

Coagulation circulating proteins in Multiple Sclerosis

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BBB permeability /disruption

Pericyte

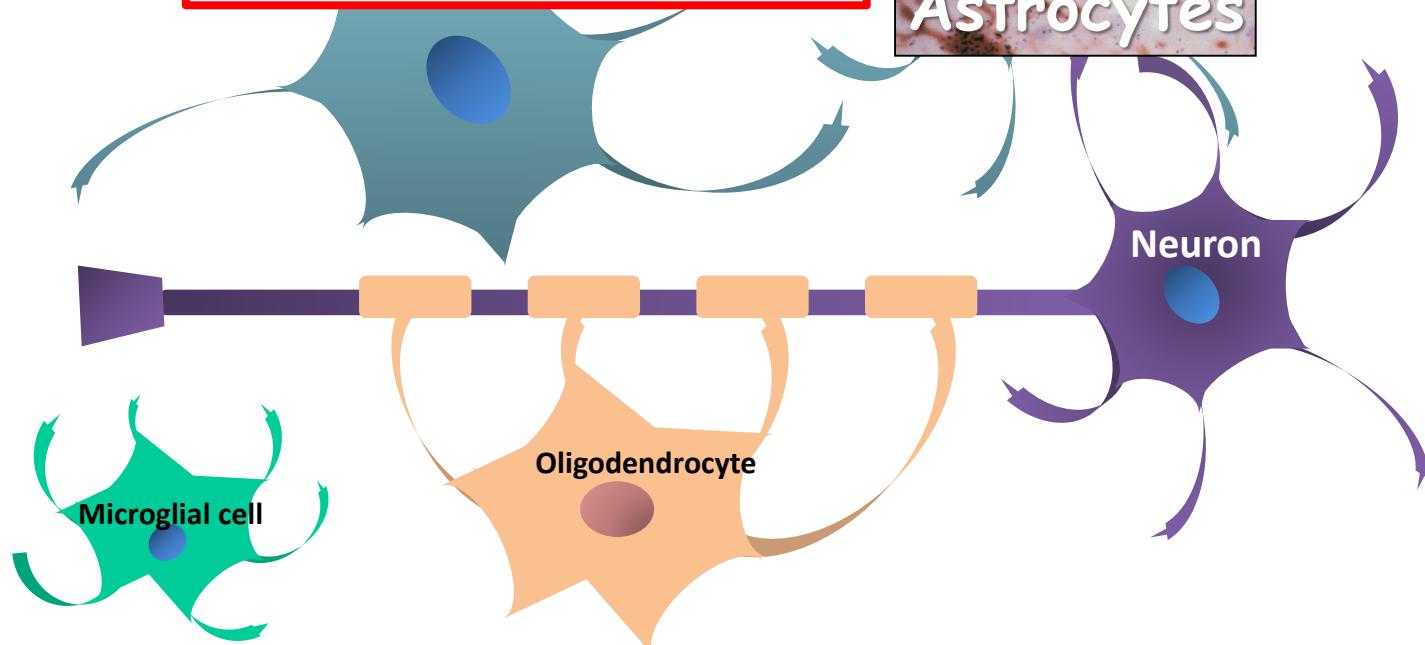
Blood

Blood Brain Barrier

Leakage and Activation
of Coagulation factors

Tissue Factor

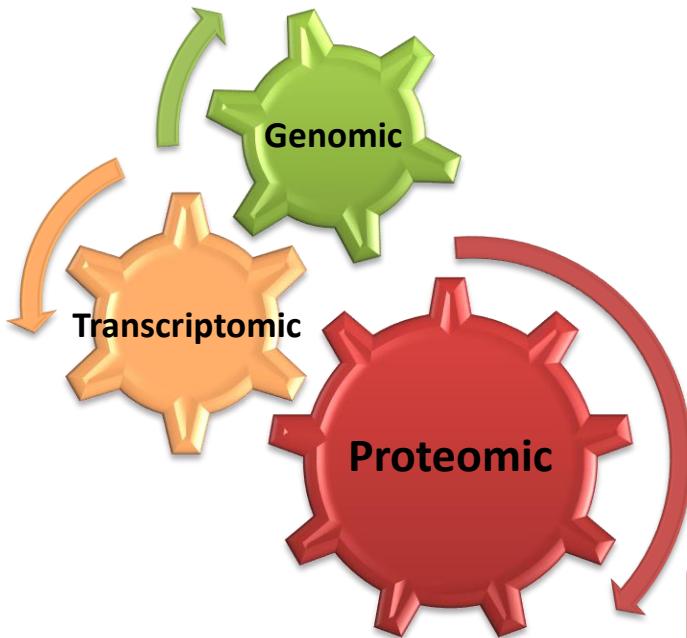
Astrocytes



Clotting factor number	Clotting factor name	Plasma half-life (h)	Plasma concentration (mg/L)
I	Fibrinogen	90	3000
II	Prothrombin	65	100
III	TF Tissue Factor	-	-
V	Proaccelerin, labile factor	15	10
VII	Stable factor, proconvertin	5	0.5
VIII	Antihemophilic factor A	10	0.1
IX	Antihemophilic factor B or Christmas factor	25	5

Experimental approaches

- highlight candidate genes/proteins in MS pathophysiology
- investigate disease progression biomarkers



WES in selected families for GWAS candidate genes
Analysis of low-frequency variants

Microarrays for expression profiling of internal jugular vein (extracranial venous compartment)

Proteomic survey by simultaneous measurement of proteins in plasma multiplex assays

Coagulation FXII activity in the “intrinsic” pathway

Intrinsic (Contact) pathway



FXII may drive adaptive immunity during neuroinflammation via uPAR/CD87-mediated modulation of dendritic cells

Gobel et al, Nat Comm 2016

- Lower FXII activity/antigen ratio (MS vs HS P <0.001)
- Lower FXII response to contact activation in thrombin generation in part of MS patients, particularly in Primary Progressive

Hemostasis biomarkers in multiple sclerosis.

