University of Virginia: Swineford 2024
A Brief MAST CELLS GONE CRAZY Tryptase

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Distinguished Professor of Medicine

Virginia Commonwealth University

Background

Mast cell activation (MCA) & anaphylaxis (ANA)

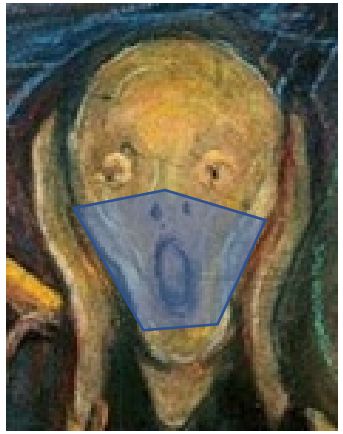
Acquired &/or inherited mutations ~ MCA

D816V-KIT & TPSAB1 CNV

MCA syndrome(s)

Vaccination-associated reactions

Disclosure Slide
Lawrence B. Schwartz, MD, PhD



Employment

- VCU/VCUHS-Self

Research Grants

- Blueprint Medicines, NIH

Consulting

- Blueprint Medicines, GLG, Celldex, Invea, Third Harmonic, Hycor, Jasper, TerSera

Speaker Bureau

- Blueprint Medicines

DSMB

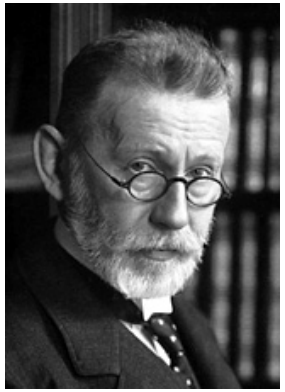
- Astra-Zeneca

Other Financial Interests

- VCU Royalties/Licensing Fees:
  ThermoFisher-Phadia (tryptase test); Millipore, Santa Cruz, BioLegend, Hycult Biotech (MC & BAS mAbs); Genentech (anti-tryptase inhibitor)
- Up-To-Date Card (royalties)
- Goldman-Cecil Medicine: Anaphylaxis (royalties)

Mast Cells (MCs) and Anaphylaxis

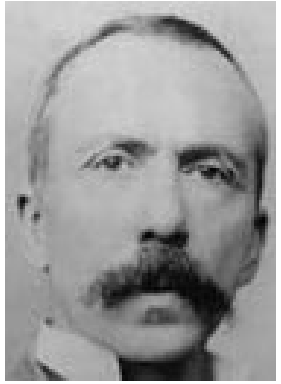
Paul Ehrlich



Nobel Laureate-Immunology, 1908
Royal Institute of Experimental Therapy, Frankfurt

Discovered & Named
Mast Cells

Charles Richet



Nobel Laureate-Anaphylaxis, 1913
Sorbonne University, Paris

Discovered & Named
Anaphylaxis

>50 years to realize mast cell activation causes anaphylaxis!

My story in translational research

1978: Introduced to **rat mast cells** (MCs) & the guinea pig ileum bioassay for histamine

"Necessity is the mother of invention"... Plato

1979/1981: **β-hexosaminidase**, MC degranulation biomarker *in vitro; replacing biassay*

J Immunol 123:1445-50, 1979 (rat MCs); J Immunol 126:1290-1294, 1981 (human MCs)

1981…: discovery/naming/cloning/expression/characterization of human **α & β tryptases**

J Biol Chem 256:11939-43, 1981; J Clin Invest 84:1188-95, 1989; 86:864-70, 1990; 97:988-95, 1996

1985…: **Anti-tryptase mAbs**, MC biomarker IHC (~200-fold >BAS); ghost MCs RIP

J Immunol 134:526-31, 1985; Proc Natl Acad Sci 83:4464-68, 1986; J Immunol 138:2184-89, 1987

1987: Serum **tryptase immunoassay** as a biomarker for mastocytosis and anaphylaxis

New Engl J Med 316:1622-126, 1987 …. MCAS & HαT, MC cytoreduction

1997/2006: B12 anti-tryptase mAb is a selective **allosteric tryptase inhibitor**

J Immunol 159: 3540-48, 1997; 176:3165-72, 2006

2019: **α/β-tryptase** heterotetramers, activators of PAR2 & EMR2

J Exp Med 216:2348-61, 2019

Clinical Criteria for Diagnosing Anaphylaxis

Brighton[1]	NIAID[2]	WAO[3]
• sudden onset AND rapid progression of signs and symptoms AND • involving multiple (≥2) organ systems		
• Dermatologic AND • Cardiovascular OR Respiratory OR GI OR acute serum tryptase	• Dermatologic OR • Cardiovascular OR • Respiratory OR • Gastrointestinal	• Dermatologic AND • Cardiovascular OR • Respiratory OR • Gastrointestinal
	OR after exposure to known allergen	
	• Hypotension alone	• Hypotension alone OR • Respiratory alone

1. https://brightoncollaboration.us/anaphylaxis-case-definition-pictorial-algorithm/ (3/2021)
2. Sampson H et al. JACI 117:391, 2006
3. Cardona et al. WAO J. 10.1016/j.waojou.2020.100472
4. Wood RA et al JACI **133**: 461-7, 2014

Adult lifetime prevalence[4] ~ 2-8%

But the differential diagnoses of such presentations might include…

Differential Diagnosis of Clinically-diagnosed Anaphylaxis

Stress-related response (vasovagal, panic, VCD)

Autonomic dysfunction (POTS, benign flushing)

Flushing disorders (carcinoid syndrome, neuroendocrine tumors)

Angioedema: Plasma prekallikrein activation → bradykinin (HAEI,II;AAE; HAEIII)

Complement activation: C3a, C5a

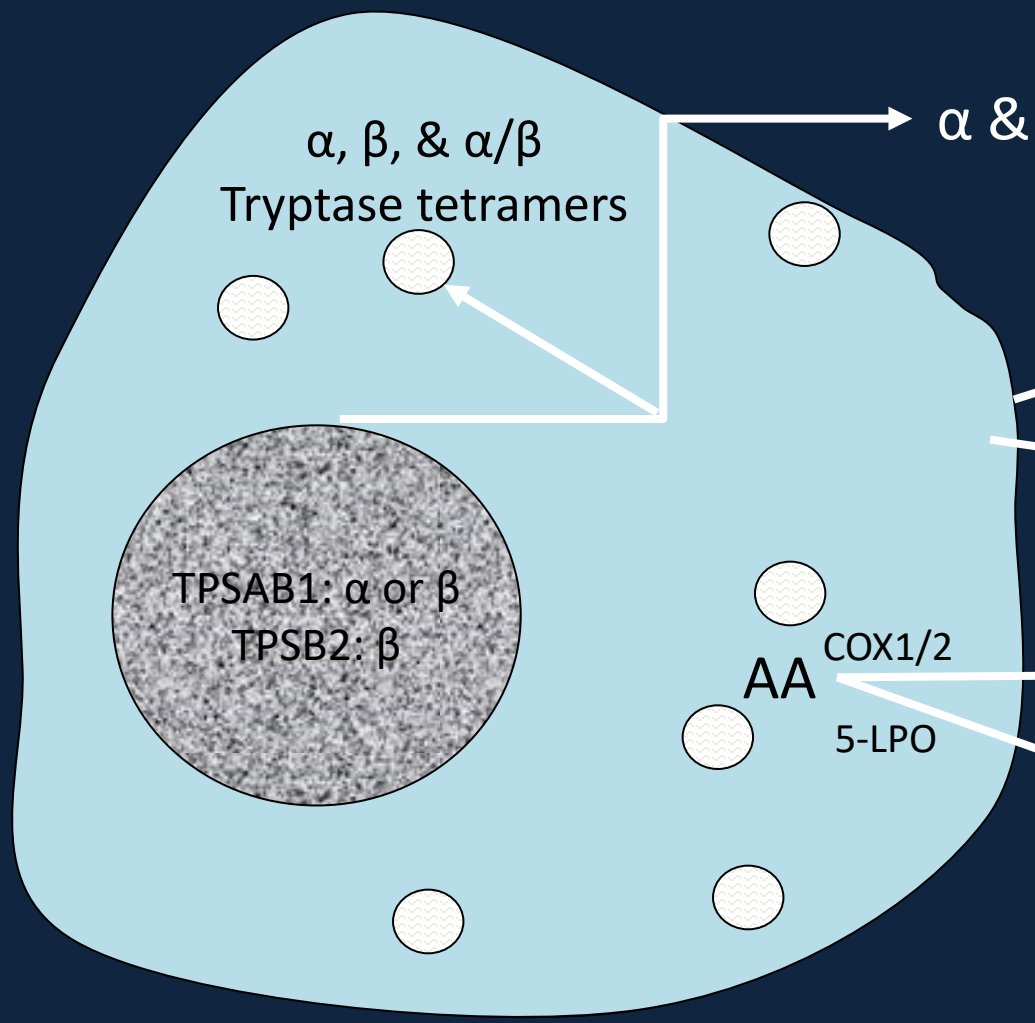
Scombroidosis: ingested histamine

Pulmonary/Cardiogenic disorders

Other shock syndromes (septic, toxins, …)

Can we be more precise with biomarkers?

