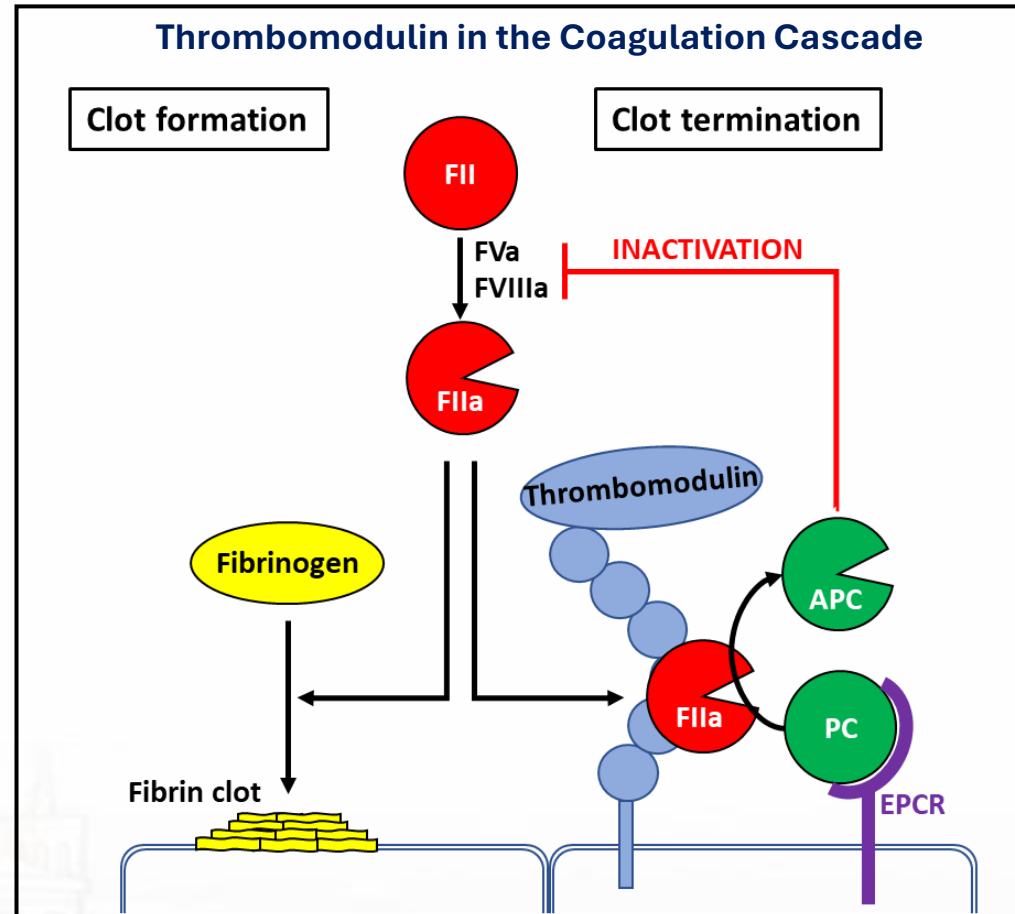


Functional study of **7 *THBD* variants** identified in families with a history of venous thromboembolism

Clara BERNARD – PhD student

Supervisor : Dr Frank PEIRETTI

The role of Thrombomodulin in the Coagulation



Thrombomodulin is at the center of the coagulation inhibition system

Identification of 16 *THBD* variants



CEHT Timone
Marseille

DVT/PE
<60 years

Negative
thrombophilia
screening

37-gene
NGS panel

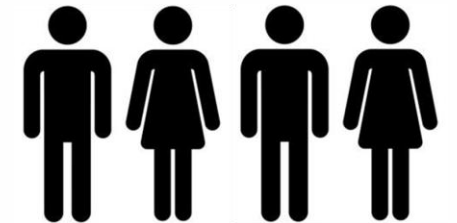
355 patients

12 *THBD* variants

P. Suchon et al., J Thromb Haemost.2025



PILGRIM



MARTHA

WGS

n = 400

2 *THBD* variants

2 *THBD* variants

Objective

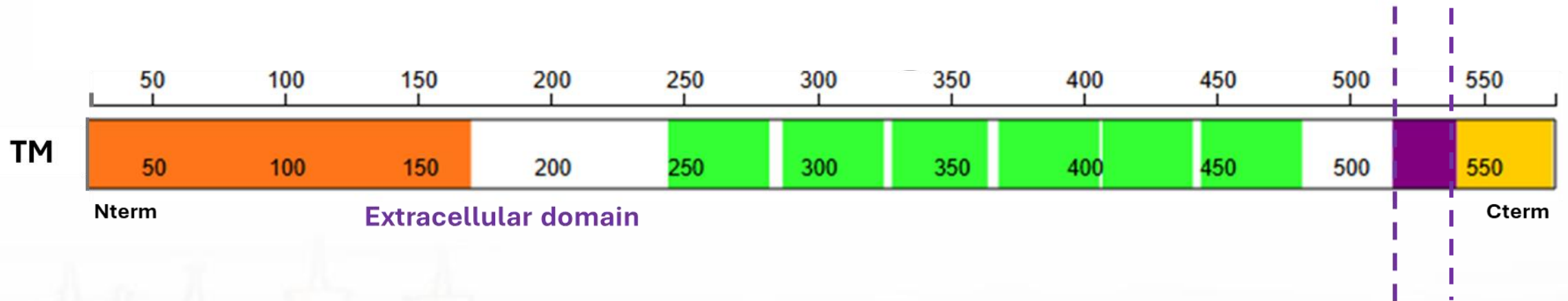
Evaluate the functionality of *THBD* variants