Ziliotto N et al

Eur J Neurol. 2018

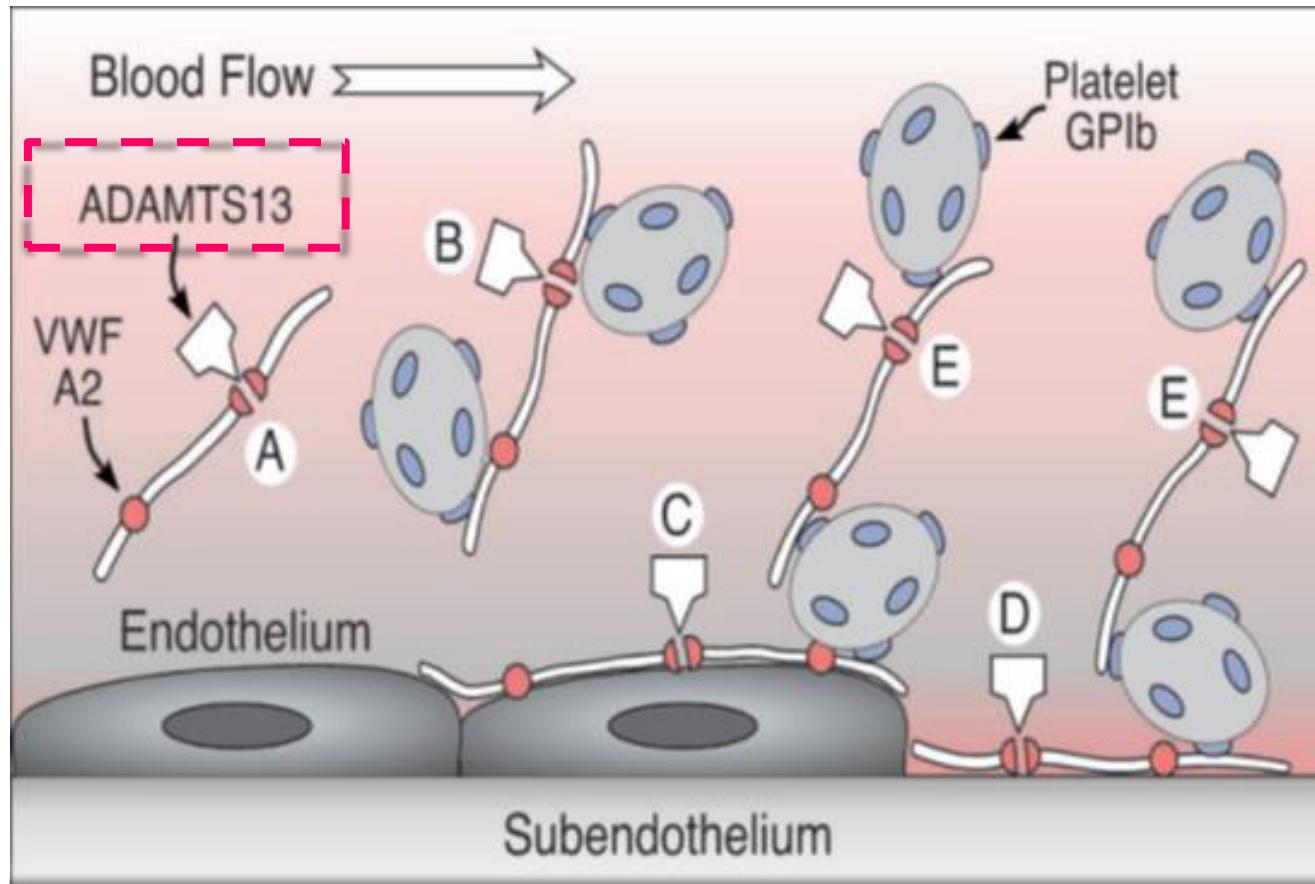
Coagulation Factor XII Levels and Intrinsic Thrombin Generation in Multiple Sclerosis

Nicole Ziliotto¹, Marcello Baroni¹, Sofia Straudi², Fabio Manfredini^{2,3}, Rosella Mari⁴, Erica Menegatti⁵, Rebecca Voltan^{5,6}, Paola Secchiero^{5,6}, Paolo Zamboni⁵, Nino Basaglia², Giovanna Marchetti³ and Francesco Bernardi^{1*}