Commercially-Available Biomarkers Secreted in Response to Mast Cell Activation



Total Tryptase

α & β Protryptase monomers (baseline)

Mature α, β, & α/β Tryptase tetramers (acute)

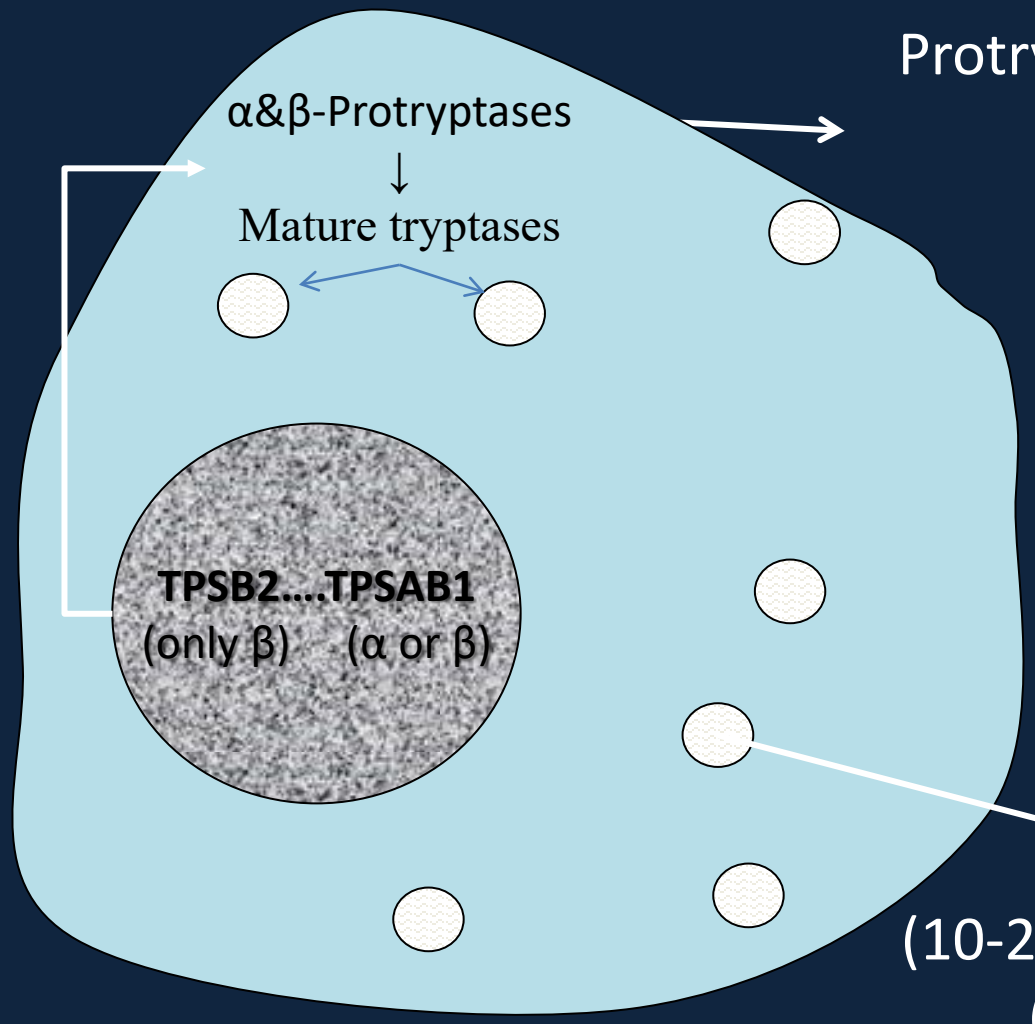
Histamine - - - - ▸ N-Methylhistamine

PGD_2 - - - - ▸ (±2,3-dinor-)11β-$PGF_{2\alpha}$

LTC_4 - - - - ▸ LTE_4

Weiler, C. R., et al. AAAAI Mast Cell Disorders Committee Work Group Report: Mast cell activation syndrome (MCAS) diagnosis and management. J Allergy Clin Immunol 144(4): 883-896, 2019.

Tryptase Secretion: tissues→serum
Unstimulated and Stimulated Mast Cells



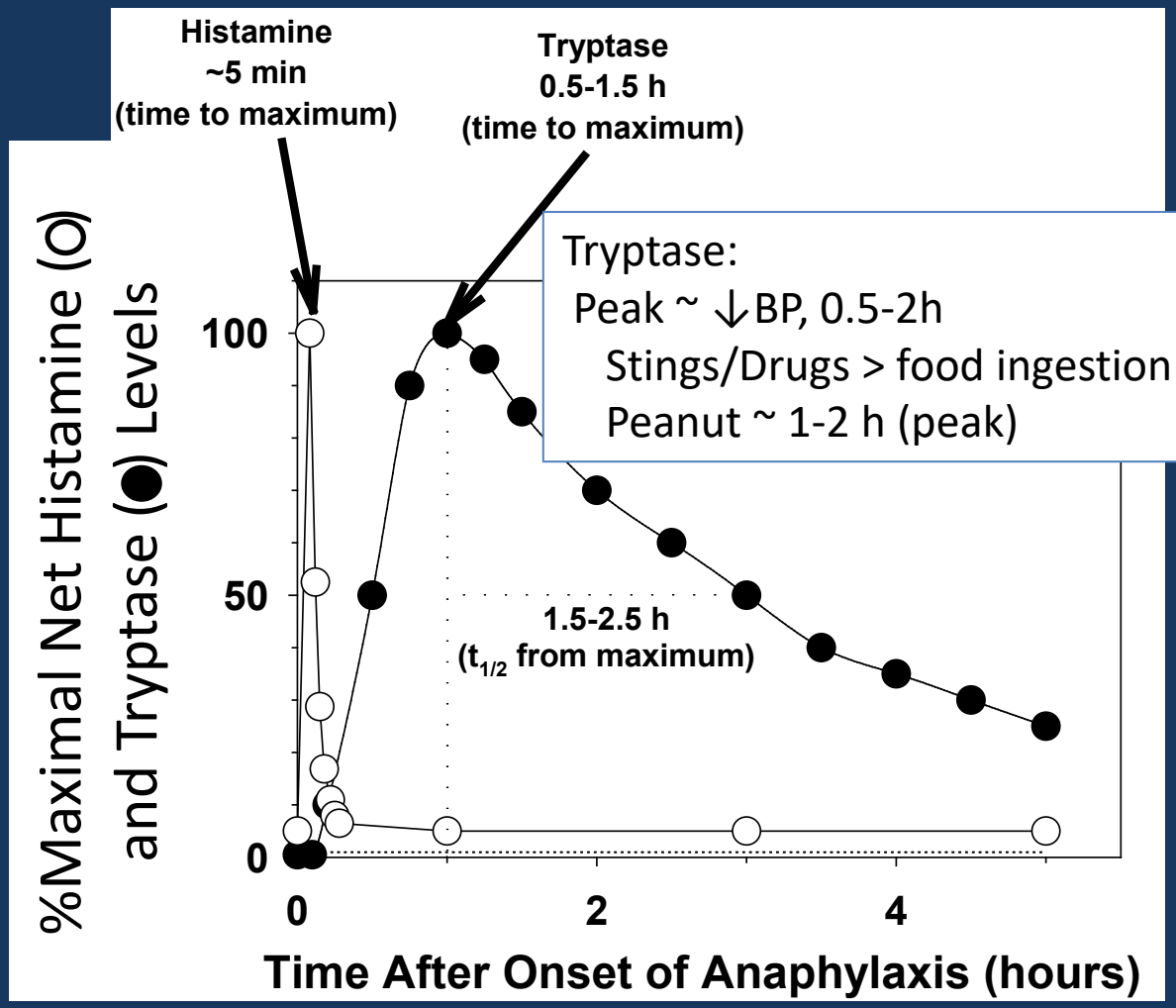
Protrypase(monomers): ≤11 ng/mL, median ~ 4 ng/mL (genetic)
(baseline serum tryptase (BST))
↑BST: HαT, RF, clonal myelocytic disorders (SM)

Heparin: Mature Tryptase (tetramers) ↑

- Anaphylaxis
- ECNM: AST> 2 + 1.2*BST
- NIH: AST > 1.685*BST
- (pro + mature = total tryptase)
- (AST, acute serum tryptase)

Mature Tryptase
(10-20% MC protein, <1% Bas protein)
(≥95% intracellular tryptase)

Net Tryptase (& Histamine) Levels in Serum (Plasma) During Insect Sting-Induced Anaphylaxis



Schwartz et al. J Clin Invest 83:1551, 1989

Clinically significant rise in tryptase:

AST > 2 + 1.2*BST

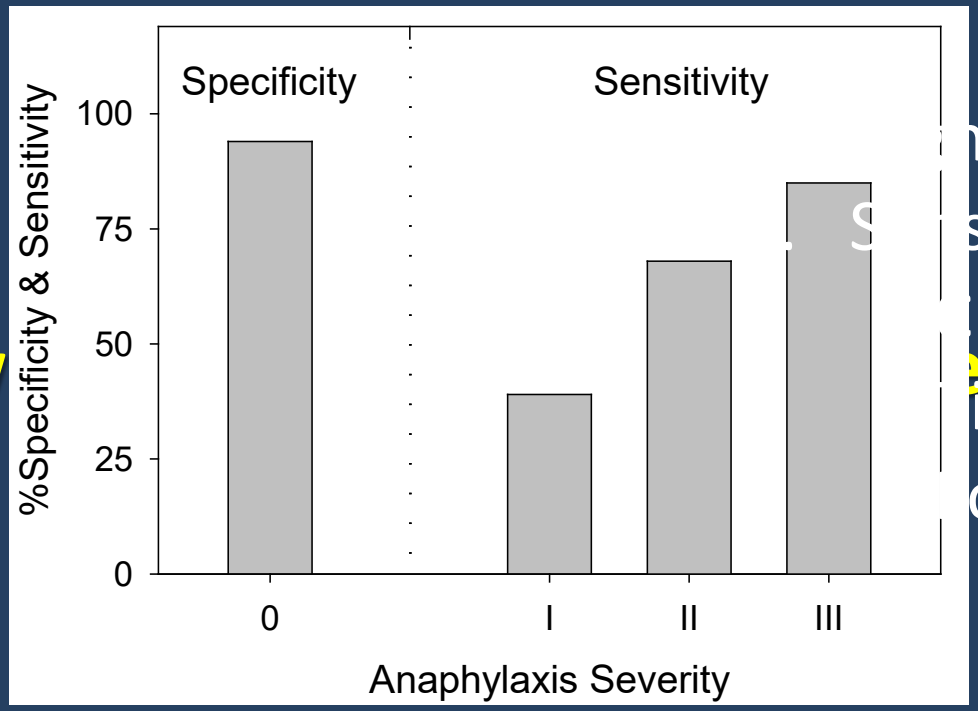
(regardless whether AST ≤ or > ULN)

