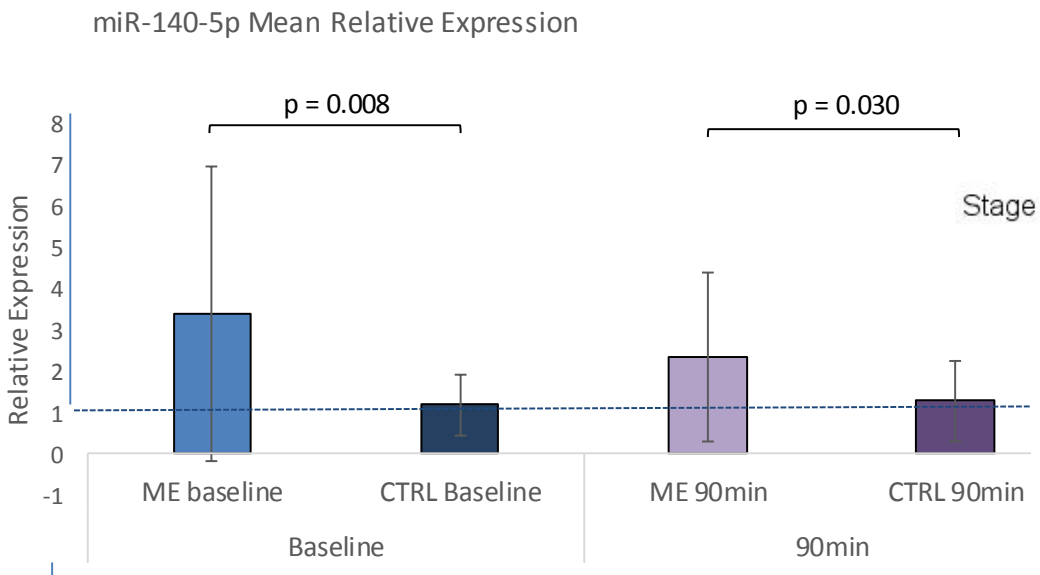
Bita Rostami

21 RITUXIMAB EFFECTS IN ME/CFS?

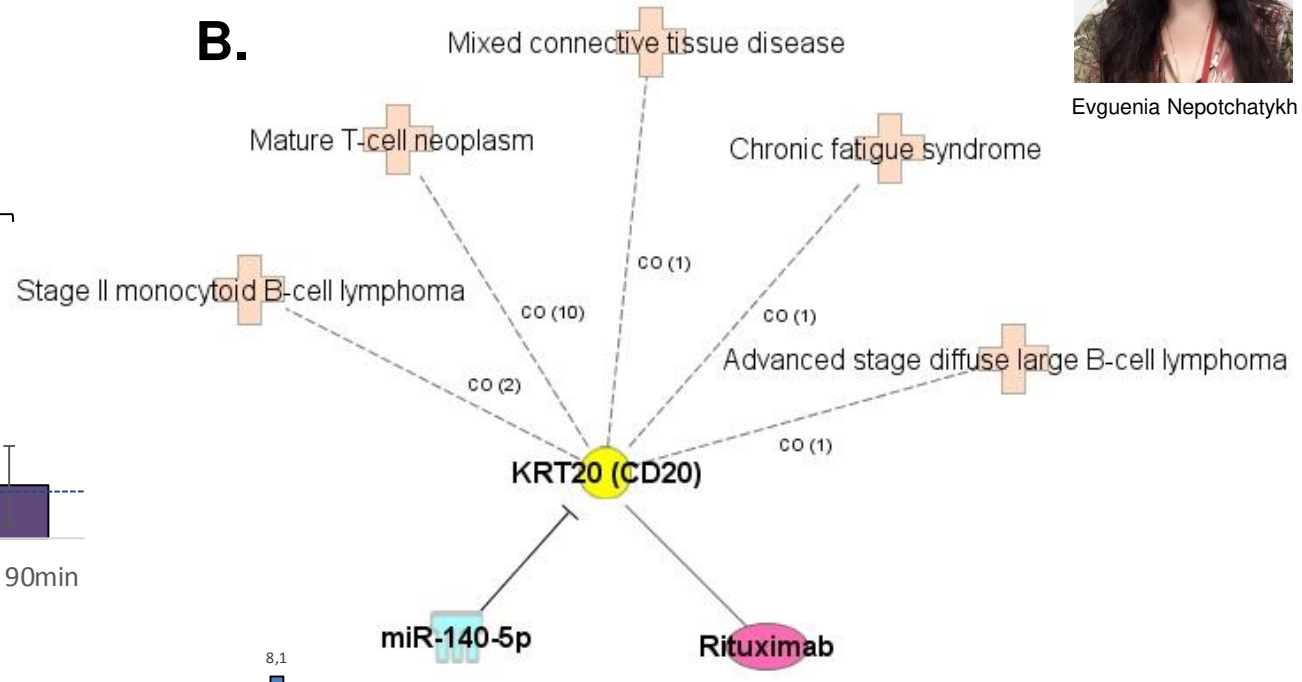
MiR-140-5p expression could influence Rituximab bioavailability