Front Neurol. 2018

ADAMTS13

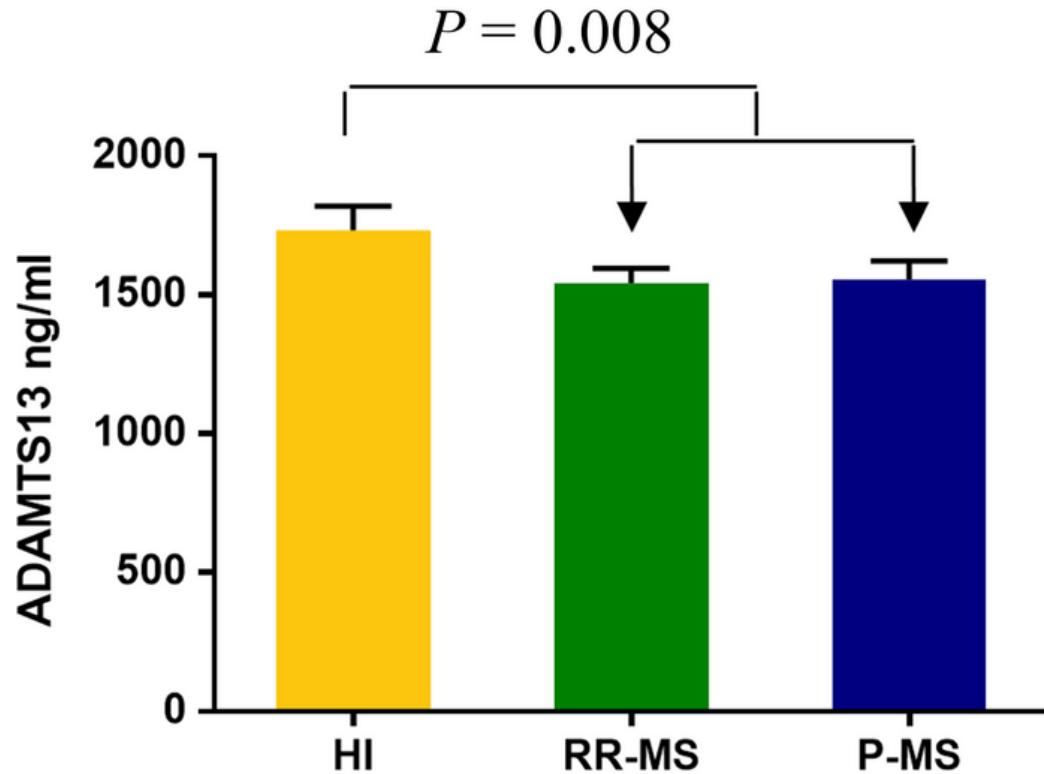
regulator of Von Willebrand Factor and platelet adhesion



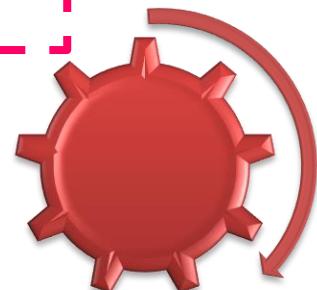
ADAMTS13 cleaves vWF multimers
→ inhibitory effects on hemostasis and inflammation

Lower plasma ADAMTS13 levels in MS

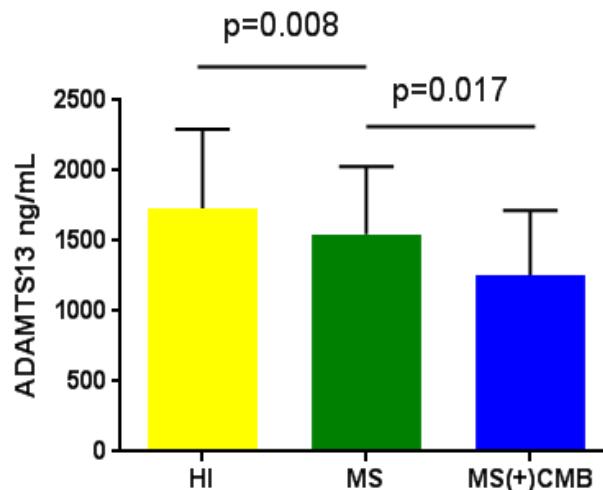
CEG (cardiovascular, environmental and genetic risk factors) study



Decreased inhibitory effects on hemostasis and inflammation?



Lower ADAMTS13 levels in MS: Association with cerebral microbleeds (CMB)



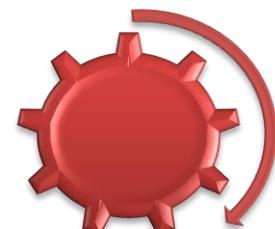
The lower levels of ADAMTS13 do not fit with increased «bleeding tendency»

European Journal of Neurology 2018

ORIGINAL ARTICLE

Hemostasis biomarkers in multiple sclerosis

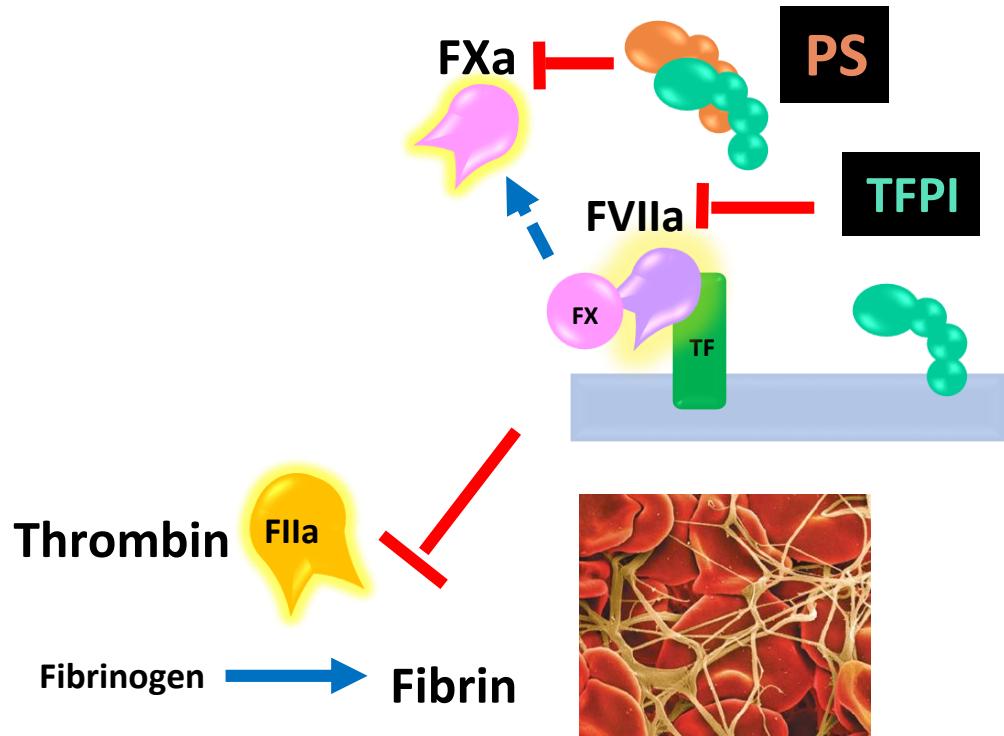
N. Ziliotto^{a,b} , F. Bernardi^a, D. Jakimovski^b, M. Baroni^a, G. Marchetti^c, N. Bergsland^b ,
D. P. Ramasamy^b, B. Weinstock-Guttman^d, F. Schweser^{b,e}, P. Zamboni^f,
M. Ramanathan^g and R. Zivadinov^{b,e} 



