Allergia ai farmaci, dalla diagnosi alla clinica

Come gestire nella pratica clinica
le reazioni ai farmaci.

Sabato 21 febbraio
Dalle 9.00 alle 16.30

Aula Magna Mangiagalli
Via della Commenda, 12

Meccanismi patogenetici delle reazioni da ipersensibilità a farmaci e ruolo del recettore MRGPRX2

Patrizia Pignatti

Laboratorio di Immuno-Allergologia Clinica
Istituti Clinici Scientifici Maugeri, IRCCS
Pavia

Different mechanisms involved in immediate drug hypersensitivity reactions

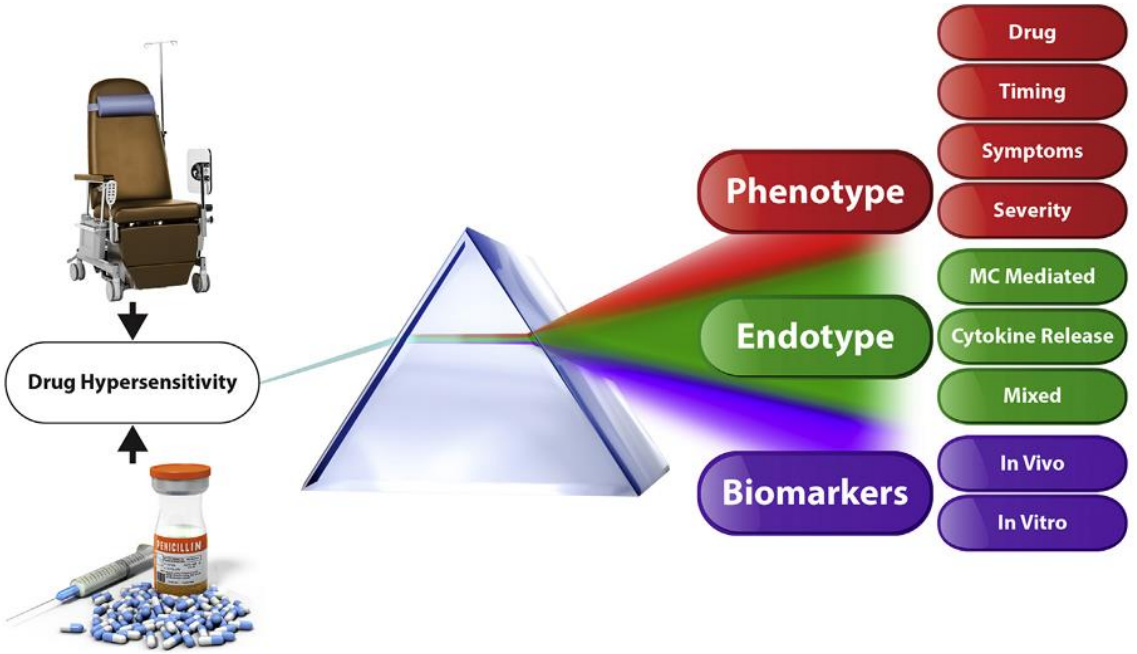
Allergy. 2024;79:601–612.

Phenotypes are characterized by:

- Clinical features,
- The time elapsed between drug exposure and the onset of symptoms,
- Appearance after the first administration or repeated doses.

Endotypes that accompany these phenotypes are defined by:

- Mechanisms involved
- Molecular mediators released by the effector cells used as biomarkers.



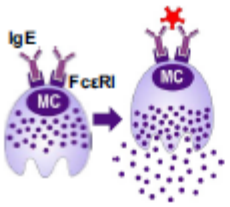
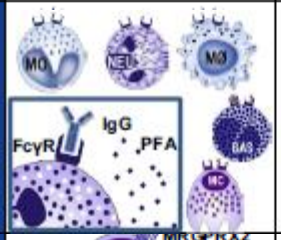
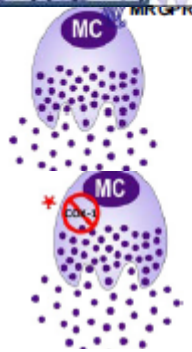
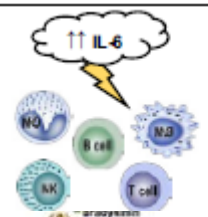
/ Ann Allergy Asthma Immunol 124 (2020) 566–572

Allergy. 2024;79:601–612.