**Check the link between these variants and the risk
of venous thrombosis**

THBD variants

Variants identified at CEHT

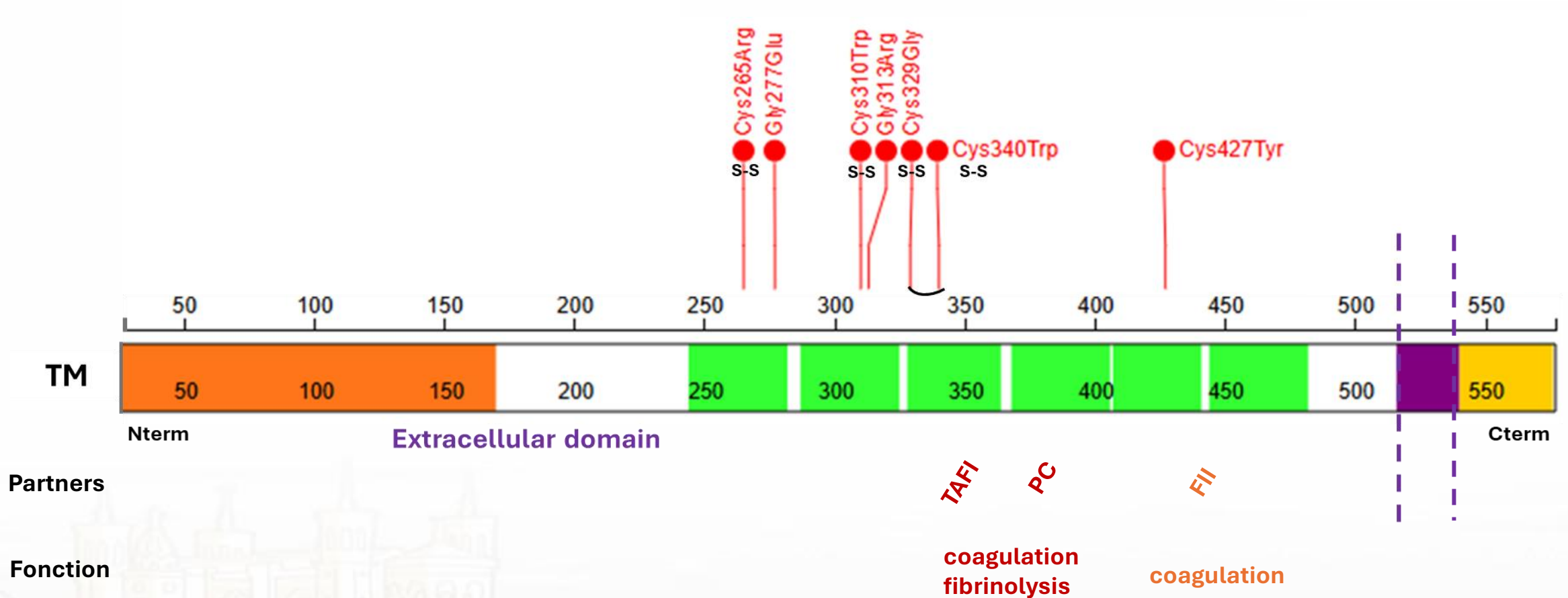
S-S Disulfide bond



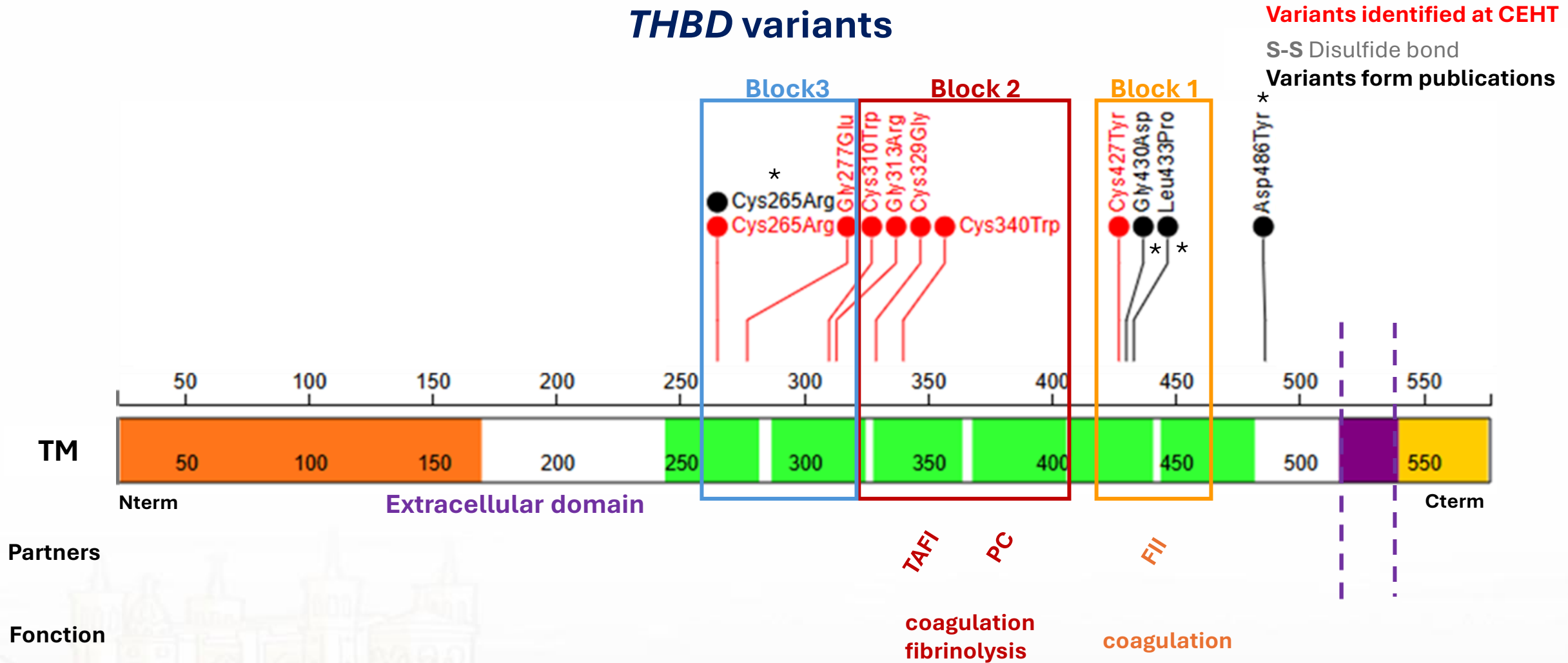
THBD variants

Variants identified at CEHT

S-S Disulfide bond



THBD variants



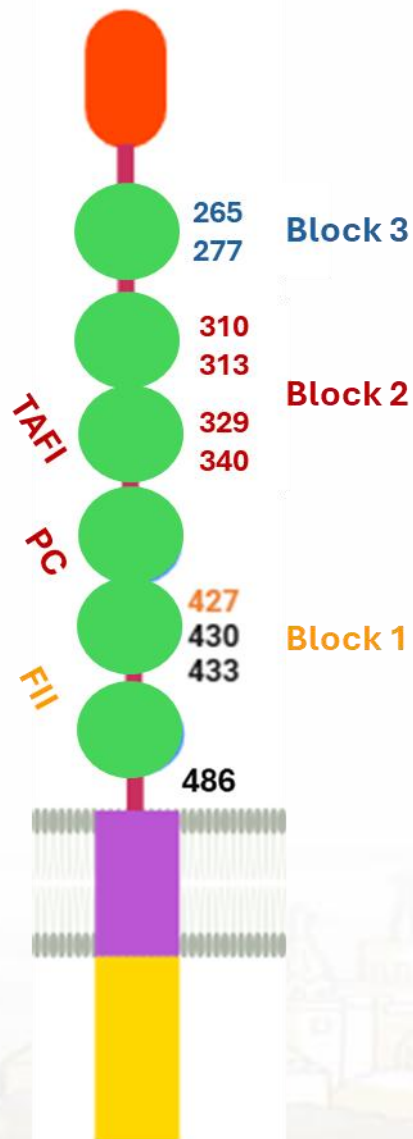
In silico and bioclinical characteristics

	Variant	Frequency in the general population (GnomAD)	ACMG class	Thrombosis	TMs (VN : 2-8 ng/mL)	ETP (TGT PPP 1pM)
Block3 EGF-1	Cys265Arg	UNK	3	Recurrent TVP	1,7	↑
	Gly277Glu	2,786.10 ⁻⁰⁶	3	TVP	3	Normal
Block2 TAFI PC	Cys310Trp	UNK	3	TVC	1,8	Normal
	Gly313Arg	UNK	3	Recurrent TVP + EP	3	Normal
	Cys329Gly	UNK	3	Recurrent TVP	2,6	Normal
	Cys340Trp	UNK	3	TVP + EP	1,6	NA
Block1 FII	Cys427Tyr	UNK	3	Recurrent TVP + EP	2,3	Normal