BST: before onset or >24 h after all signs & symptoms of anaphylaxis have resolved

AST: collect 0.5-3 h after onset, ~1-2 h optimal

*Valent et al. Int Arch Allergy Immunol **157**:215-25, 2012;*
De Schryver et al. J Allergy Clin Imm 137:1138-42, 2016 (peds ED);
Baretto RL et al. Allergy 72:2031-4, 2017 (periop) ;
Valent et al. J Allergy Clin Immunol Pract 7:1125-33, 2019

So how erform?



n specificity (>90%)

S sitivity varies

linical severity ~↓BP

iming of collection

ood vs sting/drug

Overall Non-Mastocytosis PV

53% NPV

98% PPV

*Valent et al. Int Arch Allergy Immunol **157**:215-25, 2012: European Competency Network on Mastocytosis (ECNM)*

Case MI + ANA

56 y/o stung by an insect and soon c/o dizziness, dyspnea, and chest pain; underlying HBP (HCTZ, lisinopril), . ER: hypotensive

Acute: EKG: Inferior MI
Troponin: elevated
Tryptase =15 ng/ml

} Clinically did well with MI-appropriate RX

Baseline (1 month later): Tryptase =4 (15 > 2+1.2x4=6.8)
Venom IgE skin test: positive

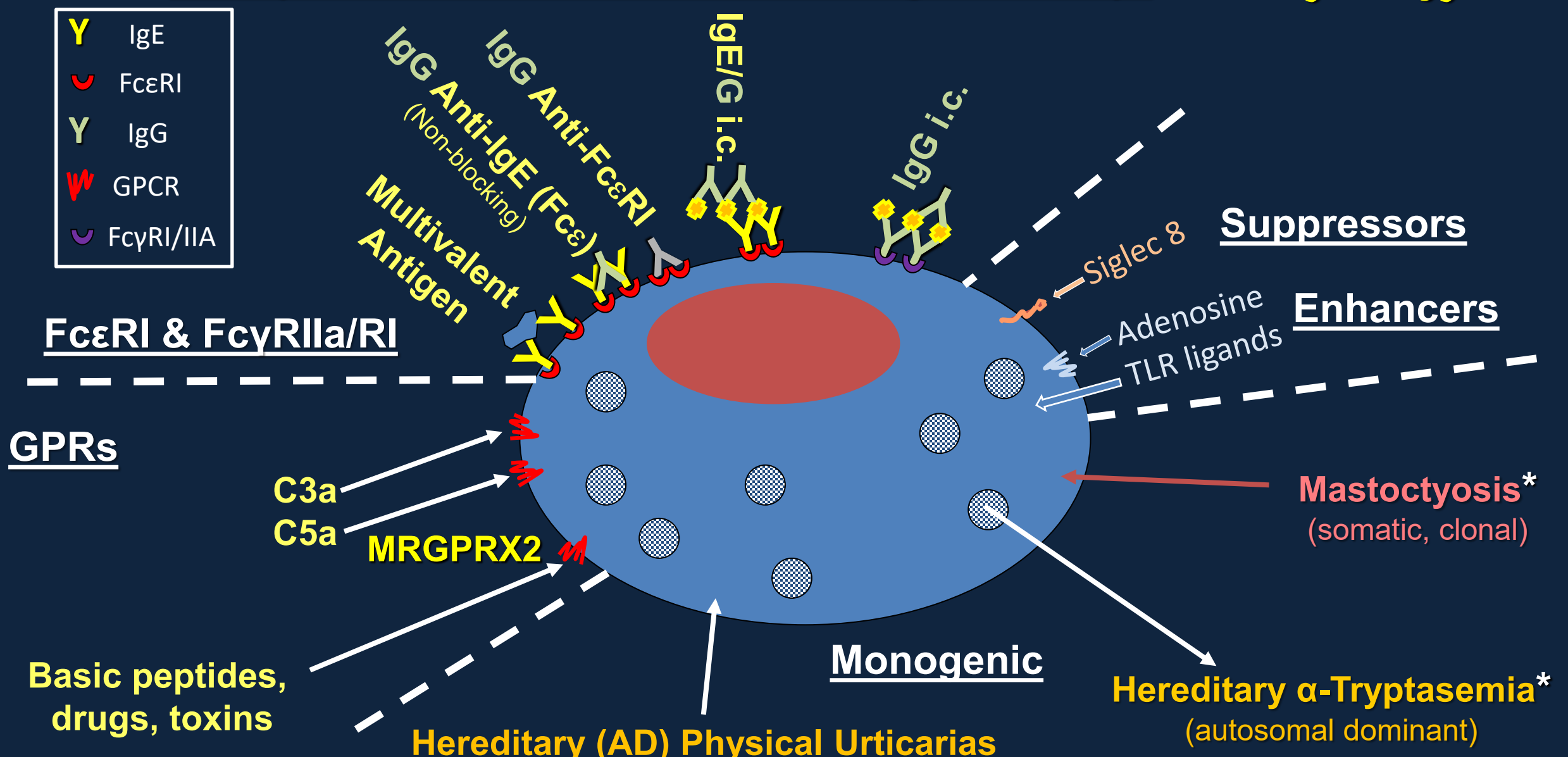
Systemic anaphylaxis to venom, which precipitated the MI.
Begin venom immunotherapy (↓risk of ANA after future stings >95%)
Consider underlying clonal or hereditary MC disorder

Acquired or inherited mutations associated with non-IgE (and IgE) dependent ANA?

D816V-KIT

TPSAB1 CNV

Receptors for Mast Cell Activating Pathways: not only allergy!



*, to be discussed

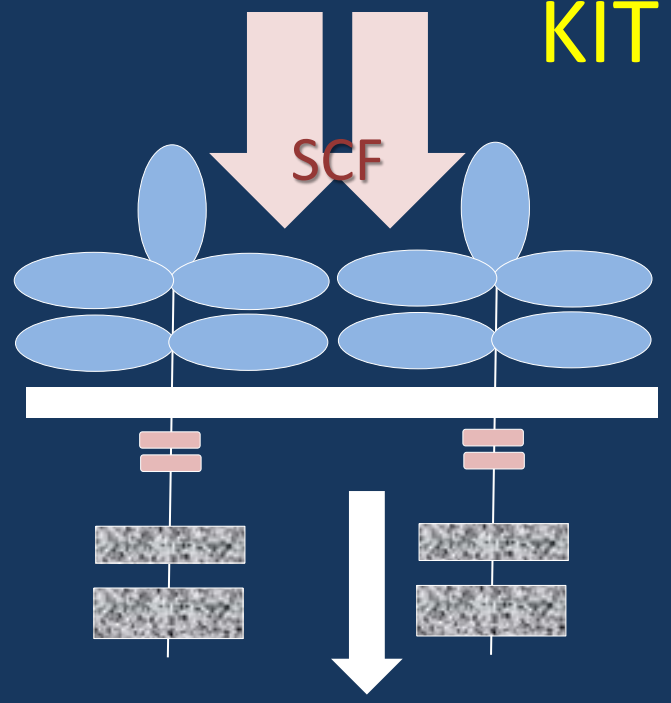
Case SM

45 y/o M: spontaneous episodes of anaphylaxis. PMH: severe anaphylaxis to wasp sting. FH: negative → suspicion of SM (40-50% prevalence of ANA)

Serum baseline tryptase (sBT) =60 ng/mL (<12); acute =95 ng/mL (>2+1.2*60=74)
D816V c-*KIT*+ (PB allele-specific PCR)

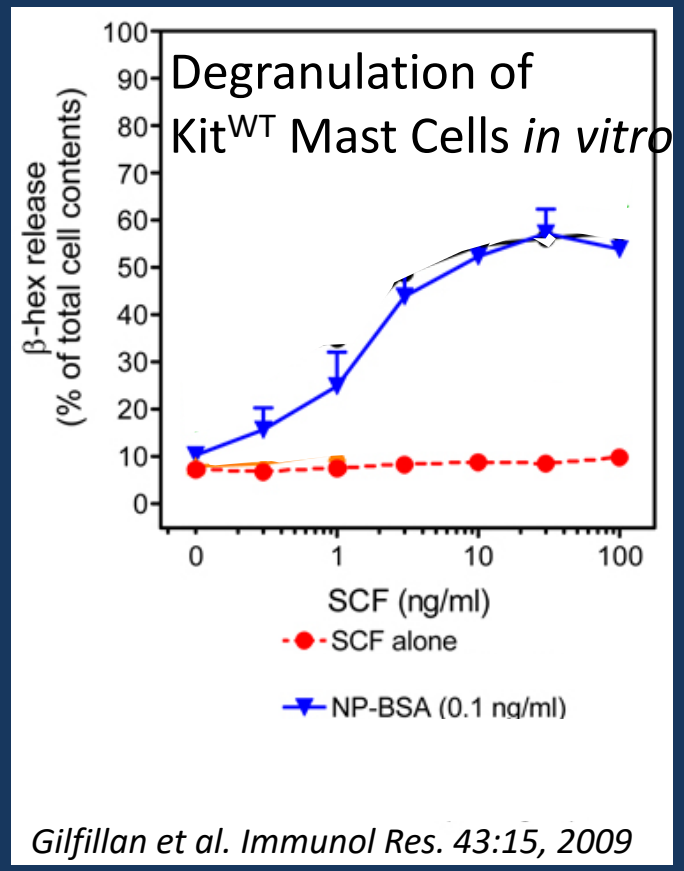
Systemic Mastocytosis (SM, WHO criteria)
Major Criterion: MC Aggregates (BM bx, >15 MC/hpf)
Minor Criteria (1) Activating c-*KIT* mutation* (2) Baseline serum tryptase >20 ng/ml*† (3) CD25$^+$, 2$^+$, or 30$^+$ MC† (4) Abnormal MC morphology (e.g., spindle)† *, peripheral blood or BM; †, not if other cause is present
Diagnosis: 1 major + 1 minor OR ≥3 minor