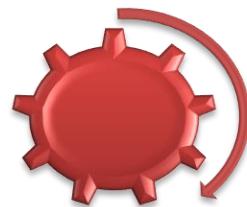
Proteomic

Inhibition of Coagulation initiation

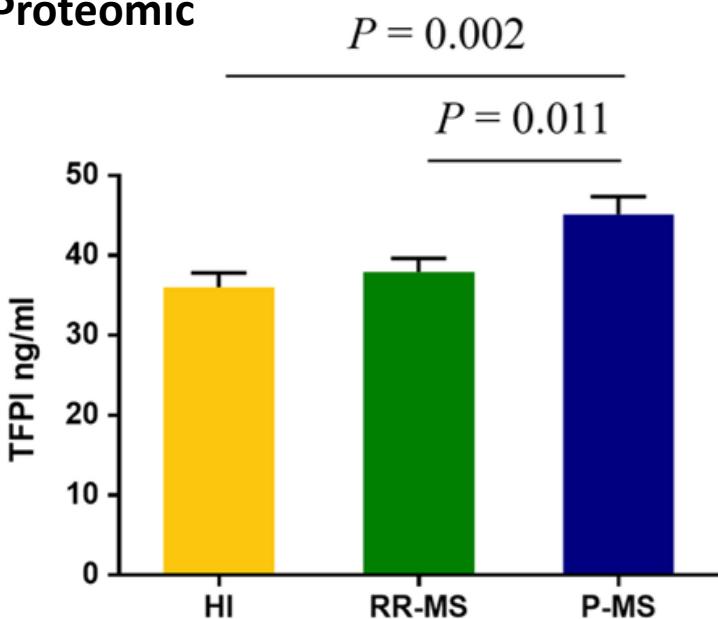
Tissue Factor Pathway Inhibitor - *TFPI*



Increased plasma levels of *TFPI* in progressive MS



Proteomic

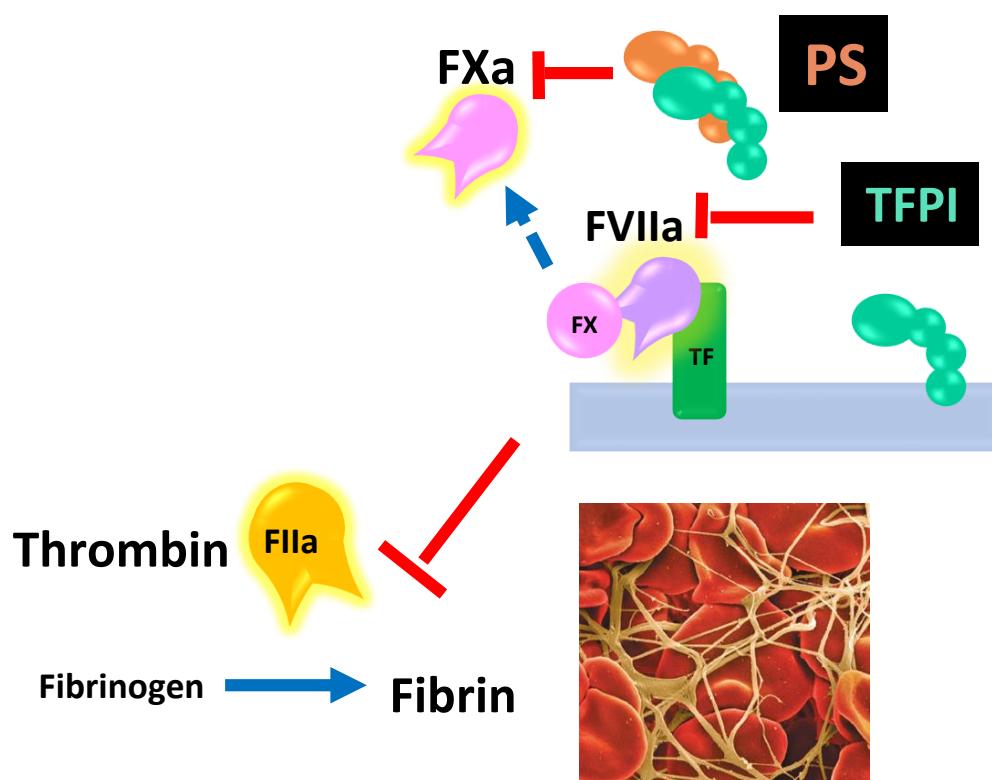


Decreased Thrombin and
Fibrin formation?
Endothelium alteration?