Different mechanisms involved in immediate drug hypersensitivity reactions

Immunologic reactions

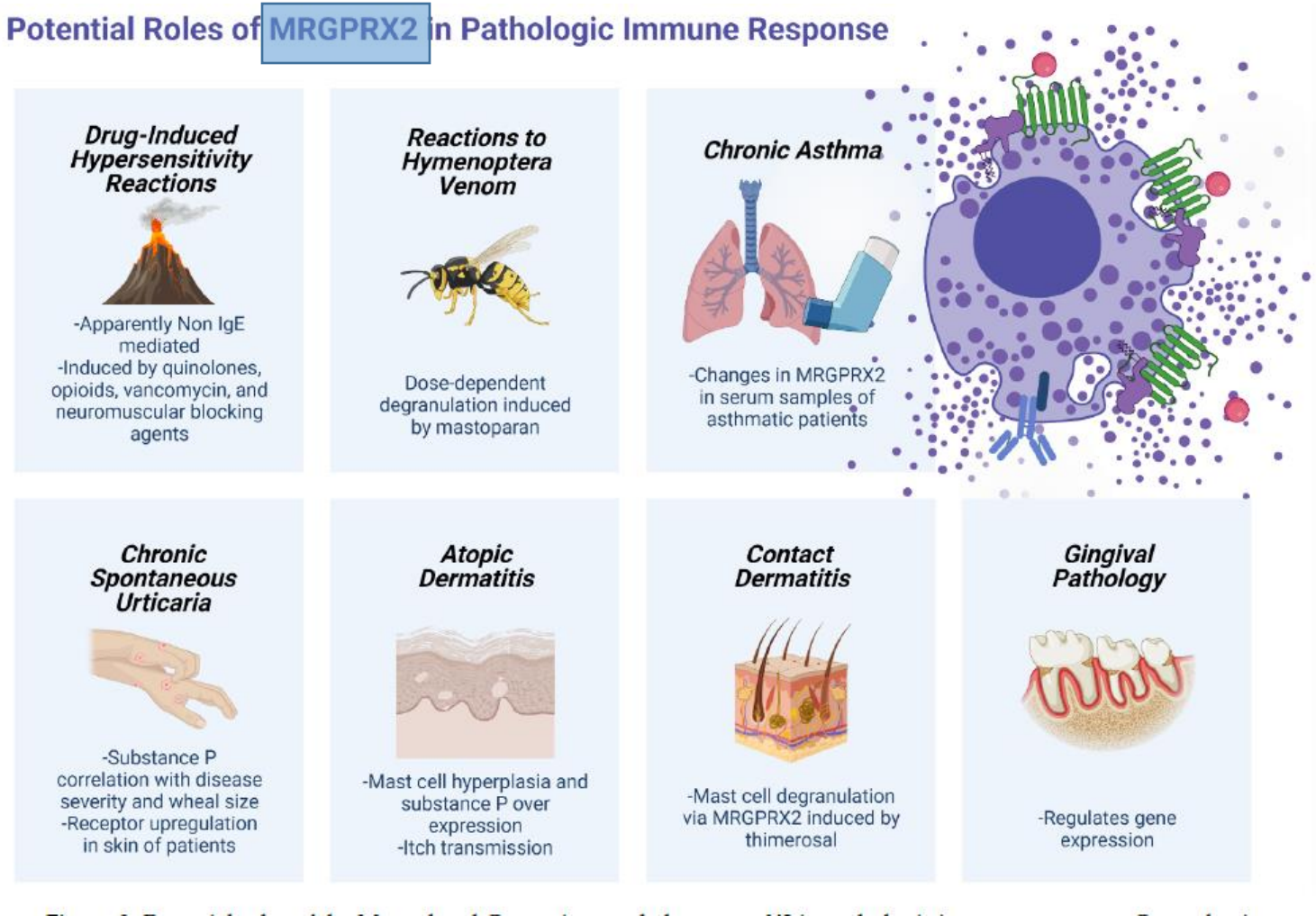
Non immunologic reactions

		Effector cells	Phenotyping biomarkers	Endotyping biomarkers	Effect of the dose
IgE-mediated reactions		Mast cells, basophils	Pruritus, erythema, urticaria, angioedema, rhinitis broncospasm, abdominal pain, vomiting.	Tryptase, histamine, histamine metabolites, sIgE, basophil activation markers; skin tests	• From second dose • Low dose
IgG-mediated reactions		Mast cells, basophils, neutrophils, macrophages, monocytes		PAF, PAF-AH, Anaphylotoxin C3a, C5a	• From second dose • Higher dose than IgE-mediated reactions
MRGPRX2 mediated reactions		Mast cells, (conditioned) basophils		Tryptase, histamine, MRGPRX2 polymorphisms Skin tests	• From first dose • Higher dose than IgE-mediated reactions
NSAIDs cross-hypersensitivity		Mast cells		LTC4, LTD4, LTE4, 9a,11b-PGF2 NERD biomarkers: IL-5, ECP, IFN-γ, TGF-β1, DDP10, SPD, foliculin	• From first dose • Higher dose than IgE-mediated reactions
Cytokine release syndrome		Monocytes, macrophages, T cells, B cells and NK cells	Fever, chills, rigor, nausea, pain, headache, hypotension, oxygen desaturation (in mixed reactions with mast cells and basophils involving, also flushing, urticaria and cardiovascular collapse)	IL-6, IL-8, IL-10, IL-1, TNF-α, IFN-γ (in mixed reactions: tryptase, histamine)	From first dose
Bradykinin mediated angioedema		Damaged tissues	Angioedema	Serum BK, plasma activity of DPP-IV protein and APP, serum endothelial-selectin, angiopoietin-2, 6-keto-PG F1α	Higher dose than IgE-mediated reactions

The Multifaceted Mas-Related G Protein-Coupled Receptor Member X2 in Allergic Diseases and Beyond

Int. J. Mol. Sci. **2021**, *22*, 4421.