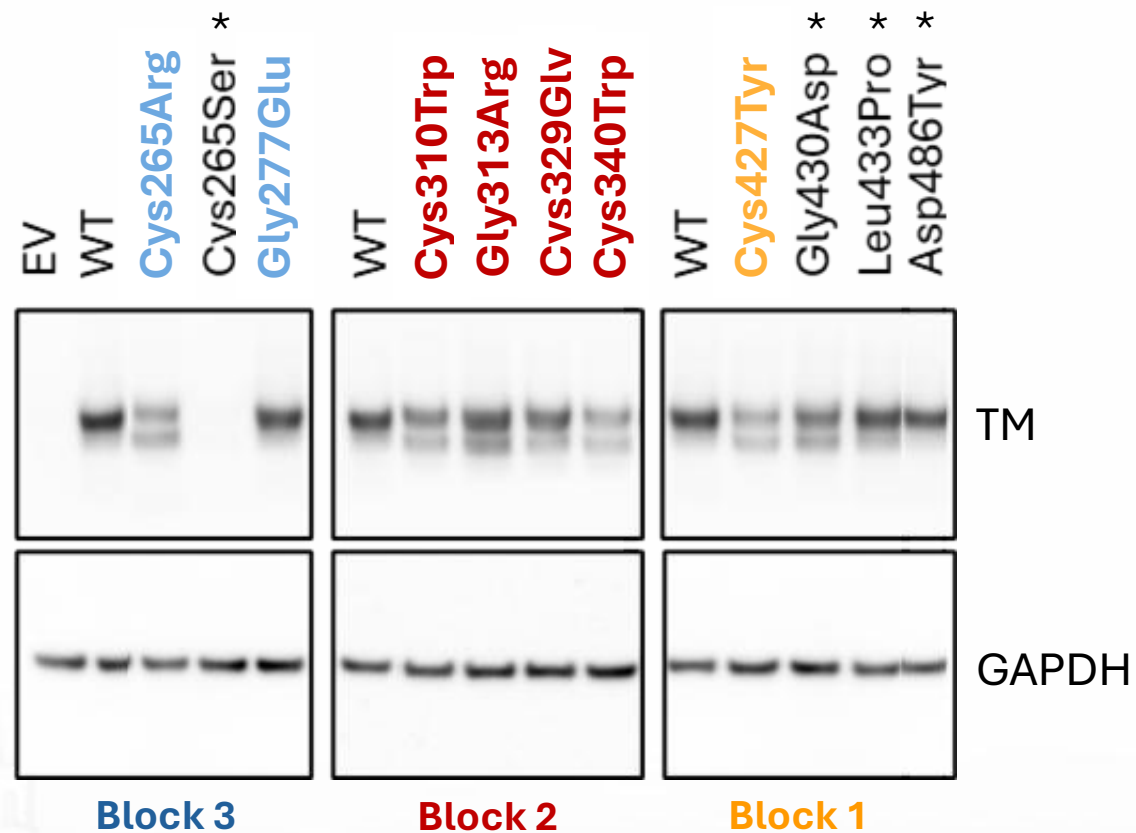
UNK : Unknown

Insufficient biological data to prove the causality of the variations

Need to continue investigations with *in vitro* studies

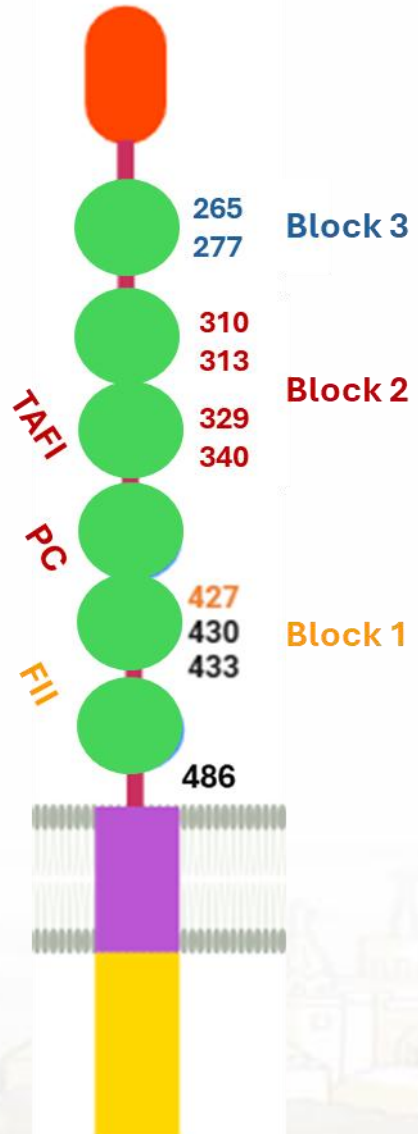


TM total cell expression

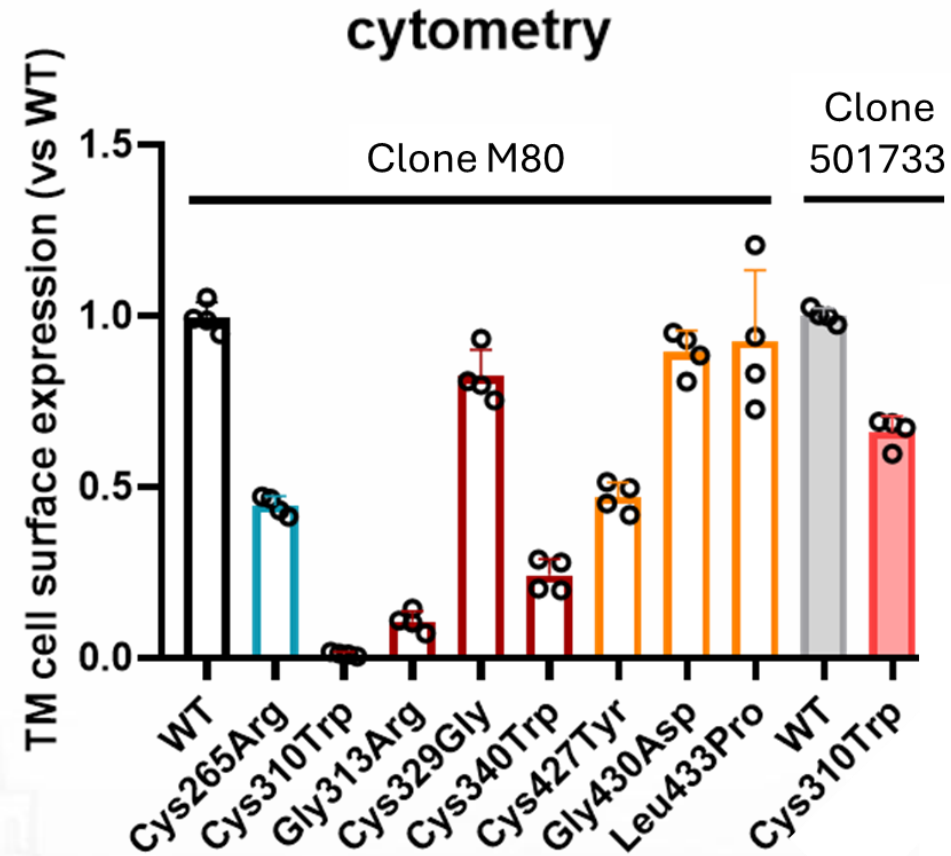


Some variants are expressed differently in lysates

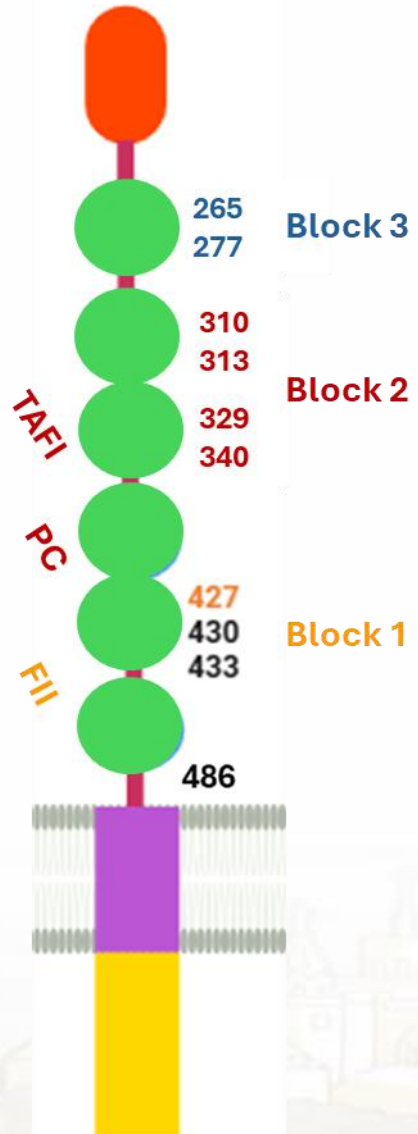
* Variants from publications



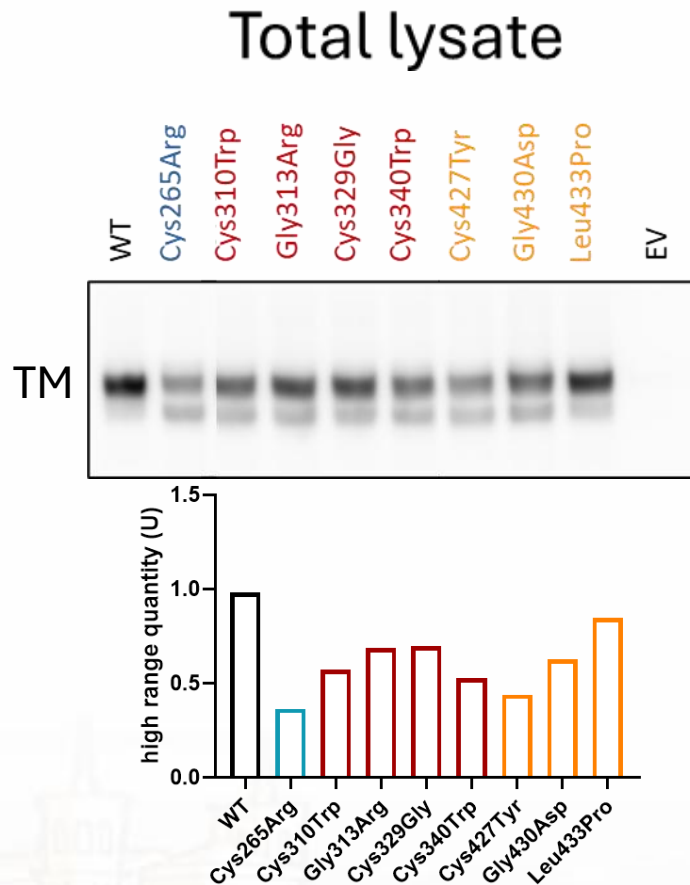
TM cell surface expression



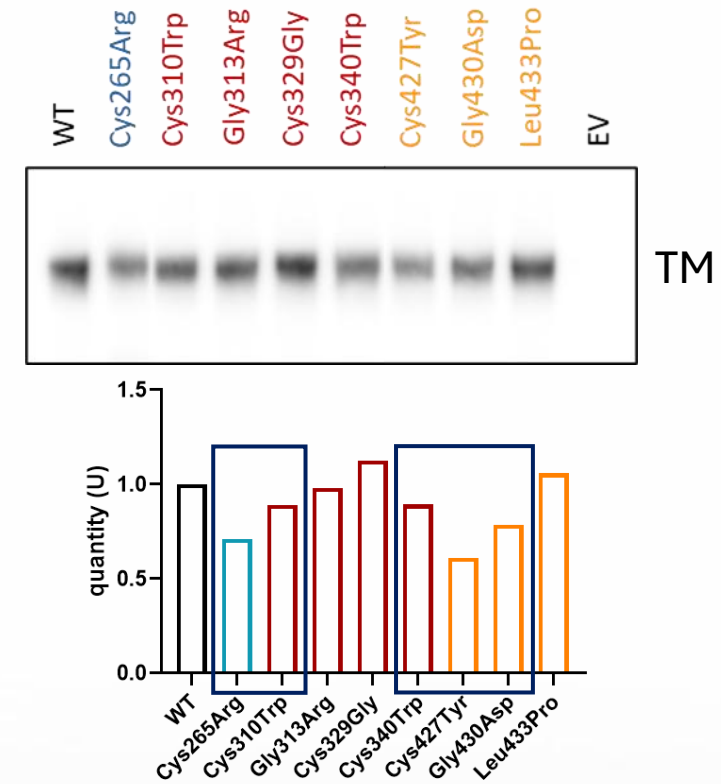
Possible impact of the variant on cytometry detection