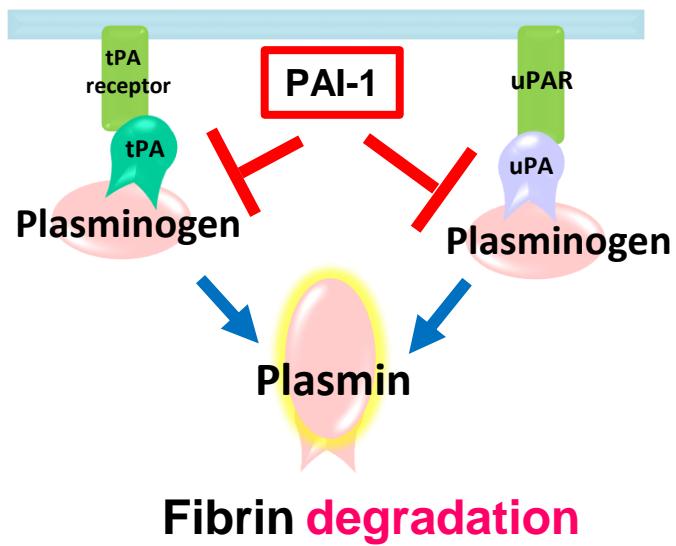
ORIGINAL ARTICLE

Hemostasis biomarkers in multiple sclerosis

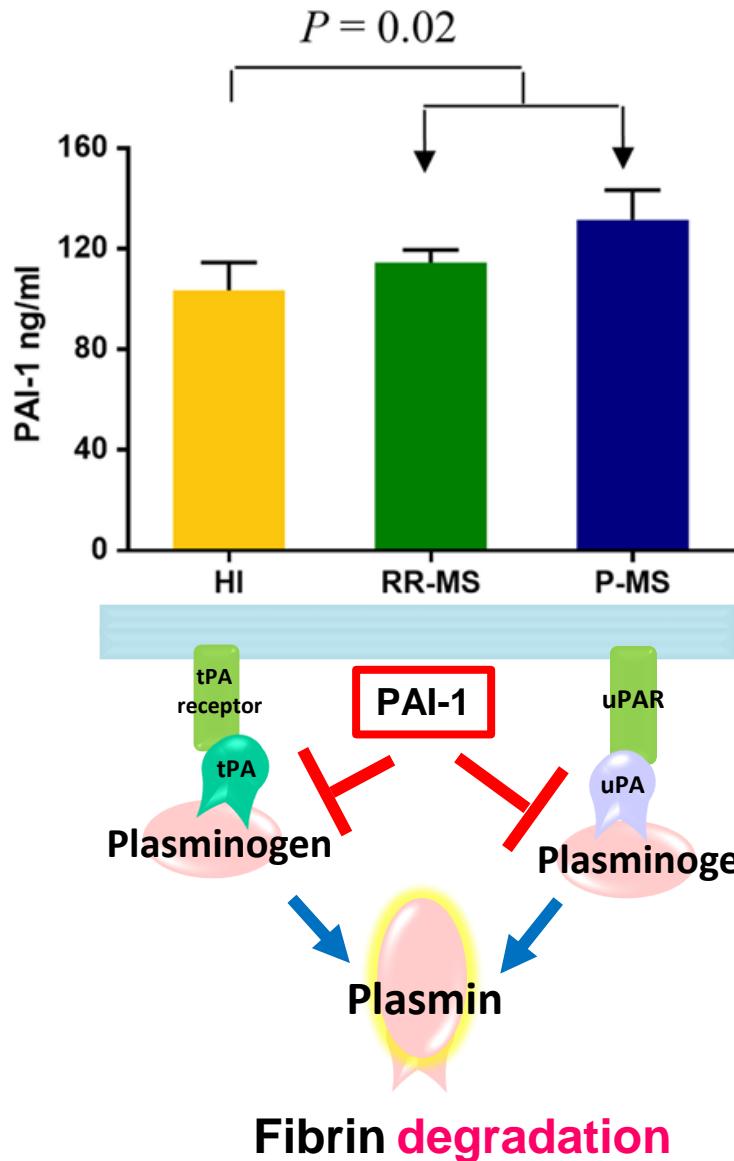
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Plasminogen Activator Inhibitor 1 (PAI-1) *fibrinolysis inhibitor*

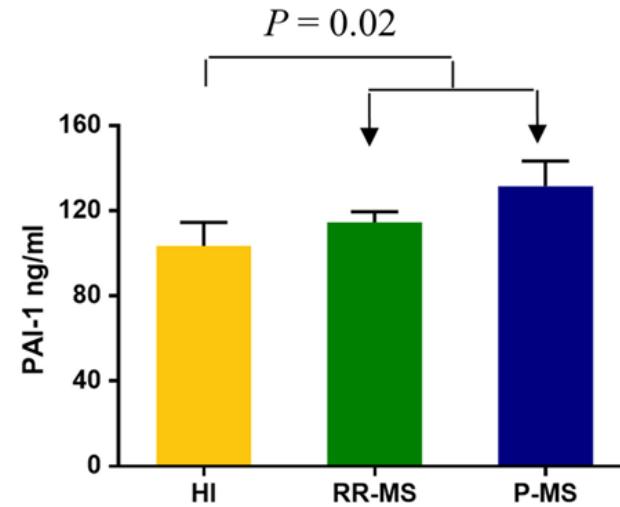
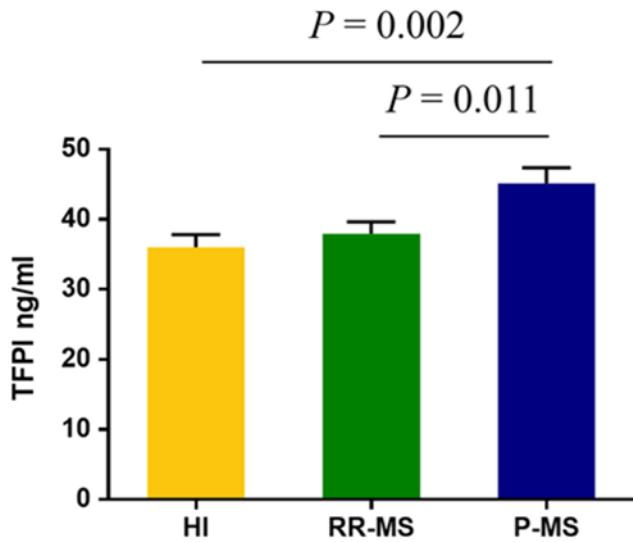