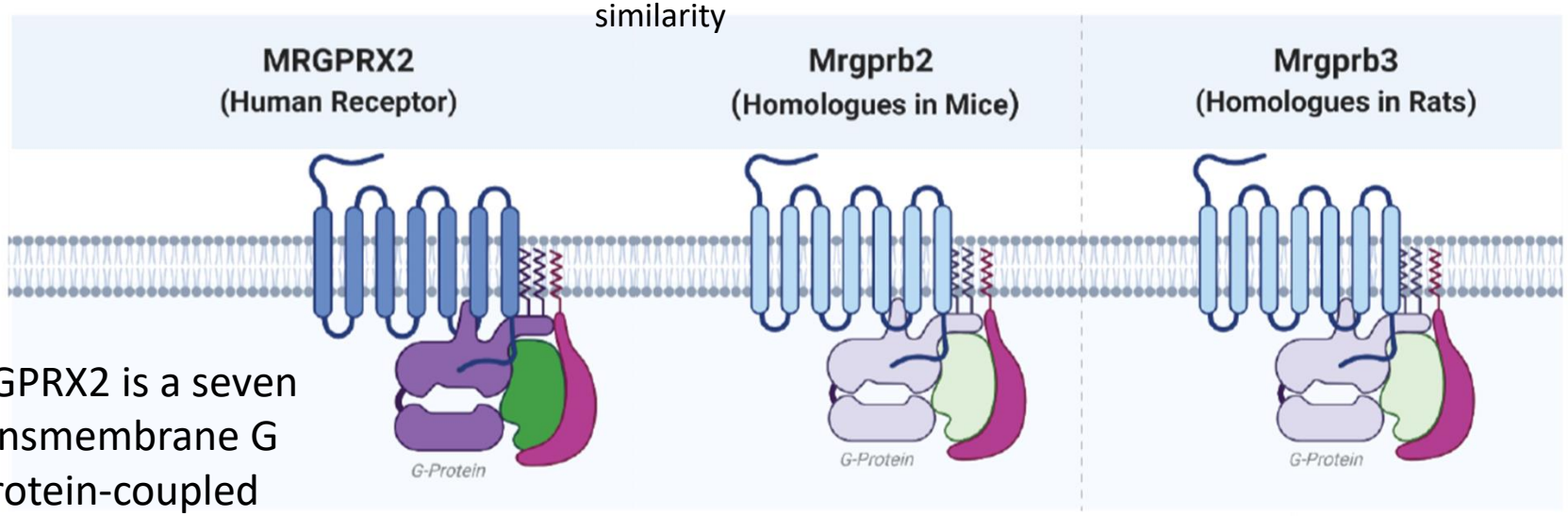
Paola Leonor Quan [1,*], Marina Sabaté-Brescó [1,2,†], Yanru Guo [3,4], Margarita Martín [3,4,†] and Gabriel Gastaminza [1,2,†]



Mas-Related G Protein-Coupled Receptors

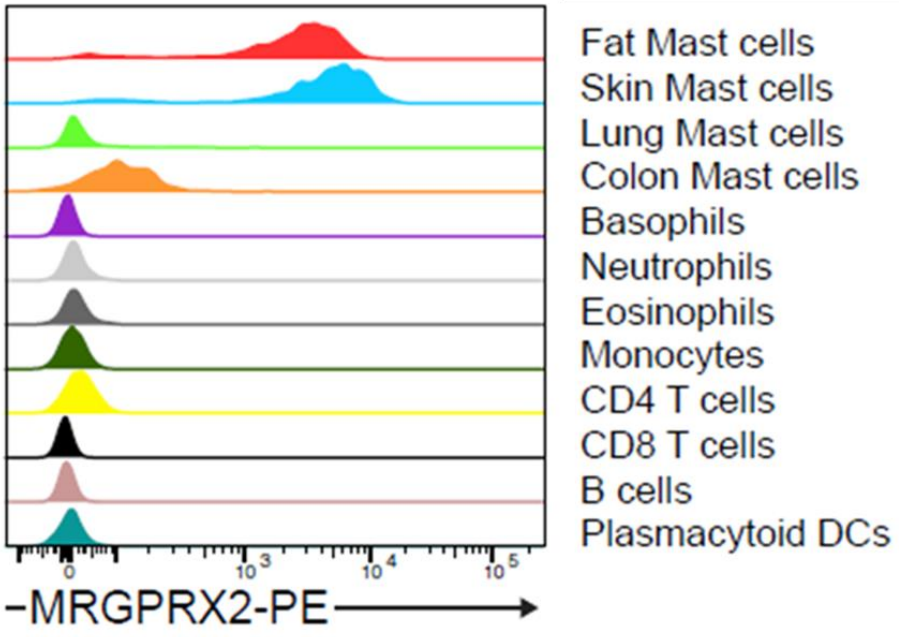
53% sequence similarity

Int. J. Mol. Sci. **2021**, *22*, 4421.