TM cell surface expression



Surface biotinylation

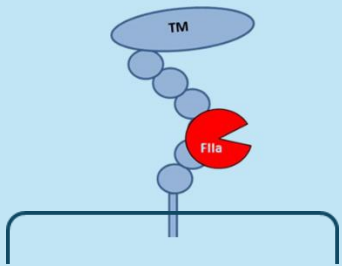


Top band in cell lysate $\approx$ TM cell surface expression

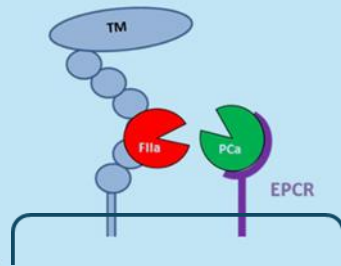
Functional investigations

On cell investigations

Binding FII on cell

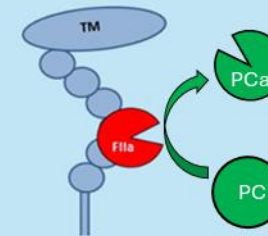


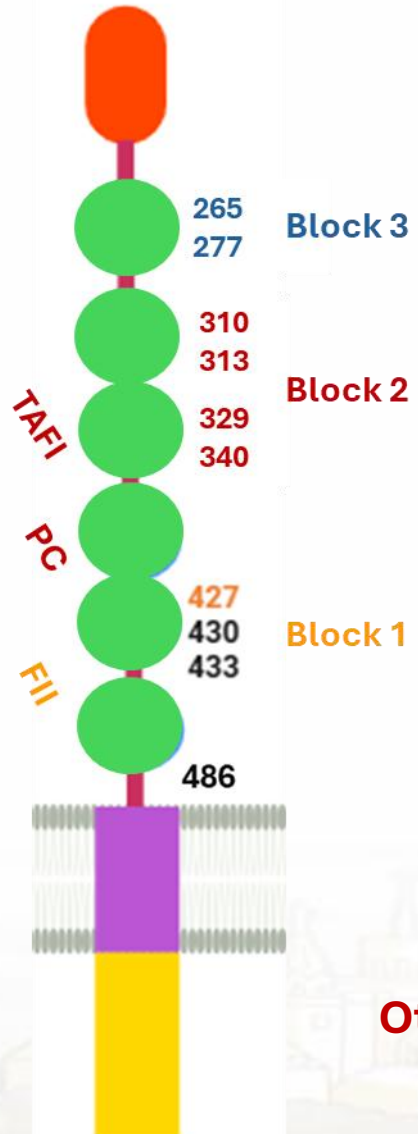
On cell PC activation



In vitro investigations

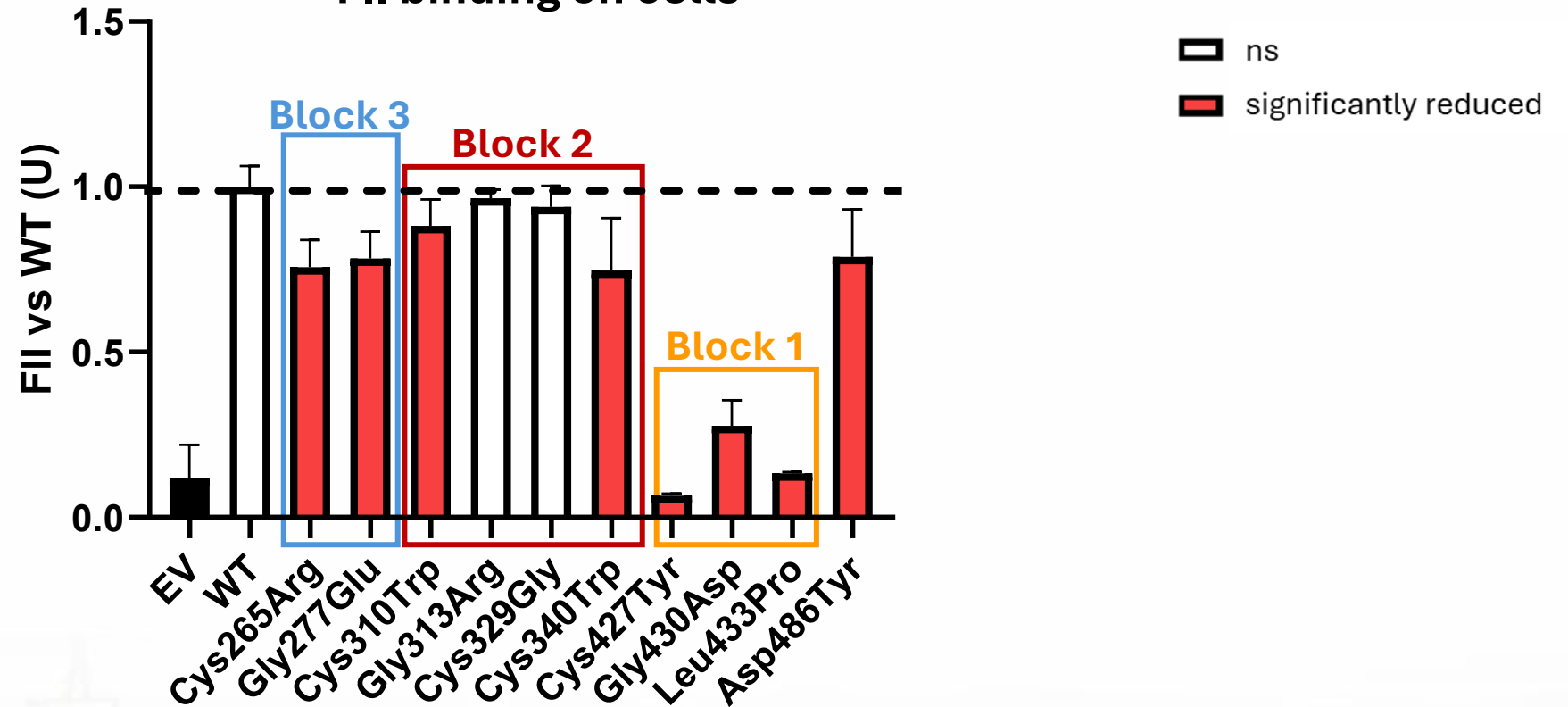
Suspended PC activation





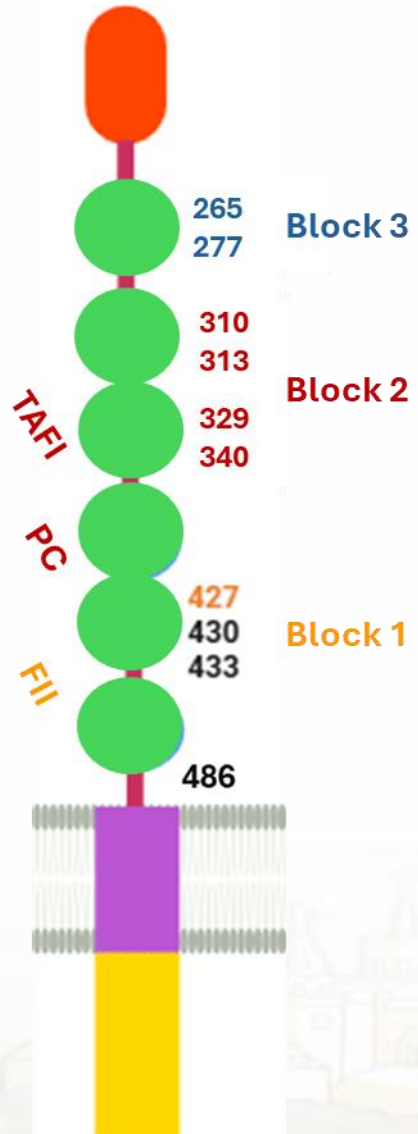
Study of the PC system on the surface of cells

FII binding on cells



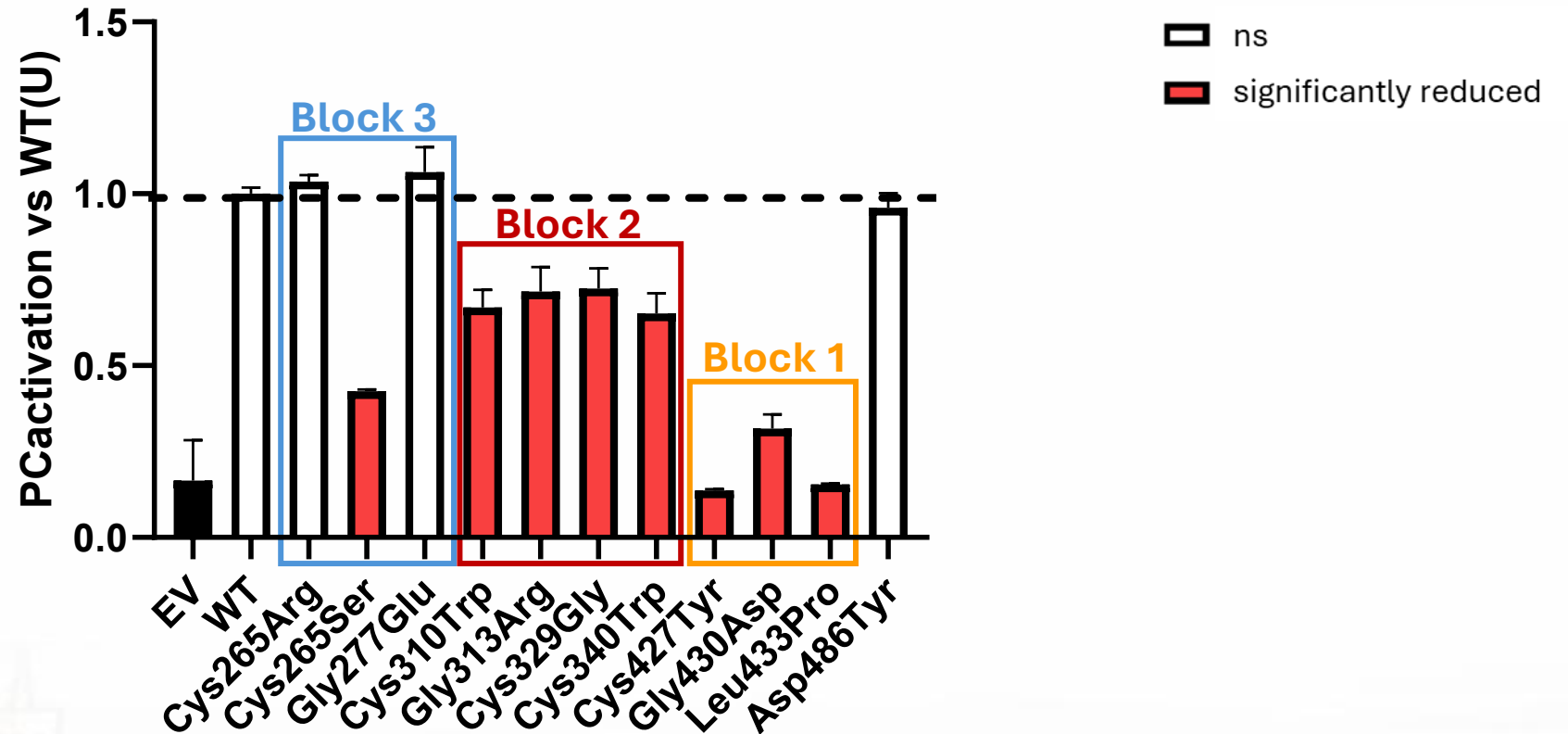
Block 1 variants : no thrombin binding

Others variants : decreased thrombin binding due to a lower surface expression ?