Increased plasma levels of *PAI-1* in MS

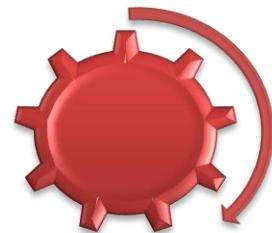


Decreased Fibrin Degradation?
Increased inflammation
Modulation of fibrin contribution to neuroinflammation?

Increased plasma levels of *TFPI* and *PAI-1* in progressive MS



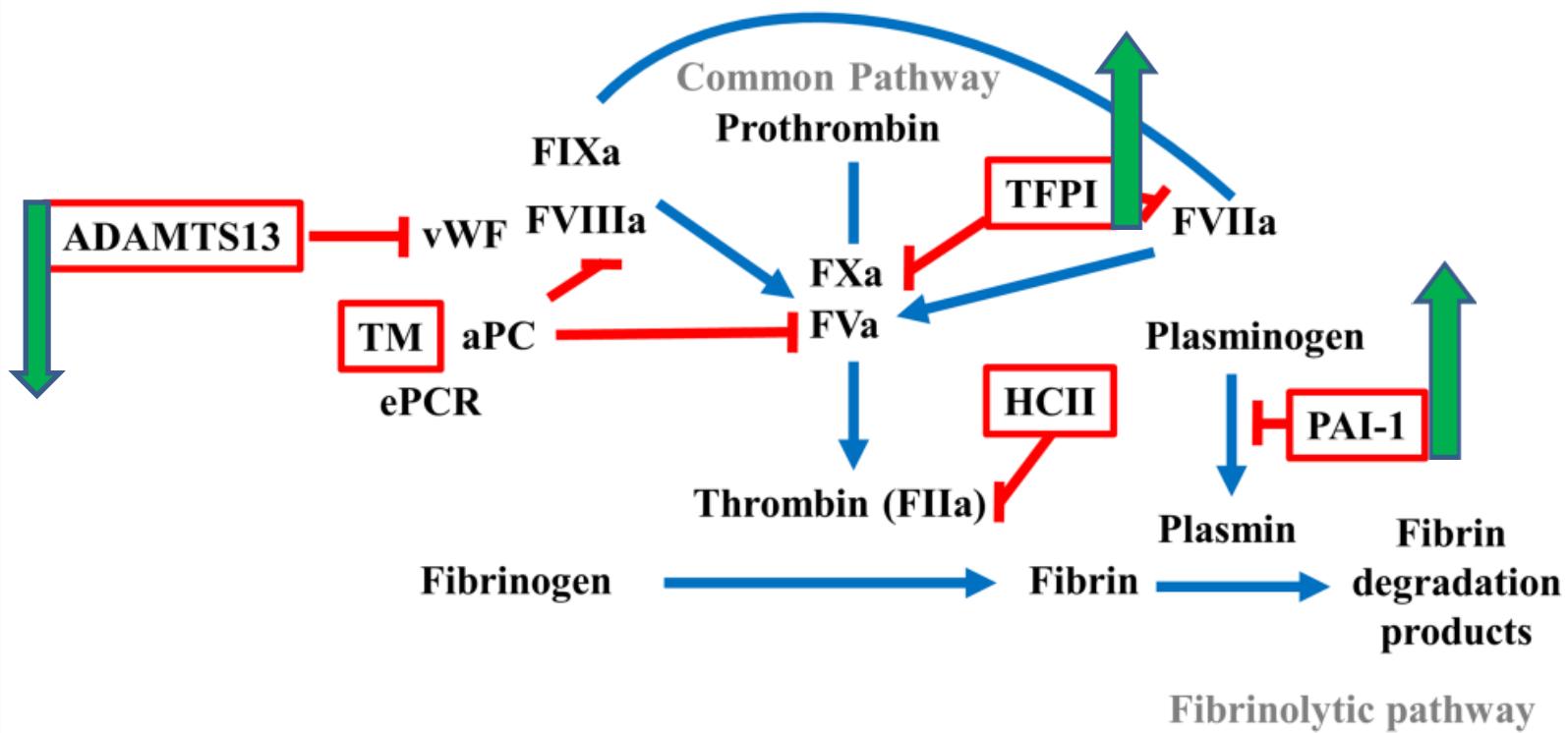
Is the hemostatic balance preserved?
Candidate Biomarkers of disease progression?