Evguenia Nepotchatykh

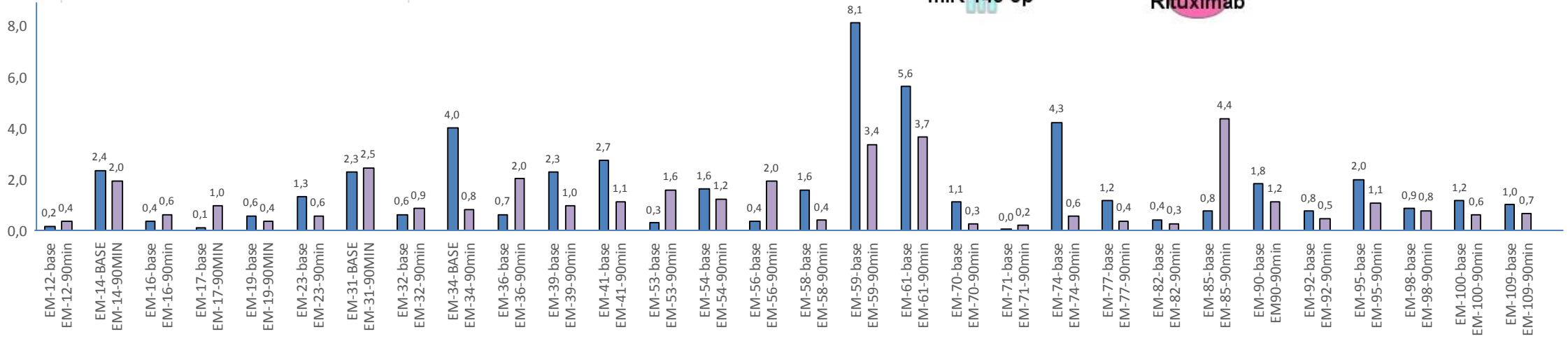
A.



B.



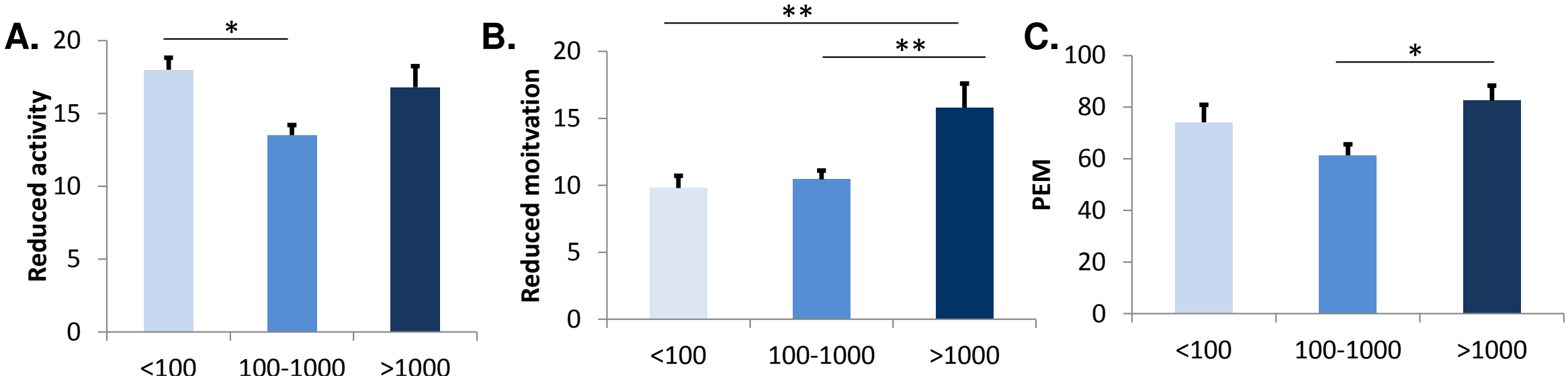
C.



22 CHANGES IN URINARY SMPDL3B LEVELS

Association of urinary SMPDL3b levels and ME/CFS symptoms

Bita 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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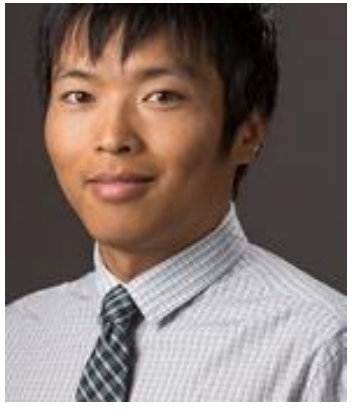
Dr. Dawei Li