Study of the PC system on the surface of cells

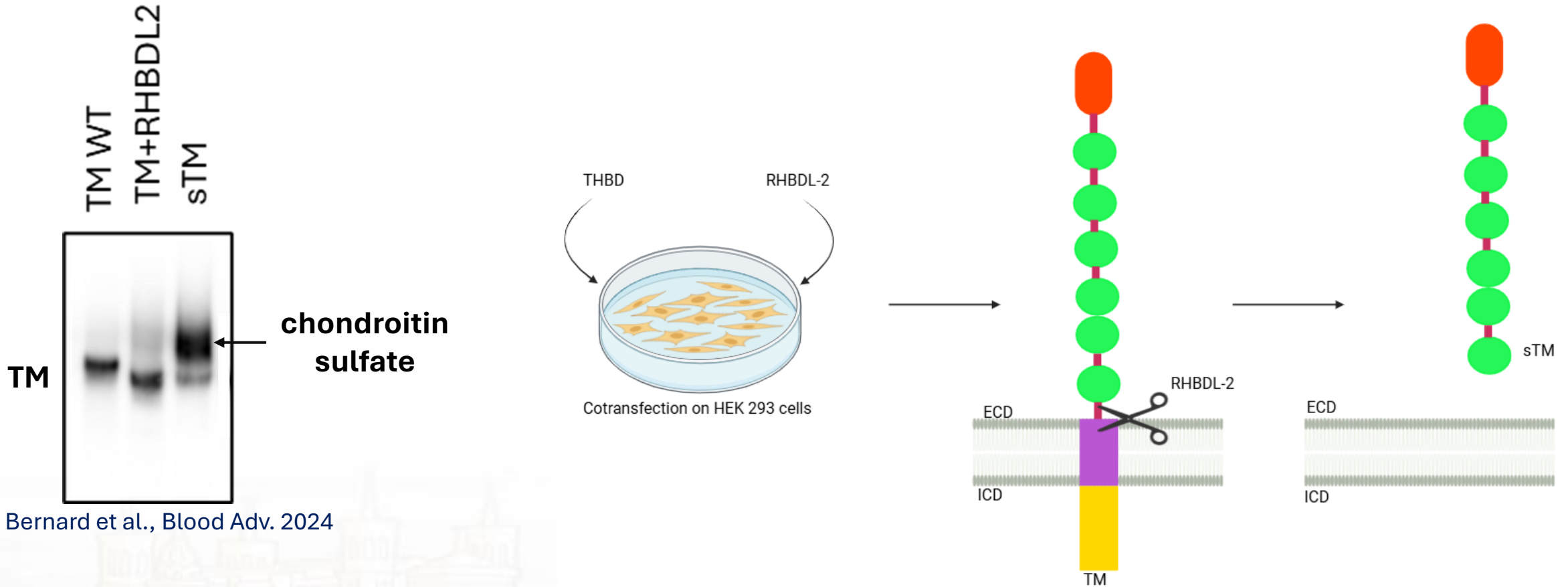
PC activation on cells



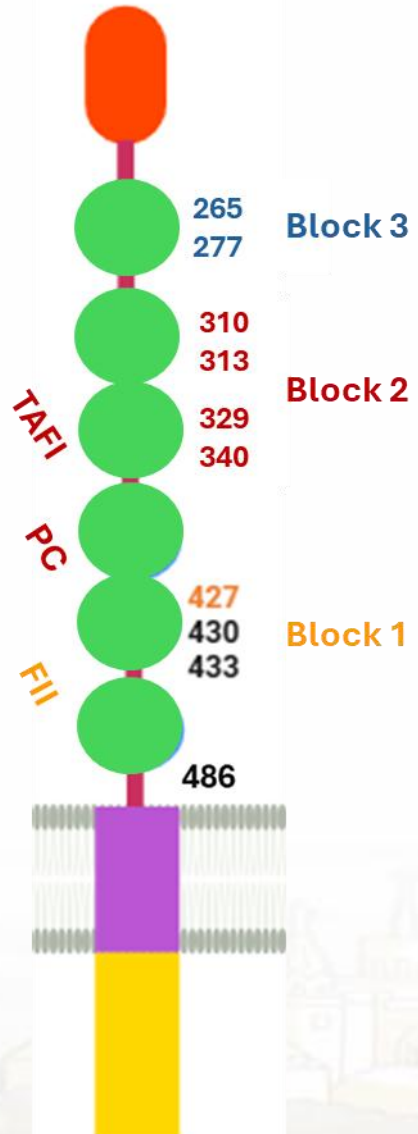
Block 1 variants : no thrombin binding → no PC activation

Block 2 variants : variants causing a functional defect

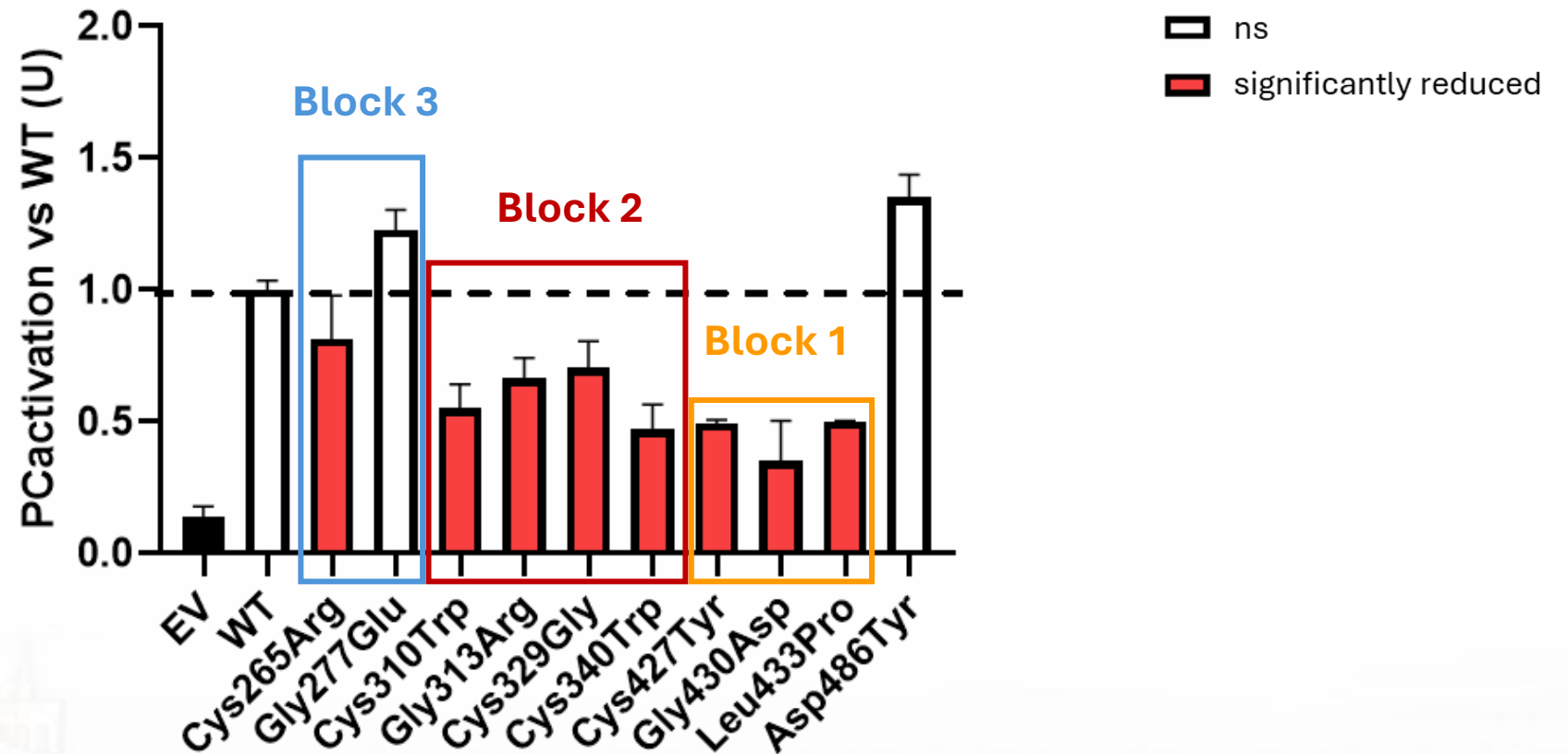
Production of soluble recombinant proteins