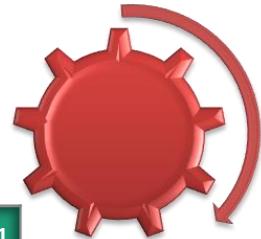
Proteomic

Plasma levels of Inhibitors of hemostasis in MS

Ziliotto N et al. Eur J Neurol. 2018



Correlations between plasma concentrations of Hemostasis/Inflammation/Adhesion proteins



Proteins		FXII µg/mL	ADAMTS13 ng/mL	HCII ng/mL	TFPI ng/mL	TM ng/mL	PAI-1 ng/mL	CCL18 ng/mL	CCL5 ng/mL	CD86 U/mL	sNCAM ng/mL	sICAM-1 ng/mL	sVCAM-1 ng/mL
ADAMTS13 ng/mL	Rho: P value:	0.029 0.732											
HCII ng/mL	Rho: P value:	0.044 0.604	-0.100 0.241										
TFPI ng/mL	Rho: P value:	0.010 0.909	0.062 0.472	-0.080 0.351									
TM ng/mL	Rho: P value:	-0.005 0.954	0.072 0.400	-0.012 0.890	0.240 0.004								
PAI-1 ng/mL	Rho: P value:	0.284 0.001	-0.060 0.484	-0.209 0.014	0.153 0.073	-0.050 0.562							
CCL18 ng/mL	Rho: P value:	0.037 0.669	-0.098 0.252	-0.345 <0.00 1	0.207 0.015	0.082 0.342	0.340 <0.001						
CCL5 ng/mL	Rho: P value:	0.089 0.302	-0.239 0.005	0.117 0.173	-0.027 0.750	-0.155 0.069	0.542 <0.001	0.047 0.583					
CD86 U/mL	Rho: P value:	0.149 0.082	-0.084 0.326	0.110 0.199	0.092 0.281	0.098 0.254	0.051 0.553	0.082 0.339	0.105 0.222				
sNCAM ng/mL	Rho: P value:	0.102 0.236	0.068 0.430	0.089 0.301	0.145 0.089	0.287 0.001	-0.017 0.841	-0.09 0.293	-0.052 0.542	0.209 0.014			
sICAM-1 ng/mL	Rho: P value:	-0.184 0.031	-0.032 0.711	-0.152 0.075	0.096 0.236	0.303 <0.001	-0.132 0.122	0.207 0.015	-0.232 0.006	0.232 0.006	-0.075 0.382		
sVCAM-1 ng/mL	Rho: P value:	0.049 0.565	-0.067 0.438	0.109 0.201	0.119 0.164	0.173 0.042	0.202 0.017	0.149 0.082	0.137 0.109	0.406 <0.001	0.264 0.002	0.197 0.021	
VAP-1 ng/mL	Rho: P value:	0.113 0.187	-0.004 0.965	0.038 0.660	0.214 0.012	0.198 0.020	0.060 0.485	0.087 0.313	-0.059 0.492	0.305 <0.001	0.385 <0.001	0.190 0.026	0.398 <0.001

Nicole Ziliotto PhD Thesis

«New» Correlations between hemostasis protein concentrations in MS?

MS
Patients

Proteins		FXII µg/mL	HCII ng/mL	TFPI ng/mL
PAI-1 ng/mL	Rho	0.284	-0.209	0.153
	P	0.001	0.014	0.073

Healthy
Subjects

Proteins		FXII µg/mL	HCII ng/mL	TFPI ng/mL
PAI-1 ng/mL	Rho:	-0.057	-0.081	0.331
	P	0.718	0.608	0.032

Disease-related correlations: «integrated» biomarkers?

Coagulation circulating proteins in Multiple Sclerosis- **Conclusions**

Dysregulation of plasma hemostasis inhibitors levels provides several candidate biomarkers to investigate MS progression



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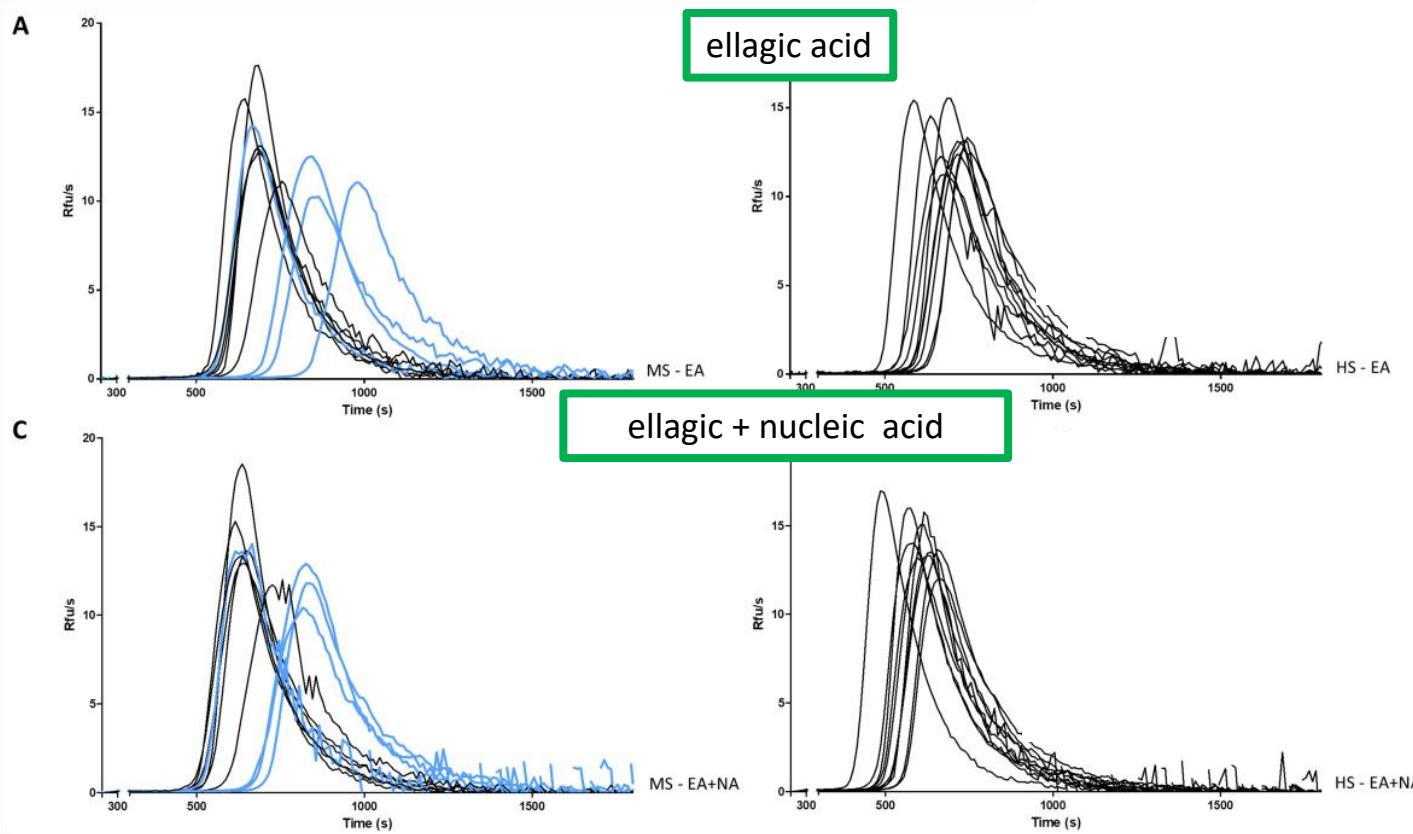