KIT and MC Biology/Pathobiology



Functionally:

1. MC differentiation
2. MC survival
3. MC activation



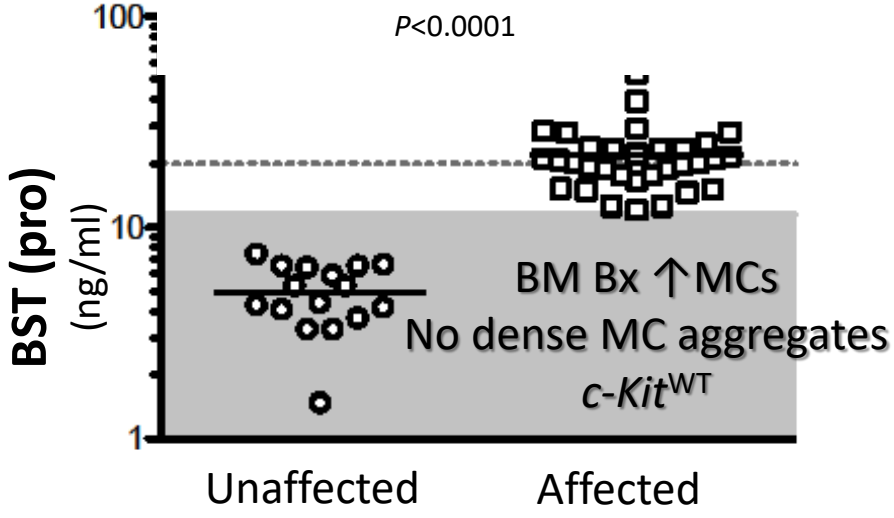
Gilfillan et al. Immunol Res. 43:15, 2009

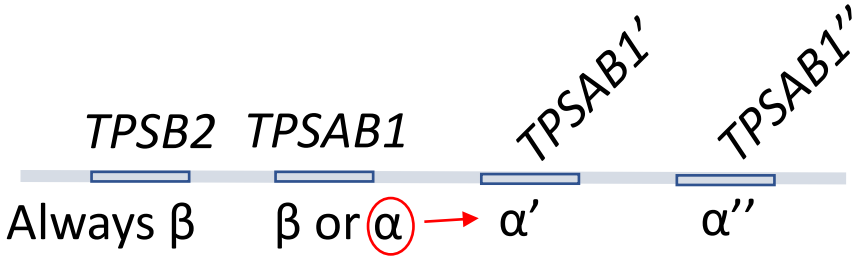
D816V *KIT*:

1. Active monomers, SCF-independent
2. Indicates MC clonality.
3. Minor criterion for diagnosis of systemic mastocytosis.
4. ↑**Risk for anaphylaxis** (severe spontaneous & insect sting triggered)

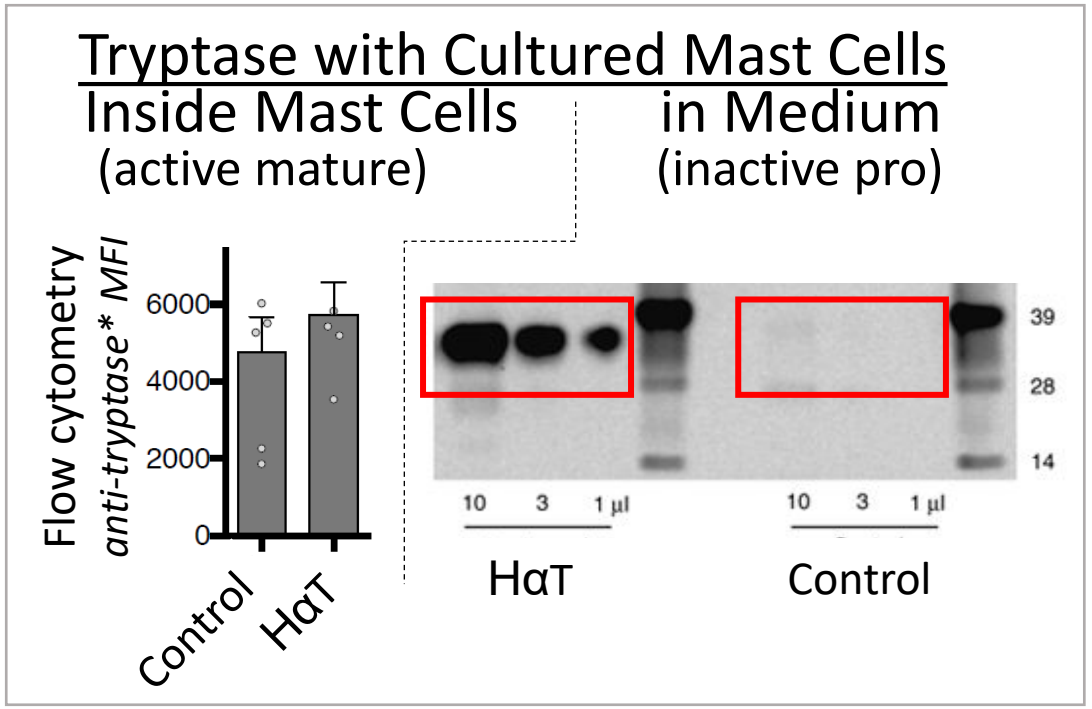
Hereditary α-Tryptasemia

(Jon Lyons et al 2014 AD ↑BST (6% European, <1% African/Asian); 2016 genetic trait)





TPSAB1 Genotype: *~80% c̄ 1 extra copy*
~15% c̄ 2 extra copies



Flushing/Pruritus/Hives (vibratory challenge)

Anaphylaxis

HαT⁺: 10% severe insect sting-triggered ANA

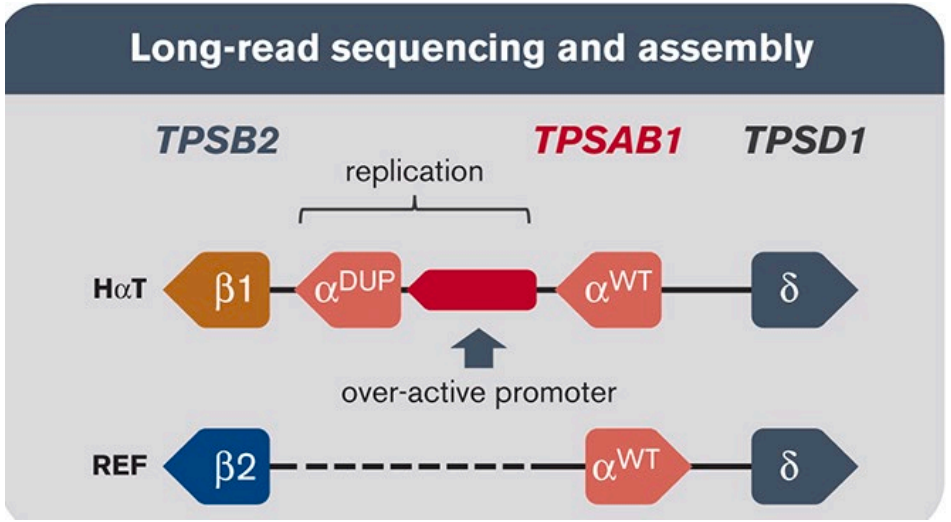
HαT⁺: 12-18% SM (↑ANA risk)

HαT⁺: 14% Idiopathic ANA s̄ cMCD

Lyons et al. JACI 133:1471-4, 2014; Nat Genetics 48:1564-9, 2016; JACI 147, 622-32, 2021