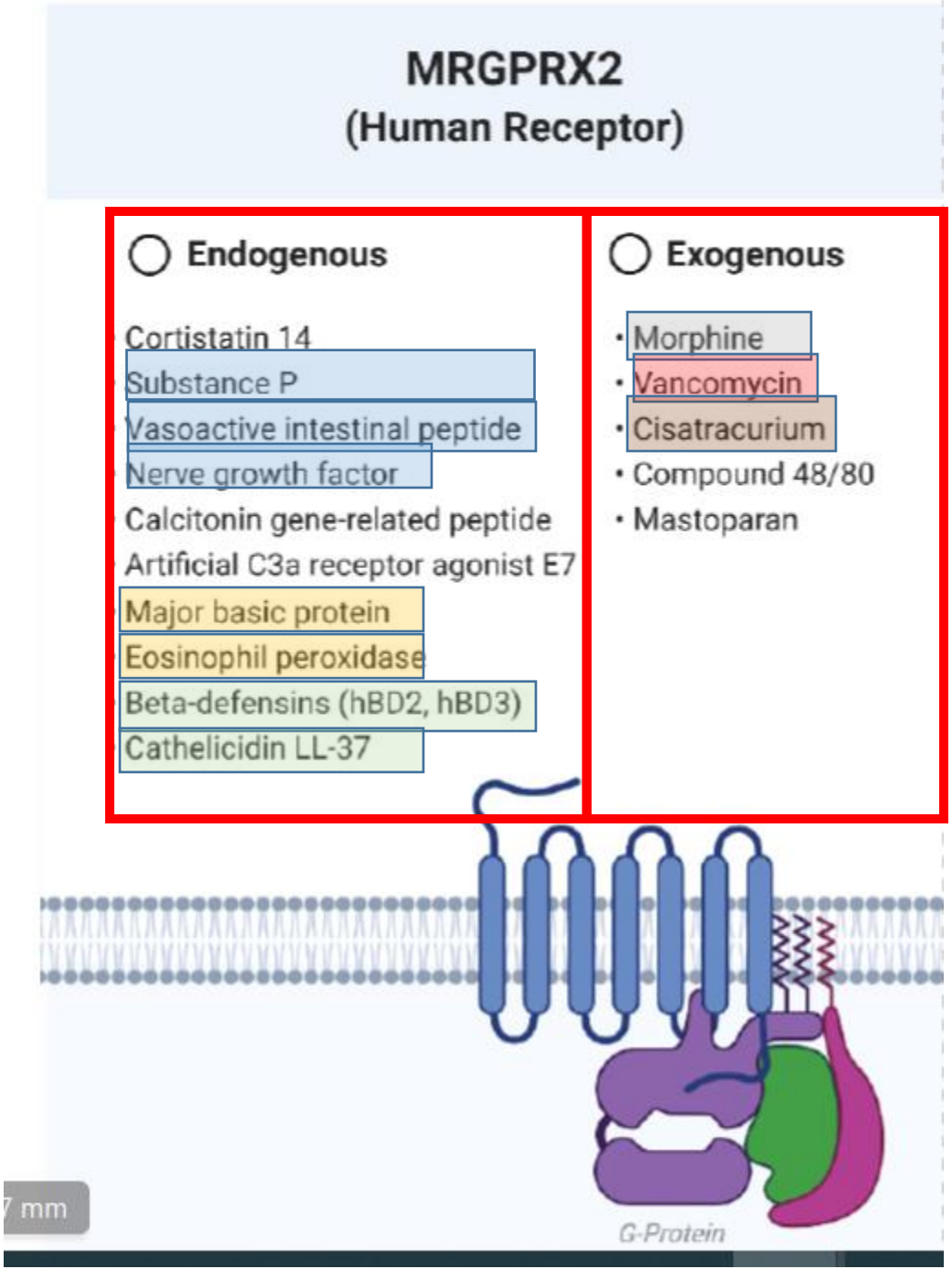


MRGPRX2 is a seven transmembrane G protein-coupled receptor

MRGPRX2 is confined to MC_{TC} (submucosal and skin localization), and is absent in MC_T (mucosal)