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Coagulation FXII activity in the “intrinsic” pathway

Multiple Sclerosis

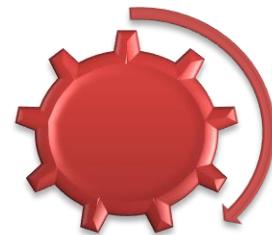
Healthy Subjects



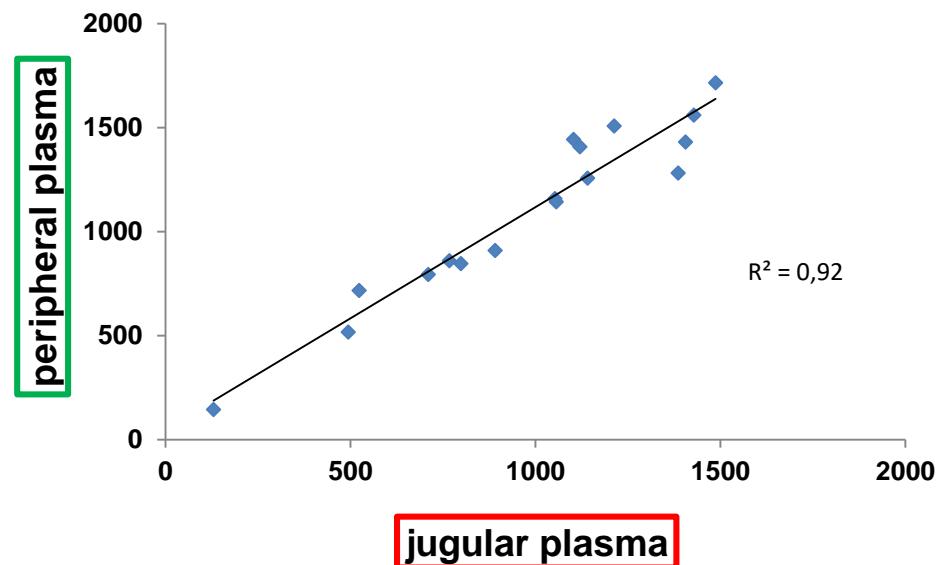
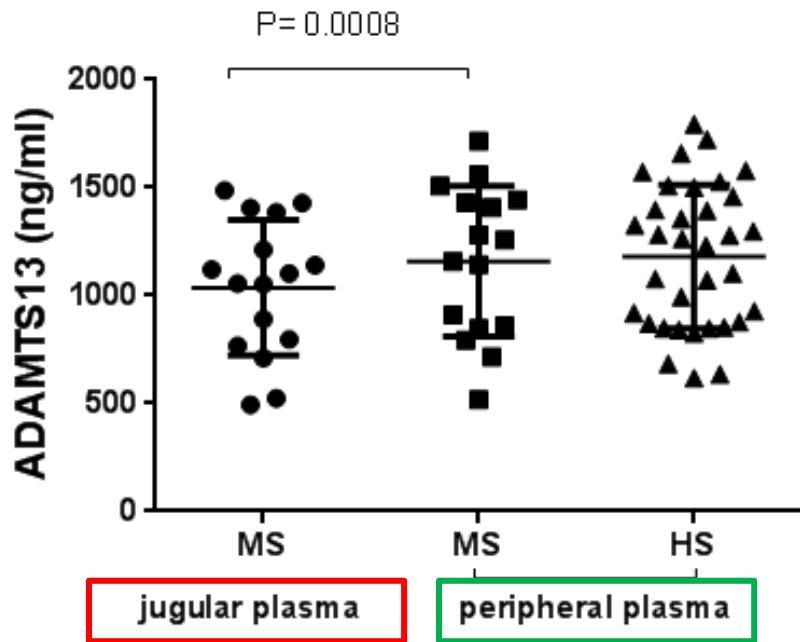
| Lower FXII response to contact activation in part of MS patients,
| particularly in Primary Progressive

Coagulation Factor XII Levels and
Intrinsic Thrombin Generation in
Multiple Sclerosis

ADAMTS13 – Plasma levels in MS/CCSVI patients



Proteomic



Reduced levels in jugular plasma

High correlation between jugular and peripheral plasma levels