Genetically-defined individual reference ranges for BST ULN

Chovenac et al. Blood Adv, 2023

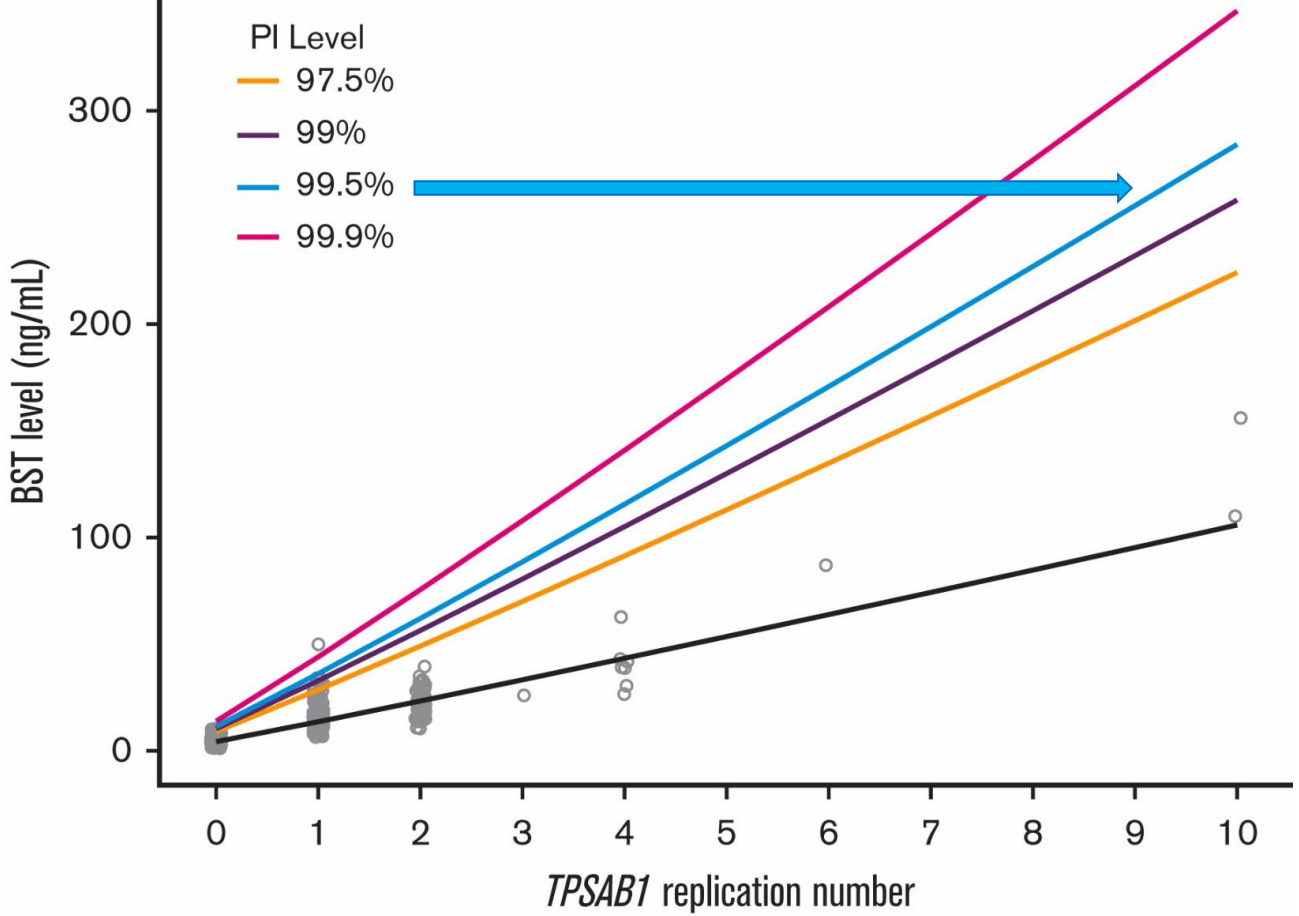


5-fold increased mRNA expression for α^{dup} vs α^{WT}

↑α-tryptase protein production

↑BST~9-10 ng/mL per α^{dup}

204 controls & 309 HαT patients w/o cMCD or RF



80% with 1, 17% with 2 CNVs

Measured & predicted BST levels based on replications of *TPSAB1*-encoded α-tryptase

(204 controls, 309 HαT patients w/o cMCD)

HαT⁻ & cMCD⁻

TPSAB1 replication #	Primary data			Predicted values (ng/mL)		
	N	BST median (ng/mL)	BST STD (ng/mL)	BST	Upper 99.5% value	
0	204	4	2	4	11	
1	247	14	6	14	36	40
2	52	22	6	23	62	60
3	1	26	N/A	33	80	80
4	6	41	13	43	116	100
5	—	—	—	54	143	
6	1	87	N/A	64	171	
7	—	—	—	74	199	
8	—	—	—	85	228	
9	—	—	—	95	256	
10	2	133	33	106	285	220

2 STD ≤8 BST

99.5% = 11.4 BST

BST> 20 + CNV#*20

Chovenac et al. Blood Adv, 2023

https://bst-calculater.niaid.nih.gov/

Prevalence of HαT is not increased in Hypermobility Disorders or POTS

Vazquez…Lyons et al. Hum Genet Genom Adv 3, 100094, 2022

%HαT: 3.8% - 5.1% hypermobility disorders vs 6% controls, NS

Huang…Lyons et al. JACI-P, in press

%HαT: 7.2% POTS vs 6% controls, NS

Case Autonomic Dysfunction

25 y/o with frequent ↓BP, ↑P, lightheaded spells; POTS dx; ±(flushing or GI or dyspnea).

Baseline: Tryptase = 4 ng/ml
Acute (<3 h after onset): Tryptase = 4 & 5 ng/ml (<6.8 (2 + 1.2x4))
Semi-acute: acute urine histamine, LTC_4 and PGD_2 metabolite levels each wnl

Autonomic dysfunction, not MCA, most likely to account for hypotensive/tachycardic spells.

Quang Le, PhD

How does α-tryptase overexpression contribute to clinical features in HaT?

homotetramers

α	α
α	α

Inactive protease
α-tryptase

β	β
β	β

Active protease
β-tryptase

heterotetramer

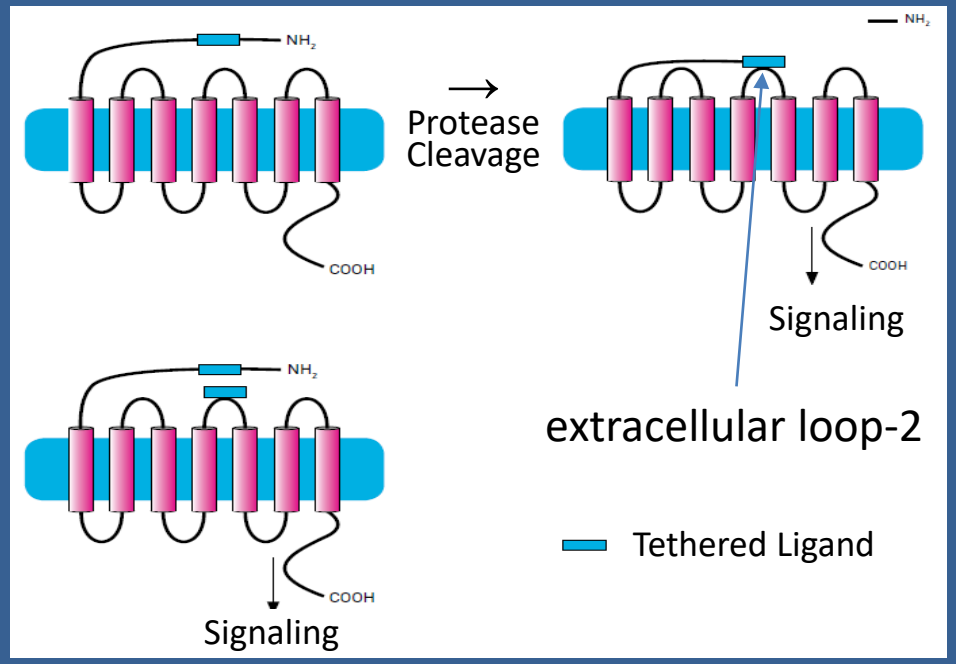
α ⇔ β
⇕ ⇕
β ⇔ α

Active protease
α/β-tryptase
?unique specificities?

$$\alpha/\beta\text{-tryptase in MCs} \sim \frac{\alpha}{\beta}\ tryptase\ gene\ ratio \sim ?\ biomarker$$

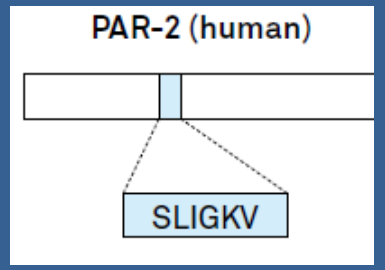
Le et al J Exp Med 216:2348, 2019; Lyons et al. JACI 147:622-32, 2021

Protease-Activated Receptor (PAR)-2



PAR2 is expressed on:

- Endothelium: ↑vasopermeability ~ ↑ANA severity
- Smooth muscle: bronchospasm, abdominal cramping
- Neurons: pruritus, hyperalgesia
- Epithelium: inflammation
- Fibroblasts: fibrosis
- Chondrocytes: arthritis
- Cancer cells: progression



Is PAR2 a target for mast cell tryptase? Discrepant literature.

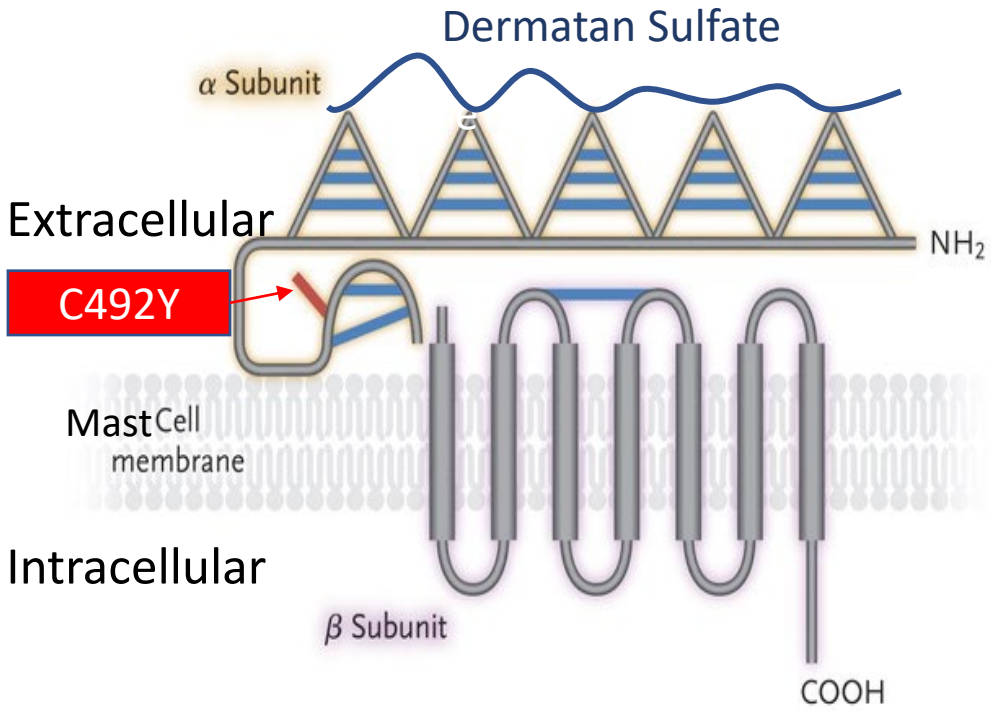
Le et al. J Exp Med 216:2348, 2019/Lyons et al JACI 147:622, 2021

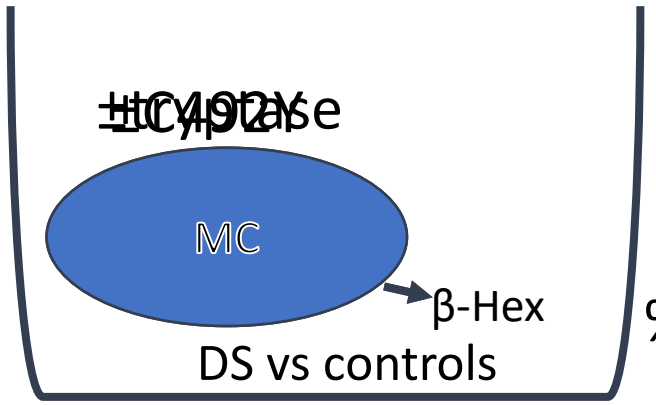
α/β-tryptase tetramers, but neither α- nor β- tryptase:

- *activates PAR2 on cell surfaces, pro-inflammatory*
- *increases permeability of human endothelial cell monolayers*
- *may explain increased ANA severity in HαT patients*

Severe Hereditary Vibratory Urticaria ~ C492Y in Adhesion GPCR (*ADGR-E2*; CD312, EMR2)

Boyden SE et al. New Engl J Med 374:656-663, 2016





%Degranulation to vibration

(750 rpm x20 min)

Le et al. J Exp Med 216:2348, 2019/Lyons et al JACI 147:622, 2021

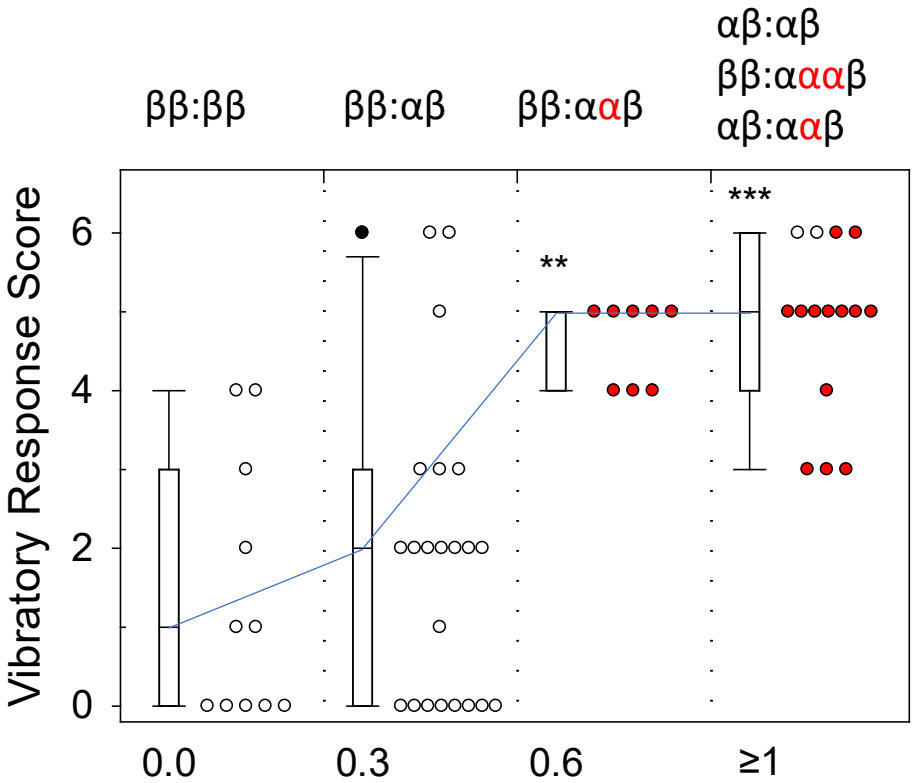
α/β-tryptase tetramers, but neither α- nor β- tryptase cleave EMR2 on MCs,

↑MCA to vibration *in vitro*

1. Skin MCs: Extrinsic signals - ?vibration/rubbing/dermographism
2. Pulmonary/GI/Vascular wall MCs: Intrinsic signals - ?stretch
3. Is the α/β-tryptase gene ratio a biomarker for predicting such responses in *in vivo*?

Quang Le, PhD

The α/β-tryptase gene ratio serves as a biomarker for clinical features due to the tryptase heterotetramers.



□/□ Tryptase Gene Ratio
(●, H□T; ○, controls)

↑α/β-tryptase heterotetramers

$\frac{\alpha}{\beta}$

Le et al J Exp Med 216:2348, 2019

2023 HαT in Clonal MC Disorders: MMAS, CM, & SM (non-Adv & Adv)

Clinical Features	Italy	France
Total Cases, n (%HαT)	444 (13%)	583 (14%)
HαT TPSAB1 CNV	85%x1, 10%x2	85%x1, 7%x2
% $\bar{c}$ D816V^{+} (+, - HαT)	87 (76, 88)	83 (81, 83)
BST (ng/mL), mean (+, - HαT)*	26 (33, 25)	63 (91, 58)

MCD (%HαT): MMAS (33%)>BMM (19%)>CM/ISM/ASM/SM-AHN (11%)>Controls (6%)
HαT more prevalent when ANA dominant diagnostic feature

Sordi B et al. JACI 151:485-93, 2023 (Italy)
Polivka L et al. JACI 2023, in press (France)

Mast Cell Activation Syndrome

Epidemic or Epiphenomenon?

(1) Recurrent episodes of **spontaneous** anaphylaxis by clinical criteria;

(2) MC **biomarker** confirmation;