Plum et al., 2020, Immunity *52*, 404–416



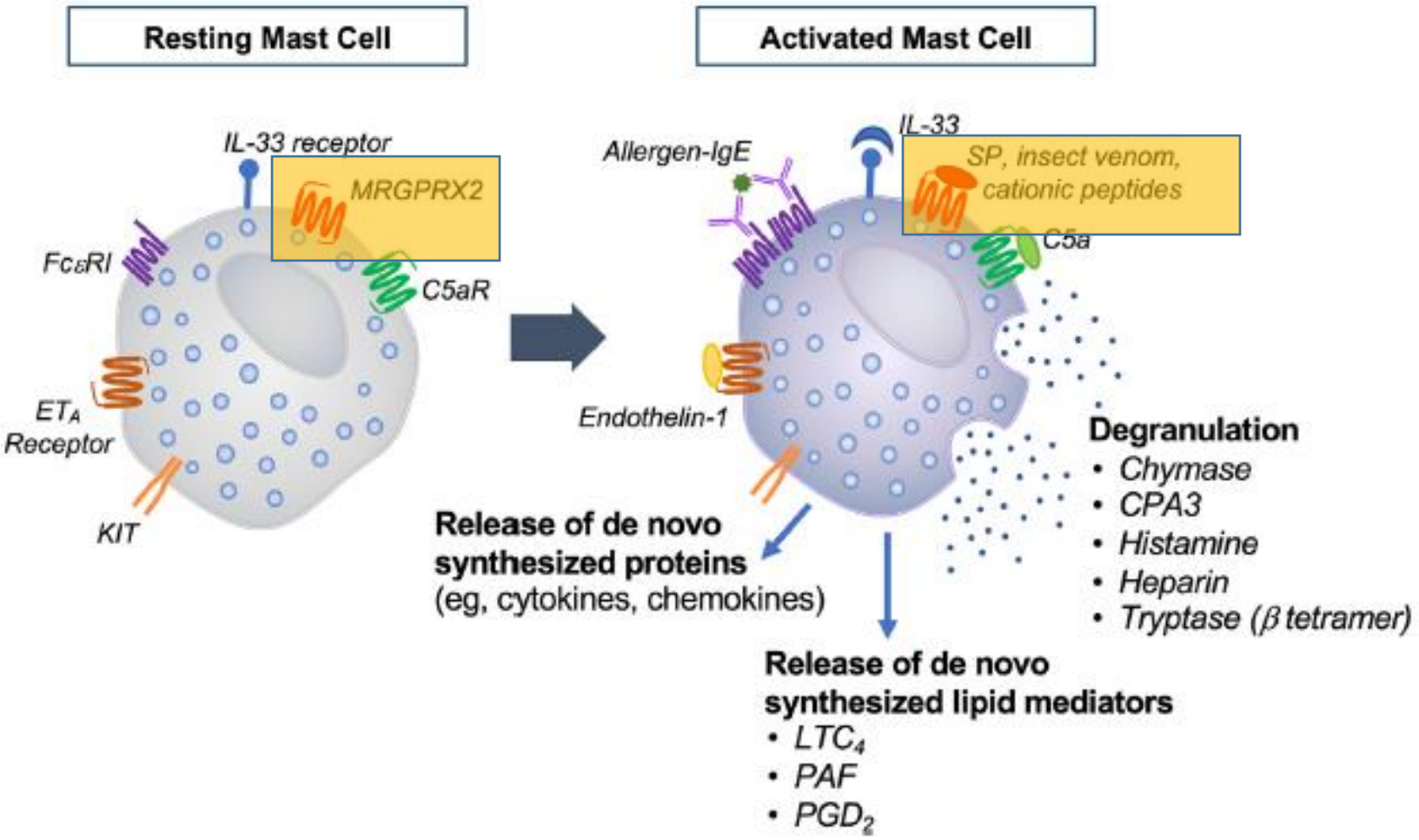
Cationic peptidergic drugs

- Dose dependent reactions
- Intravenous administration

Pseudoallergic reactions, extimated to be 30% of the anaphylactic reactions

Int. J. Mol. Sci. **2021**, *22*, 4421.