Bernard et al., Blood Adv. 2024



PC activation *in vitro*



Block 2 variants: PCa reduction. Not only due to a lower surface expression

Summary

	Variant	Total lysate	TM cell surface	FII binding	Pca on cell	Pca <i>in vitro</i>
Block3 EGF-1	Cys265Arg	2 bands	↓	↓	N	↓
	Cys265Ser*	↓↓	ND	ND	↓	ND
	Gly277Glu	1 band	ND	↓	N	N
Block2 TAFI PC	Cys310Trp	2 bands	↓	↓	↓	↓
	Gly313Arg	2 bands	=	N	↓	↓
	Cys329Gly	2 bands	=	N	↓	↓
	Cys340Trp	2 bands	↓	↓	↓	↓
Block1 FII	Cys427Tyr	2 bands	↓	↓	↓	↓
	Gly430Asp*	2 bands	↓	↓	↓	↓
	Leu433Pro*	2 bands	=	↓	↓	↓
	Asp486Tyr*	1 band	ND	↓	N	N

N : Normal; ND : Not determined

* Variants from publications

Conclusion

Identification of 16 variations on the *THBD* gene

Study of 7 variants present in EGF-like domains

Quantitative and qualitative TM protein defect for 3 of them:

-decreased surface expression

-impact of PC activation

Variant	Total lysate	TM cell surface	FII binding	Pca on cell	Pca in vitro
Cys310Trp	2 bands	↓	↓	↓	↓
Cys340Trp	2 bands	↓	↓	↓	↓
Cys427Tyr	2 bands	↓	↓	↓	↓

Reclassification of these variants as class 4 (ACMG)

Thank you for your attention

Acknowledgments

Supervision: Dr. Frank Peiretti, Pr. Pierre-Emmanuel Morange

Laboratoire C2VN, Inserm 1263 :

Camille Roze
Andrea Pin
Victoria Siles

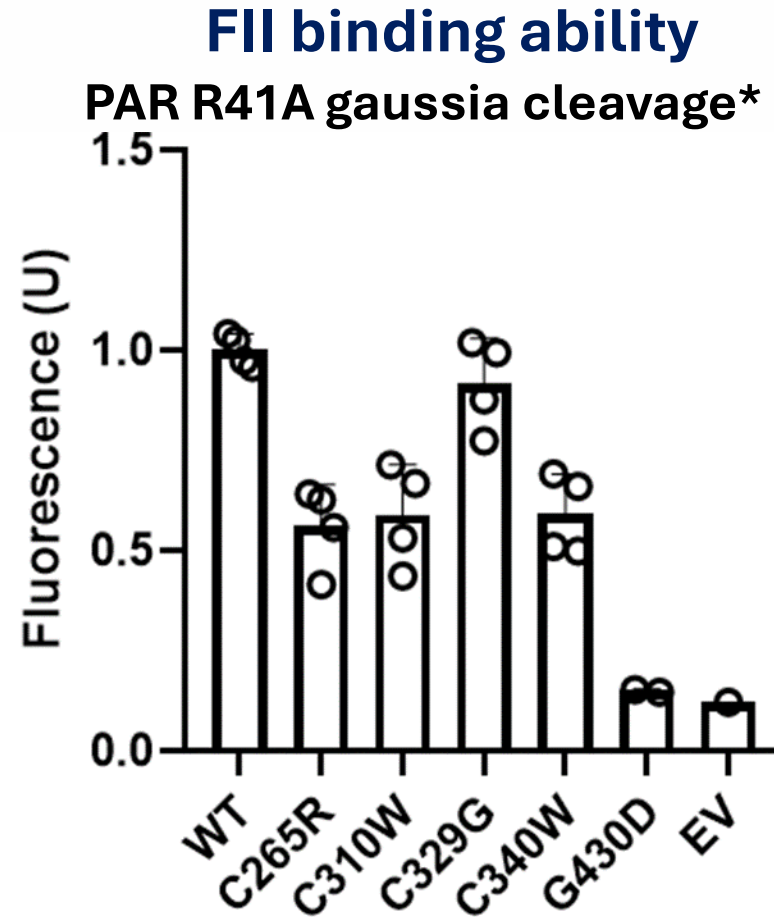
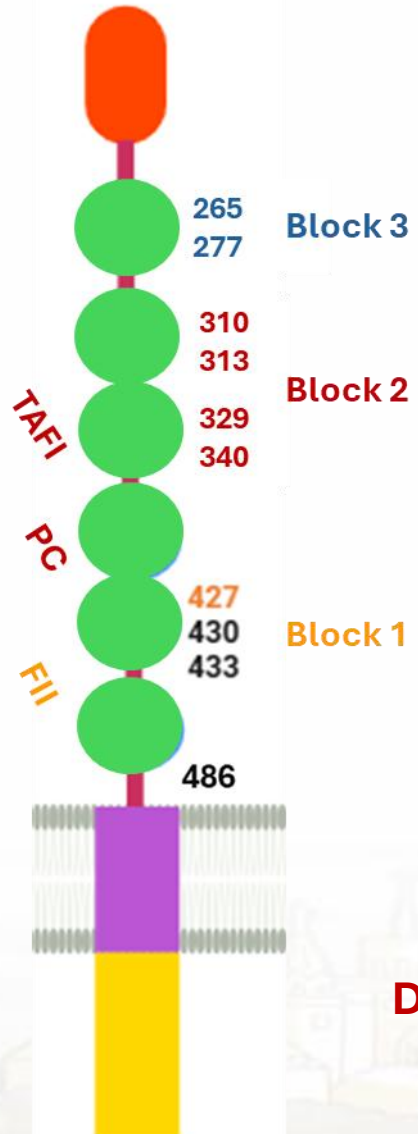
Laboratoire Hématologie, CHU Timone: Noémie Saut, Enora Tomei, Annie Vampiro

CEHT, Marseille : Marie-Christine Barthet, Vincent Ernest,
Manal Ibrahim-Kosta, Pierre Suchon

Lyon: Judith Catella

Annecy: Mathieu Bonnerot, Albane Simonnet





Decreased PAR cut by TM-FII complex with C265R, C310W and C340W variants.

* I. Biswas et al., J Biol chem. 2025

In silico characteristics

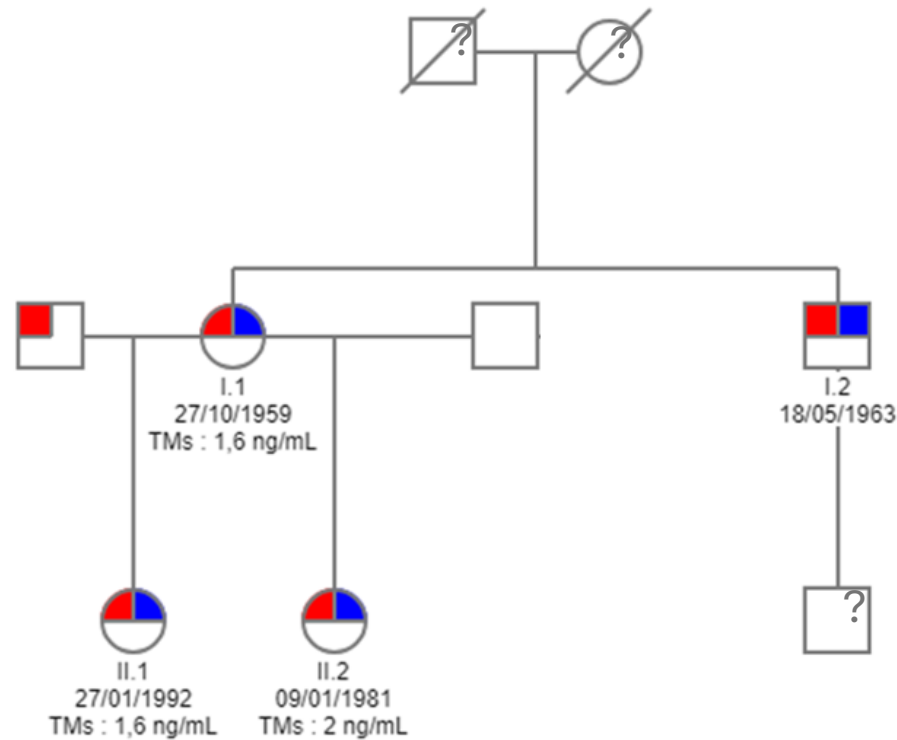
Variant	Frequency in the general population (GnomAD)	Clinvar/Intervar/Genetip classification	Pathogenicity prediction software	Publication	ACMG class
Cys265Arg	UNK	NC/VUS/LP	10/10 P	NA	3
Gly277Glu	2,786.10 ⁻⁰⁶	NC/VUS/VUS	4/10 P	NA	3
Cys310Trp	UNK	NC/VUS/LP	10/10 P	NA	3+
Gly313Arg	UNK	NC/VUS/LP	10/10 P	NA	3+
Cys329Gly	UNK	NC/VUS/LP	10/10 P	NA	3+
Cys340Trp	UNK	NC/VUS/LP	10/10 P	NA	3+
Cys427Tyr	UNK	NC/VUS/LP	10/10 P	NA	3+