(3) MC targeted **therapy response**

D816V cMC, HαT, or both; idiopathic

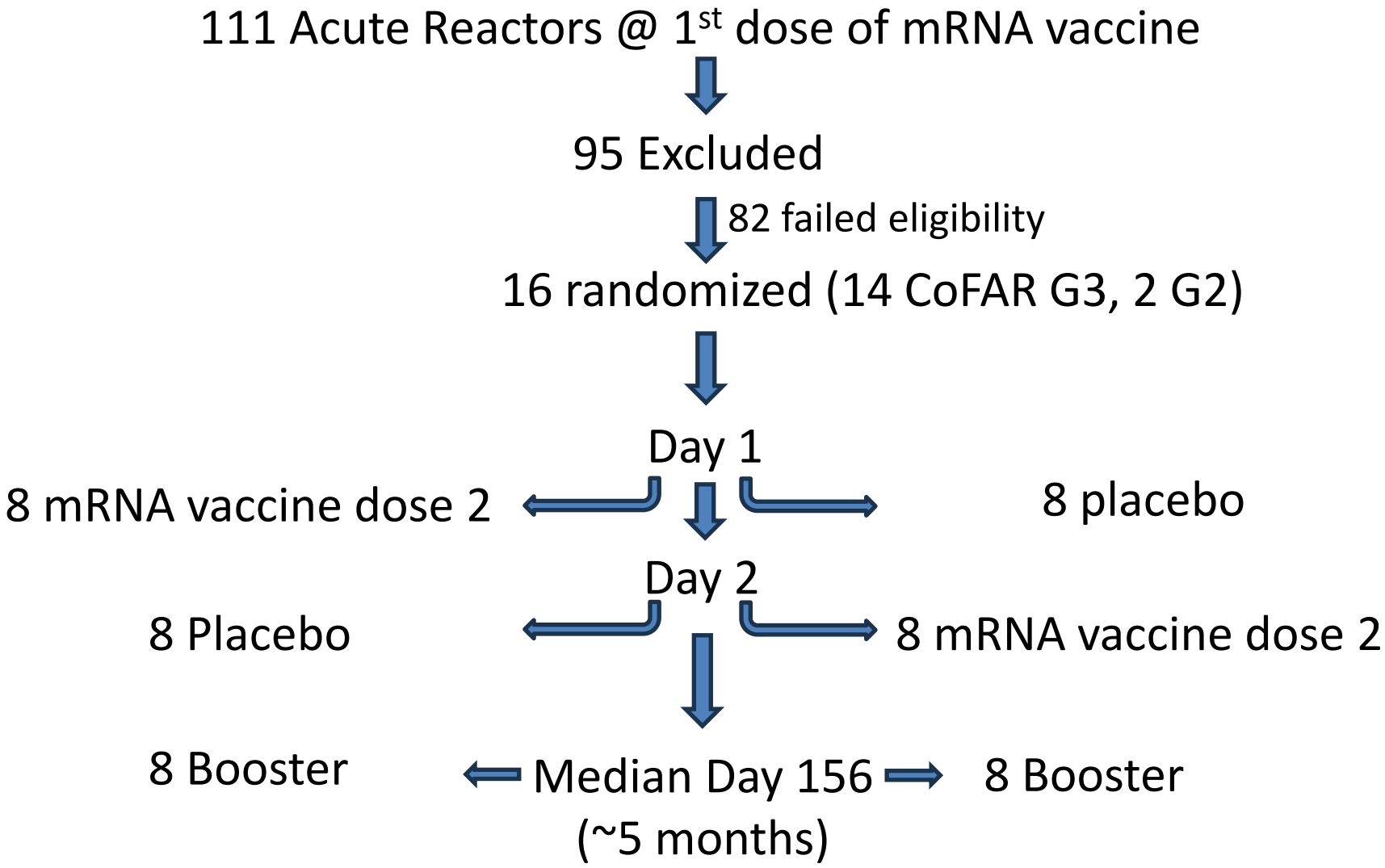
Over-diagnosis of MCAS

Symptom creep: chronic fatigue, fibromyalgia, abdominal pain …

Non-validated tests: chromogranin A, heparin pre/post veno-occlusion …

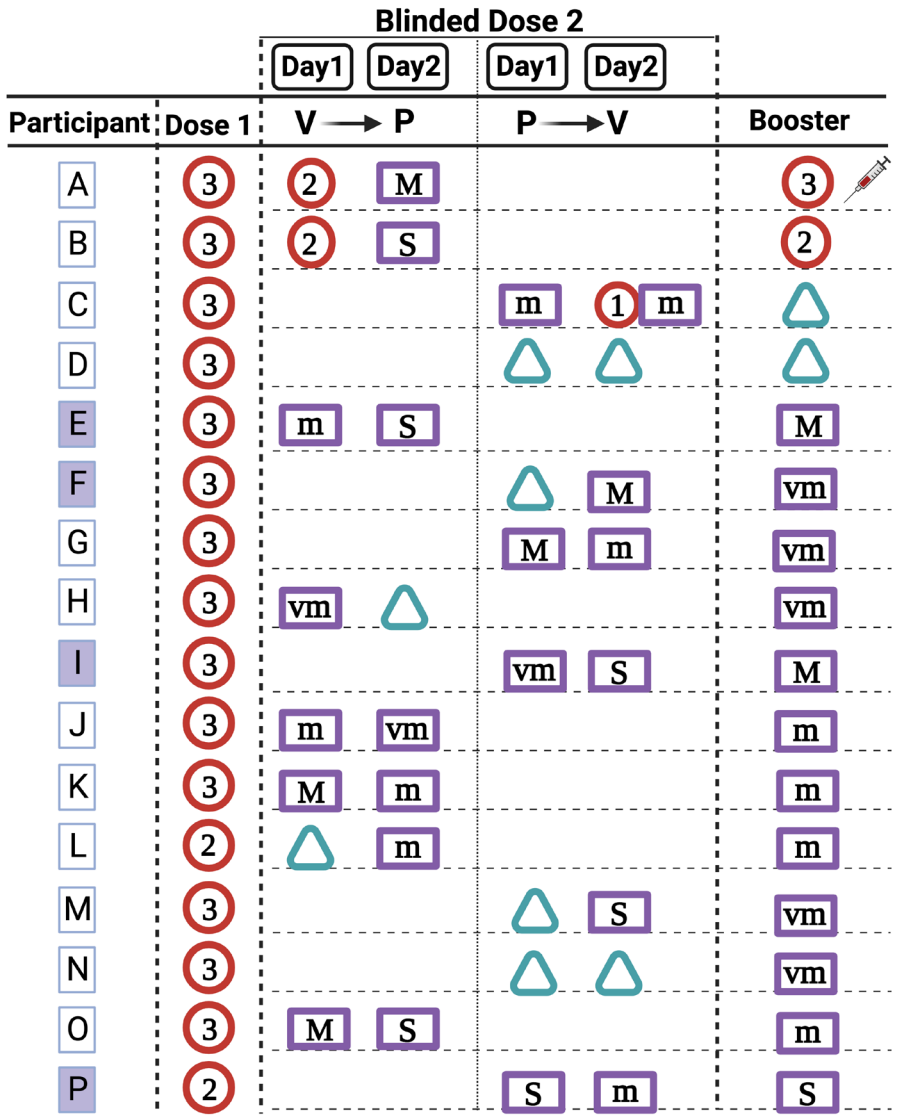
A randomized double-blinded trial to assess recurrence of systemic allergic reactions following COVID-19 mRNA vaccination

Khalid et al. JACI, in press



Allergic Response (AR) vs Immunization Stress-Related Response (ISRR)