CC BY license (http://creativecommons.org/licenses/by/4.0/). (J Allergy Clin Immunol Pract 2024;12:1727-37)



ROLE OF COMORBIDITIES

J Allergy Clin Immunol Pract 2023;11:395-404

Comorbidities can have an important role in determining the features of MRGRPX2-mediated reactions.

Enhanced MRGPRX2 expression in patients with maculopapular cutaneous mastocytosis

Deepak V, et al. J Allergy Clin Immunol Pract 2021;9:3841-3843.e1.

Heightened sensitivity to MRGPRX2 agonists such as icatibant and atracurium in chronic spontaneous urticaria.

Shtessel M, et al. J Invest Dermatol 2021;141:678-681.e2.

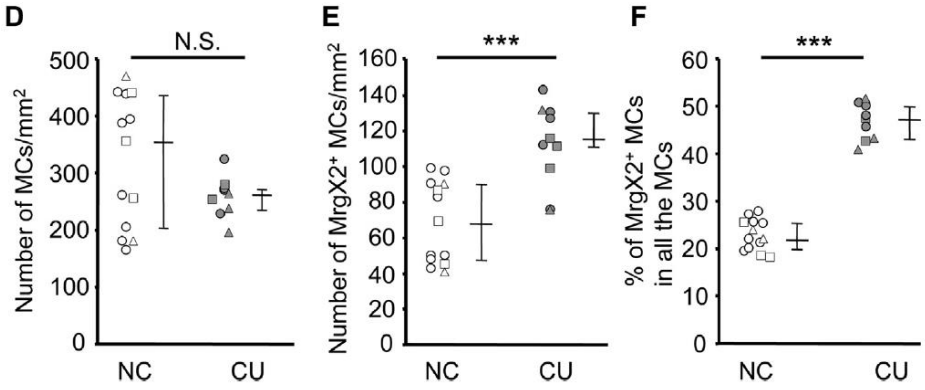
Blunting effect of infections on MC responsiveness to glycopeptides

Rybak MJ, et al. Antimicrob Agents Chemother 1992;36:1204-7.

Expression of Mas-related gene X2 on mast cells is upregulated in the skin of patients with severe chronic urticaria

Daisuke Fujisawa, MD,[a,b] Jun-ichi Kashiwakura, PhD,[c] Hirohito Kita, MD, PhD,[d] Yusuke Kikukawa, MSc,[e] Yasushi Fujitani, PhD,[e] Tomomi Sasaki-Sakamoto, BSc,[a] Kazumichi Kuroda, PhD,[f] Satoshi Nunomura, PhD,[a,b] Koremasa Hayama, MD, PhD,[a,b] Tadashi Terui, MD, PhD,[b] Chisei Ra, MD, PhD,[f] and Yoshimichi Okayama, MD, PhD[a]

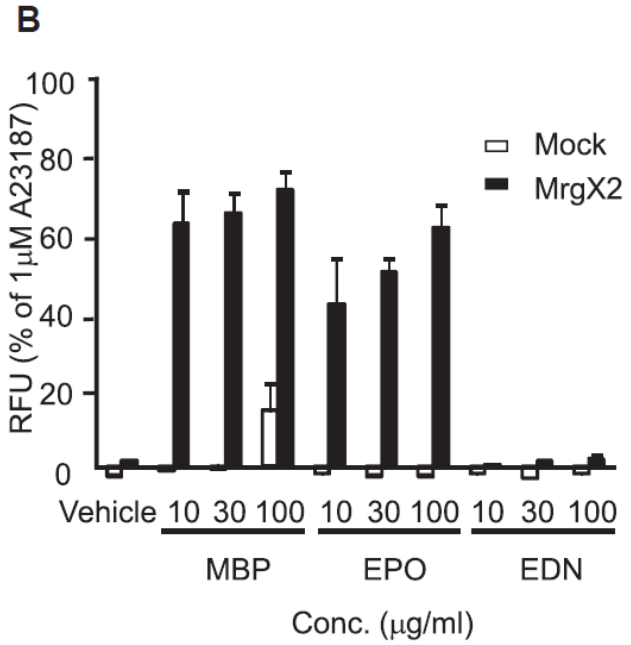
J Allergy Clin Immunol 2014;134:622-33.