UNK : Unknown ; NA : Not applicated

Bioclinical characteristics

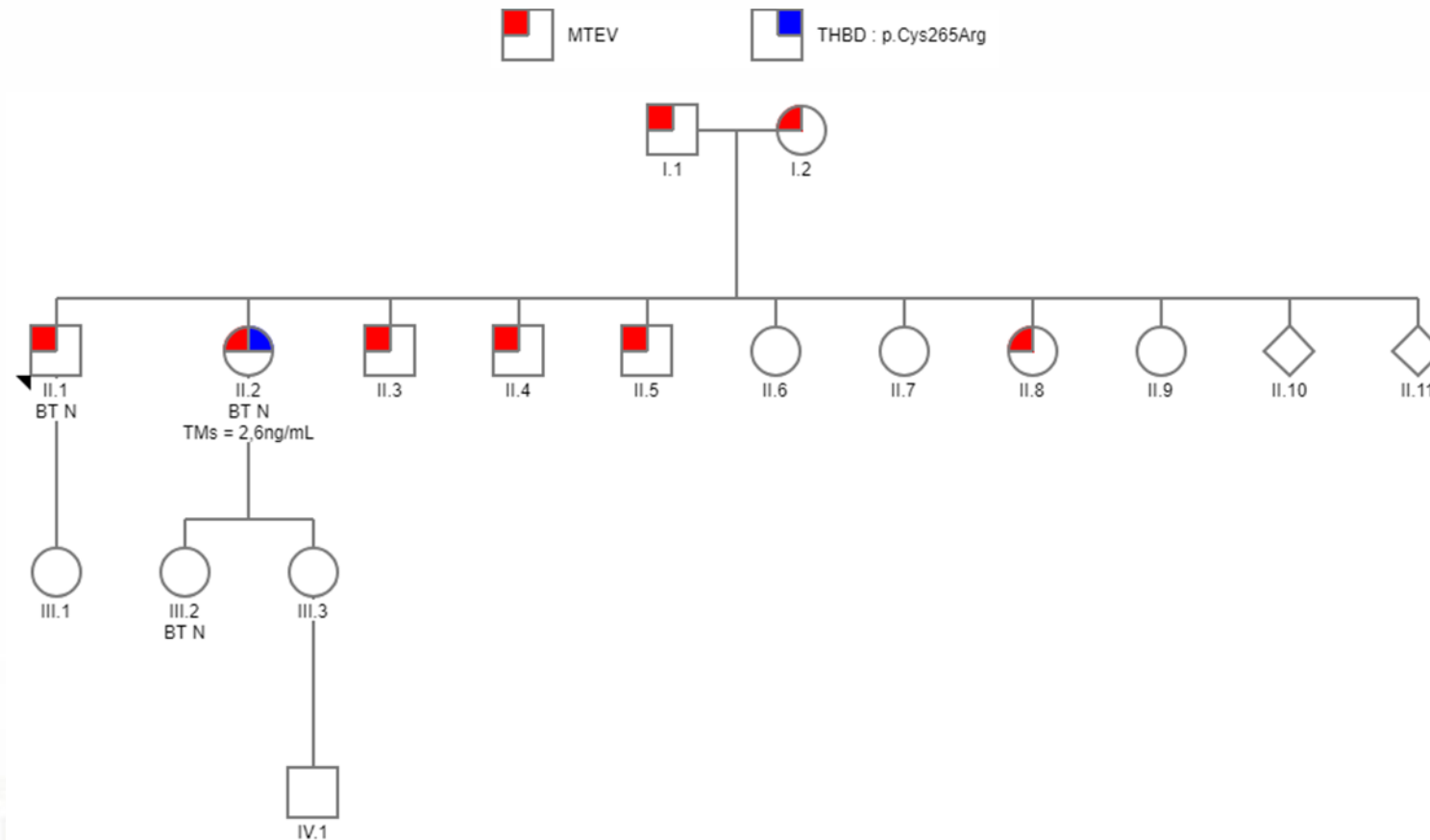
Variant	Thrombosis	Thrombophilia profile	TMs (VN : 2-8 ng/mL)	ETP (TGT PPP 1pM)	Family history	Family genetic explorations
Cys265Arg	Recurrent TVP	-	1,7	↑	Father, mother, 5/10 siblings : TVP	NA
Gly277Glu	TVP	-	3	Normal	Father : AVC	NA
Cys310Trp	TVC	-	1,8	Normal	Father : recurrent TVP	Father : Cys310Trp
Gly313Arg	Recurrent TVP + EP	-	3	Normal	Father : TVP	NA
Cys329Gly	Recurrent TVP	-	2,6	Normal	-	NA
Cys340Trp	TVP + EP	-	1,6	NA	Mother : TVP + EP Maternal uncle : EP Sister : TVS	Mother, maternal uncle, sister : Cys340Trp
Cys427Tyr	Recurrent TVP + EP	F II Leiden homozygous	2,3	Normal	-	Father, Mother, brother : FIIL htz

Focus on Cys340Trp family

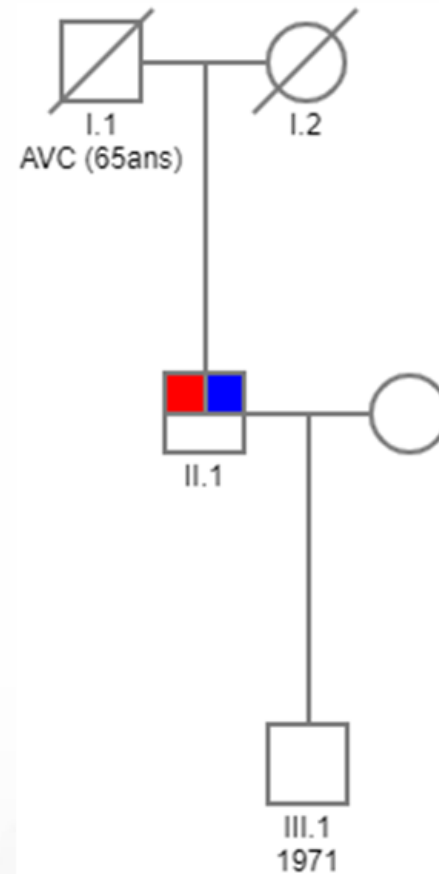


No plasma available to perform the thrombin generation test.

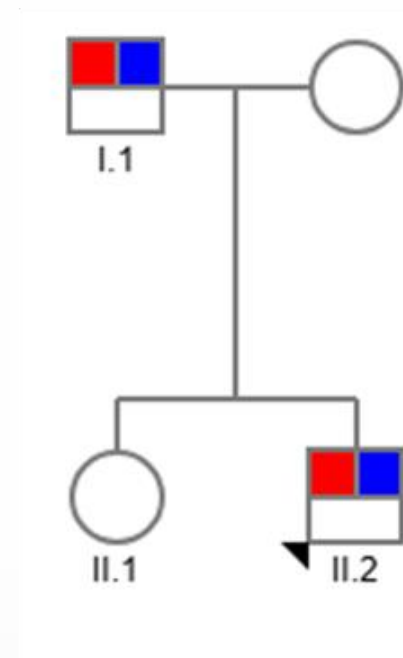
Focus on Cys265Arg family



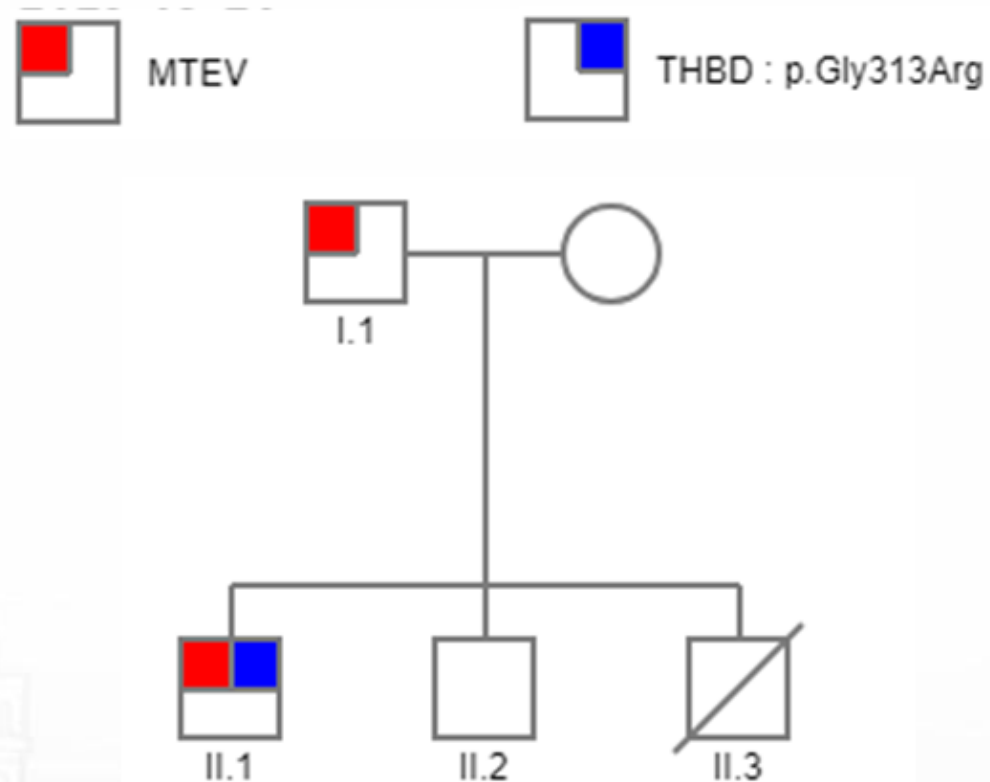
Focus on Gly277Ala family



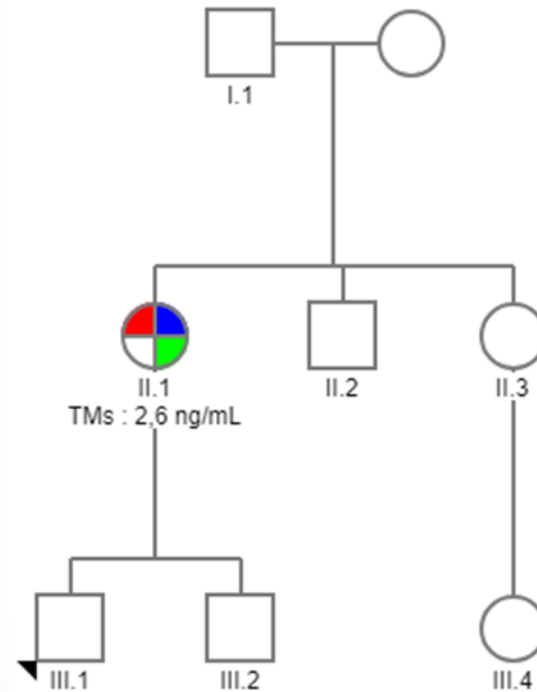
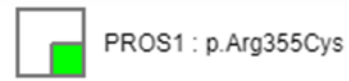
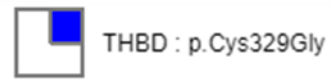
Focus on Cys310Trp family