Khalid et al. JACI, in press



= No Reaction

1 = CoFAR grade-1 AR

2 = CoFAR grade-2 AR

3 = CoFAR grade-3 AR

vm = Very mild ISRR

m = Mild ISRR

M = Moderate ISRR

S = Severe ISRR

1 m = Mixed Reaction

= Epinephrine IM

No reaction: 10/48 inj; 1/16 subj

ISRR alone: 33/48; 12/15

AR Alone: 4/48; 0/15

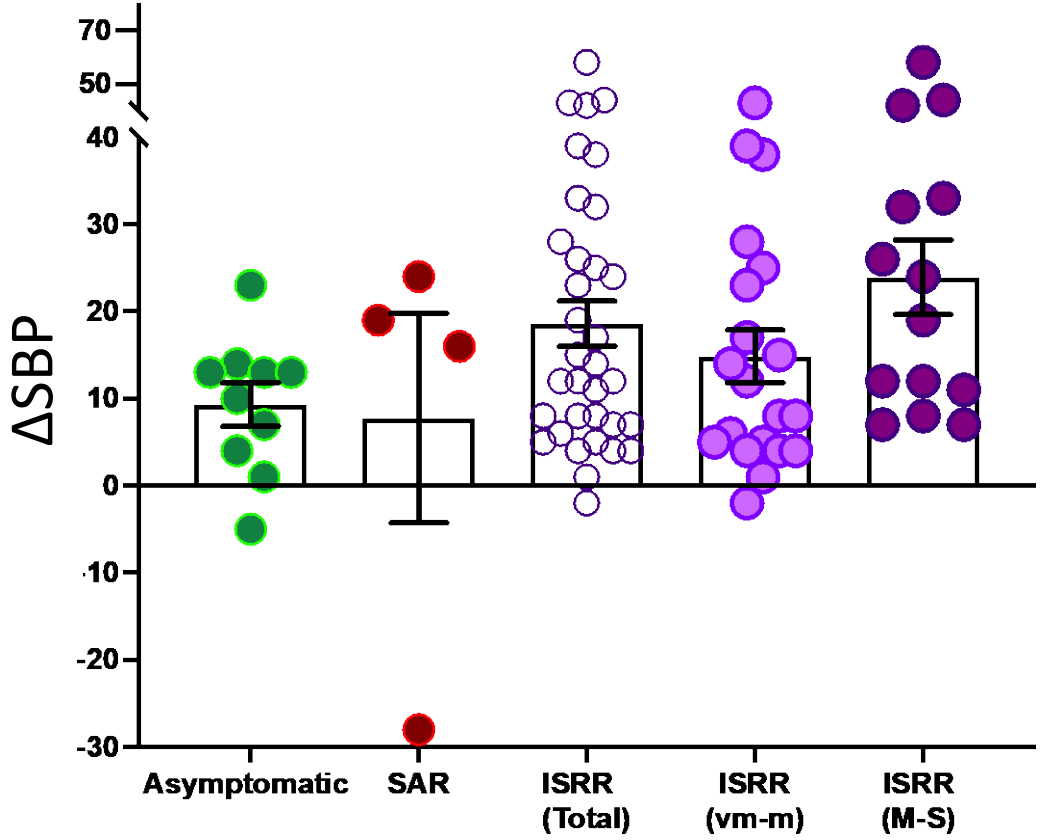
ISRR+AR: 1/48; 3/15

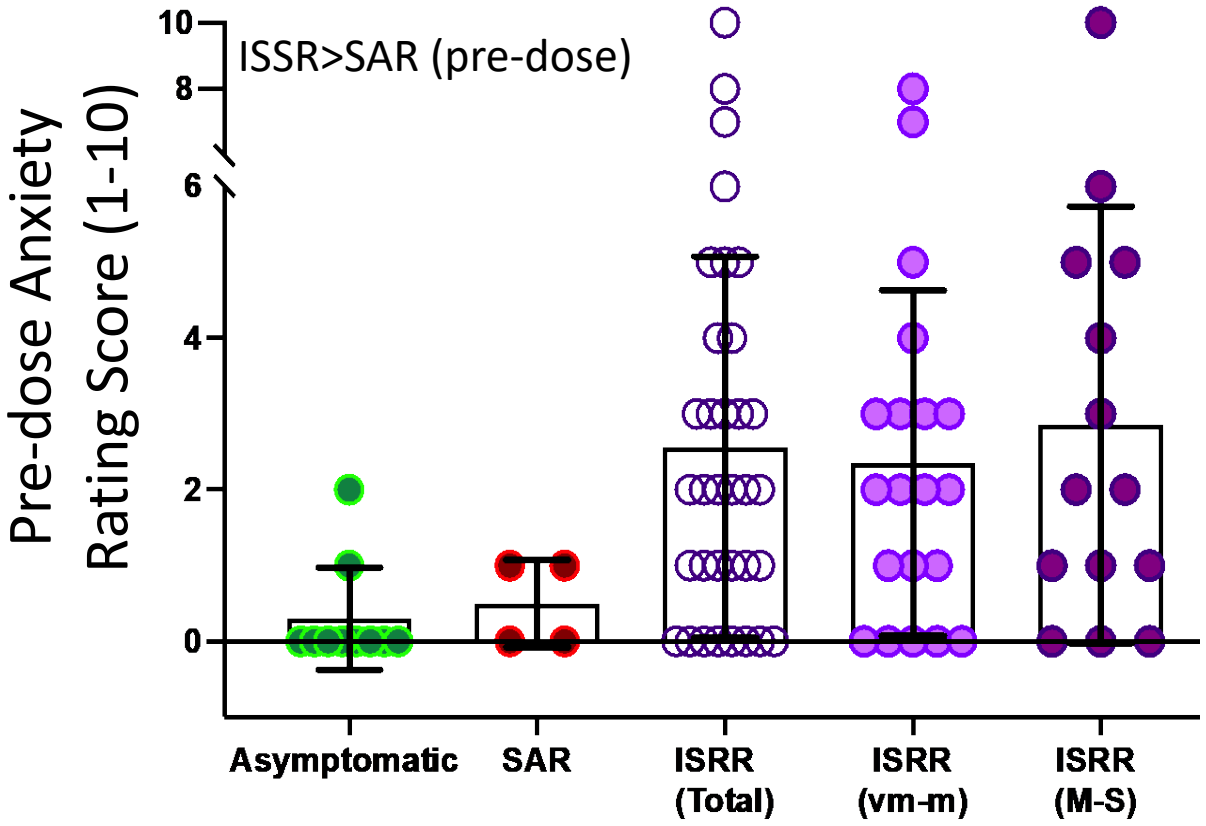
Grade 3 AR: 1

Grade 2 AR: 3

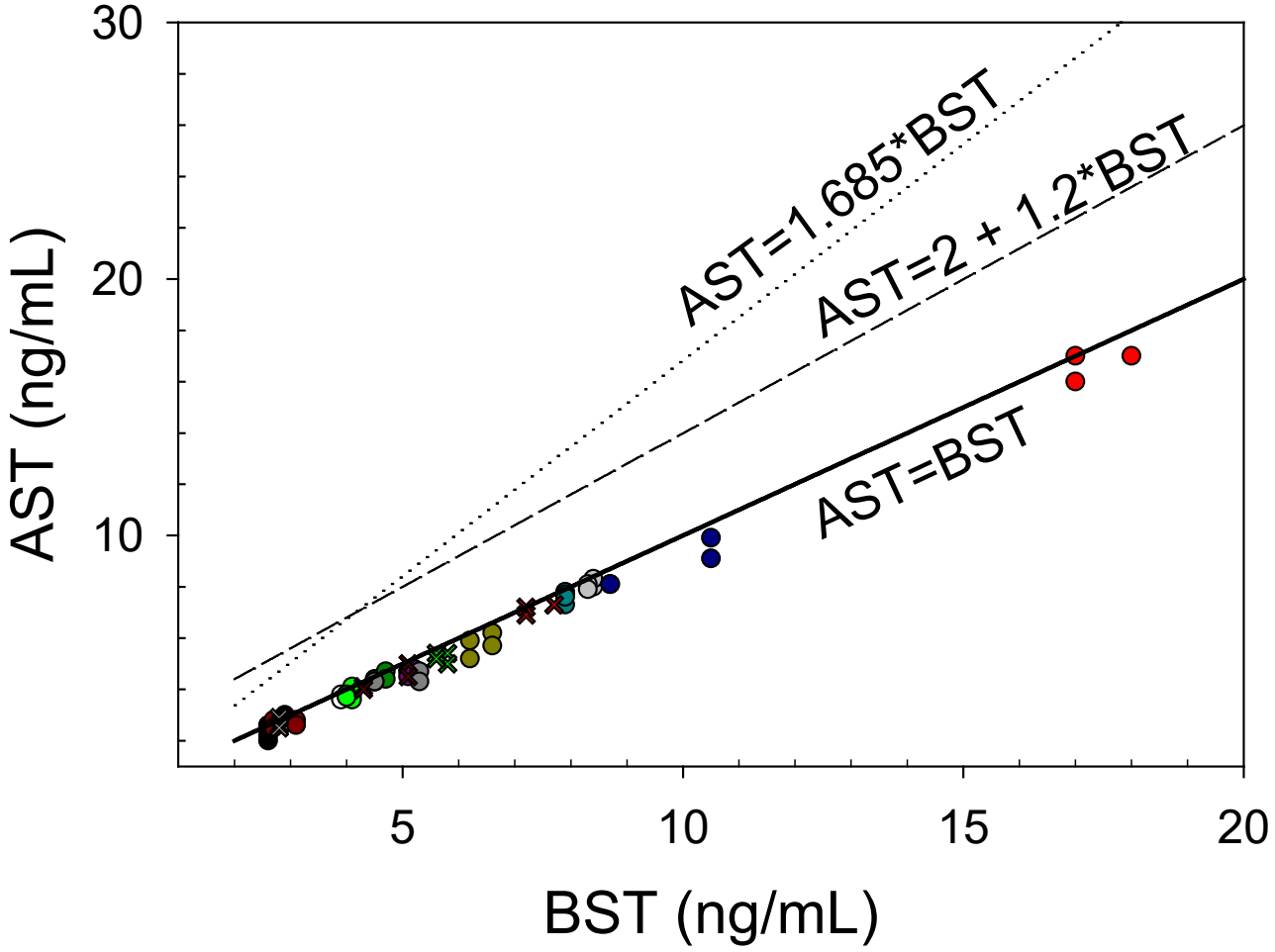
Grade 1 AR: 1

Systolic blood pressure & anxiety score (Khalid et al. JACI, in press)





Acute (AST) & Baseline (BST) Serum Tryptase Levels in COVID-19 Vaccination Study During D1 & D2 Visits (Khalid et al. JACI, in press)

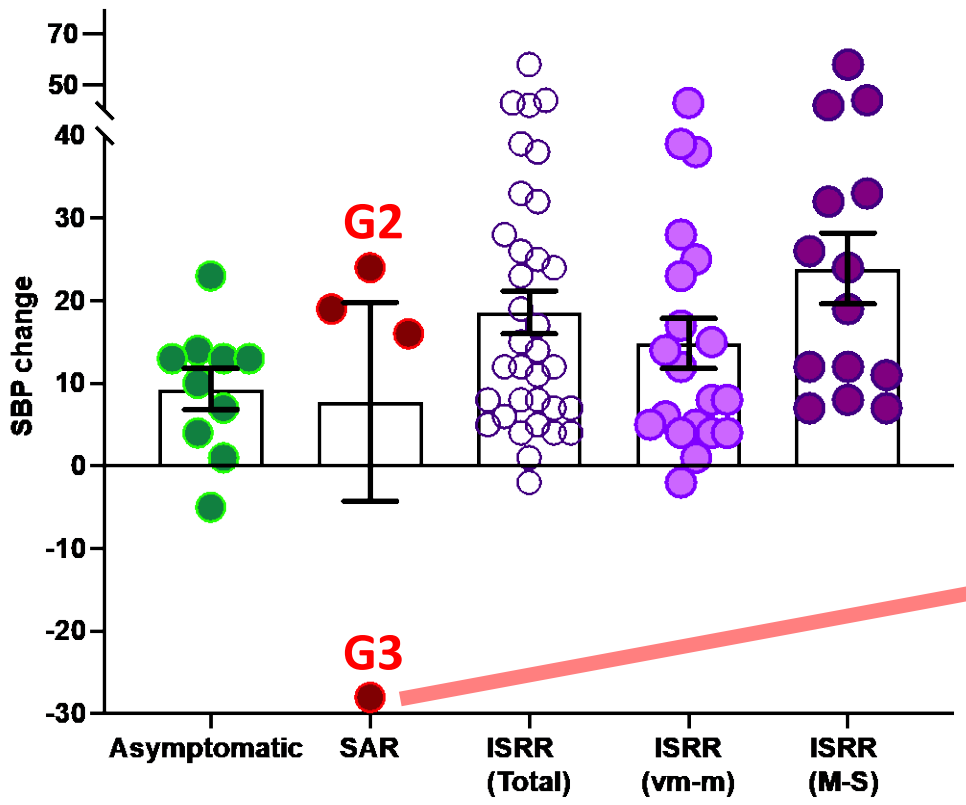


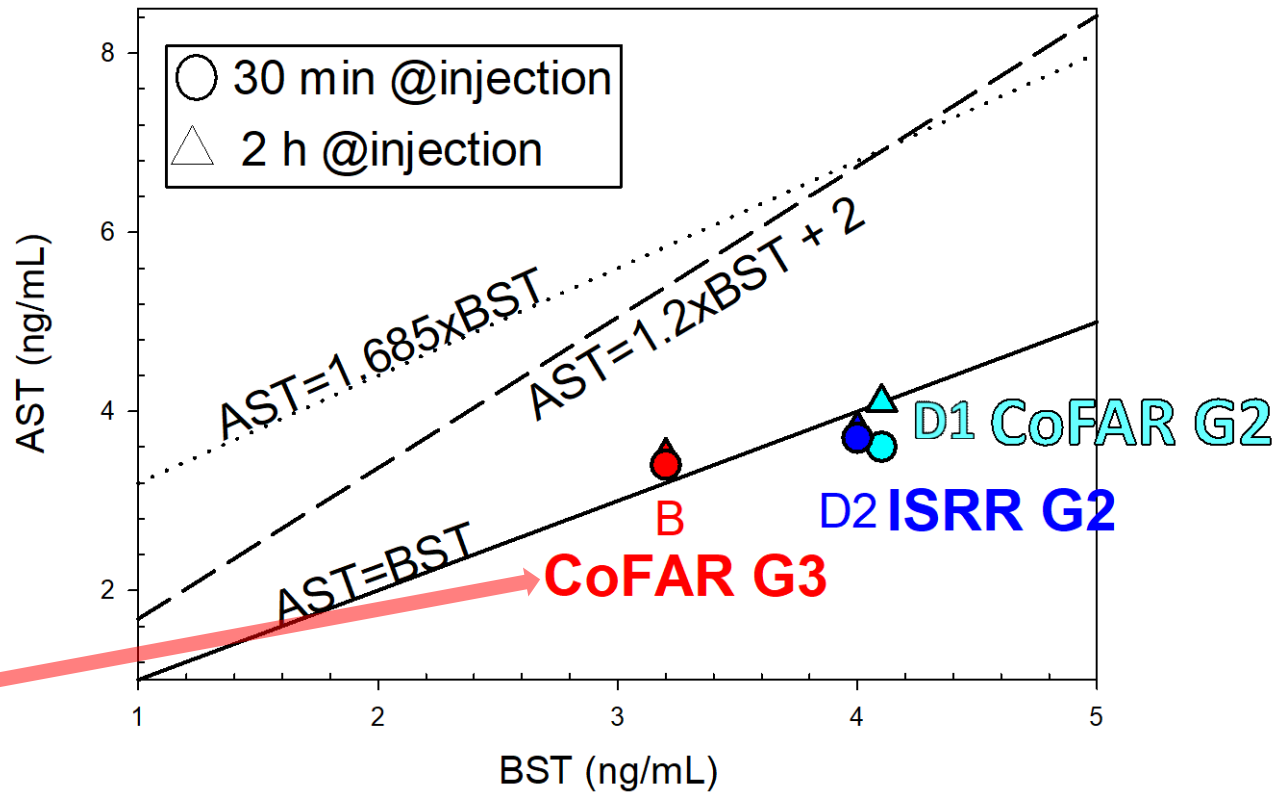
No AST level elevated to a clinically significant magnitude by either ECNM or NIH algorithm.

Between assay variability eliminated.

AST/BST in Grade 3-SAR with ↓sBP>20%

Khalid et al. JACI, in press





What is the 'gold standard' for ANA?

Clinical signs/symptoms, elevated biomarker(s), or both?

Concluding Comments

1. Tryptase serves as a biomarker for disorders of MC activation, of clonal MC hyperplasia or proliferation, the TPSAB1 CNV trait named HαT, and the effect of agents targeting MC survival.
2. Most reactions to mRNA vaccines or to POTS attacks are not MCA events
3. The differential enzymatic activities of α-, β-, and α/β- tryptase tetramers may explain clinical features of MC biology/pathobiology that have yet to be understood.
4. Whether tryptase(s) are therapeutic targets remains to be determined.

Charles Richet: "I possess every good quality,
but the one that distinguishes me above all is modesty."

Anthony Fauci: "Science, by its nature, is self-correcting;
so understanding & advice evolves based on new discoveries."