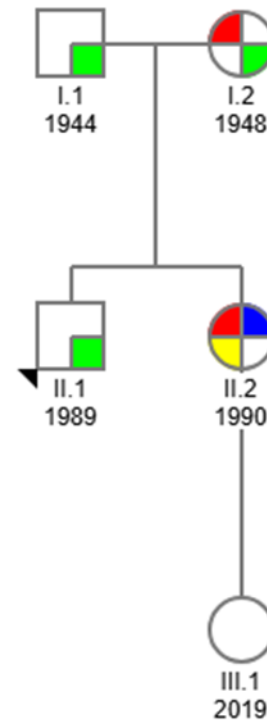
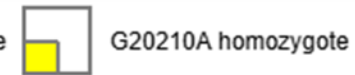
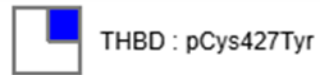
Focus on Gly313Arg family

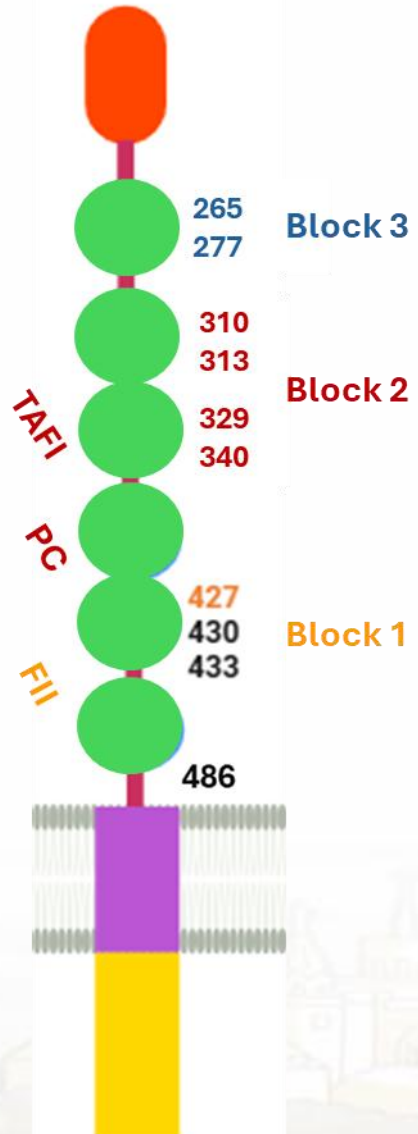


Focus on Cys329Gly family

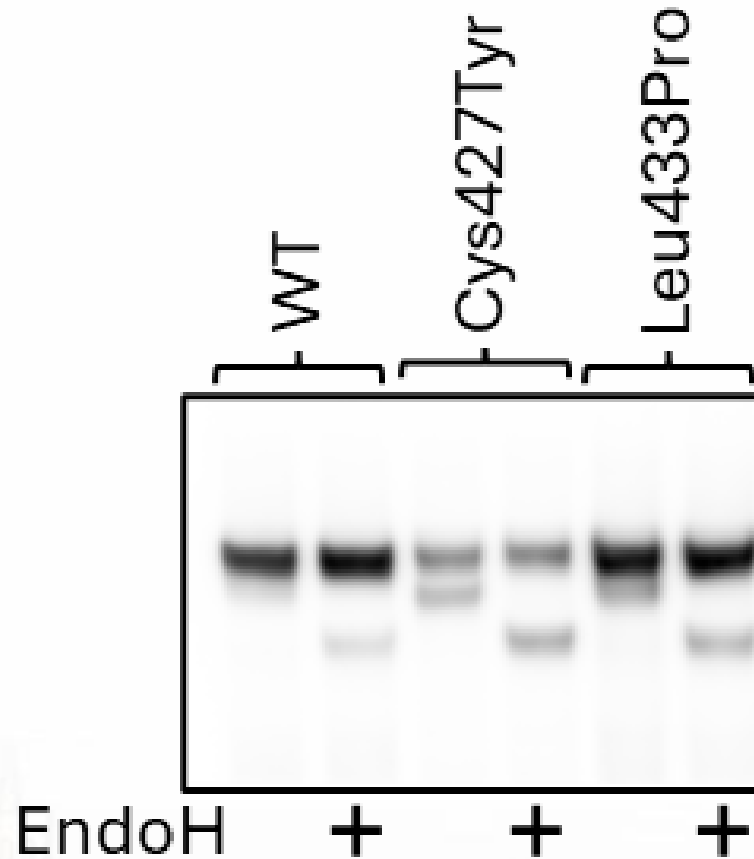


Focus on Cys427Typ family



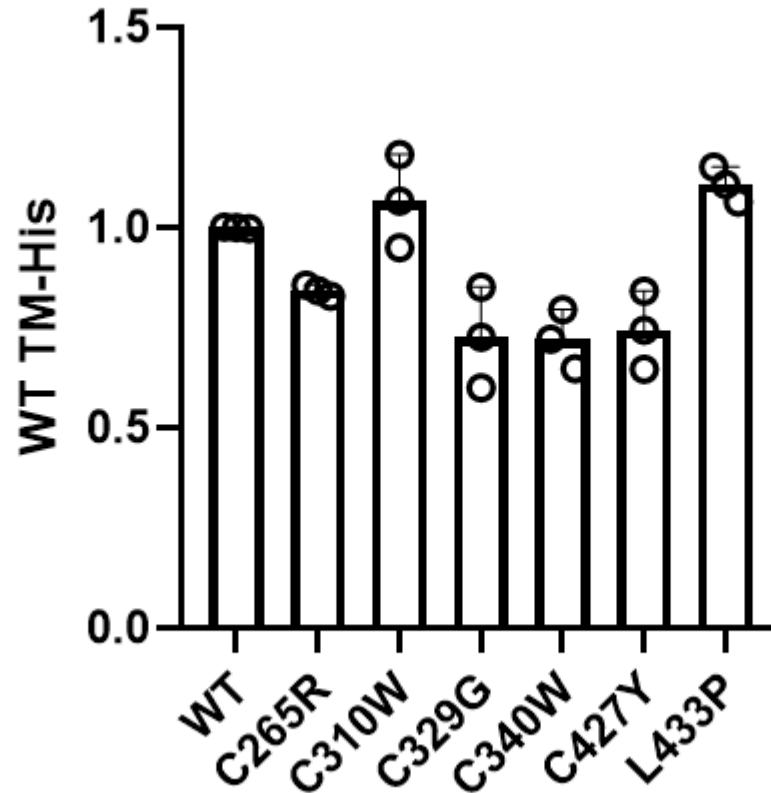
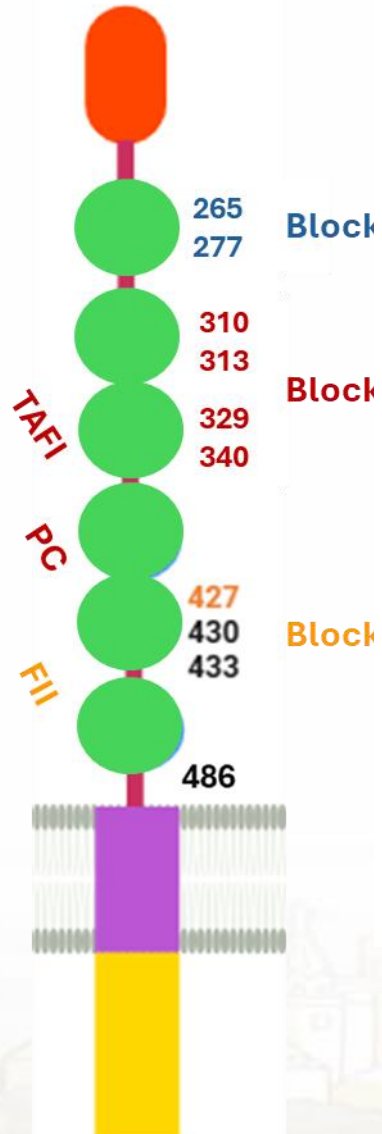


TM total cell expression



Cell retention for the form of the bottom band

Surface expression of wild-type TM as a function of variant TM



Decreased surface expression of wild-type TM by some variant