Age and CU drugs (H1 receptor antagonist olopatadine hydrochloride, H2 receptor antagonist famotidine and dexamethasone) did not affect expression of MRGPRX2 on mast cells

In patients with CU, eosinophil infiltration and MC degranulation are commonly observed in the urticarial region

MBP and EPO, but not EDN, induced Ca2+ influx in mast cells, in a concentration-dependent manner



Patient Characteristics Associated With Reactions to Mrgprx2-Activating Drugs in an Electronic Health Record–Linked Biobank

J Allergy Clin Immunol Pract 2023;11:492-9)

Dinah Foer et al.

Retrospective, observational cohort study design using electronic health record data from adults with an Mrgprx2-activating drug exposure (n=59763),

Fluoroquinolones, morphine, neuromuscular blockade agents, vancomycin, and leuprolide.

4846 with ADR

TABLE III. Patient characteristics associated with listed reaction to Mrgprx2-activating drugs*

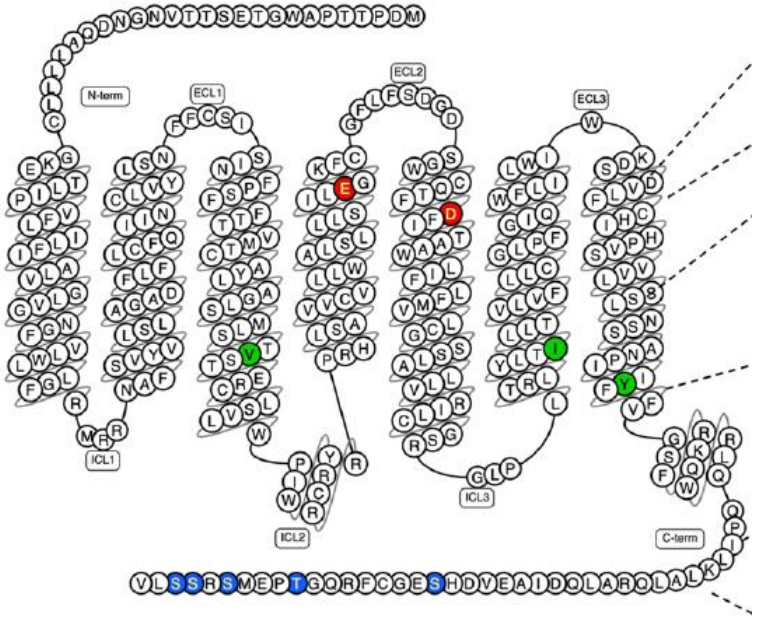
Variable	Mrgrpx2 adverse drug reaction OR (95% CI)	Mrgprx2 immediate HSR subgroup OR (95% CI)
Age†	1.03 (1.09-1.05)	0.90 (0.87-0.94)
Sex, female	2.16 (2.03-2.31)	2.38 (2.07-2.72)
Race, Black	0.58 (0.50-0.68)	0.75 (0.57-0.98)
Insurance type, commercial	0.59 (0.55-0.63)	0.62 (0.57-0.73)
PCP in health system	0.68 (0.64-0.72)	0.64 (0.57-0.73)
Allergic disease, composite	1.55 (1.45-1.65)	1.6 (1.41-1.82)
Asthma	1.81 (1.68-1.94)	1.91 (1.67-2.20)
Chronic urticaria	1.73 (1.46-2.05)	2.11 (1.56-2.84)
Atopic dermatitis	1.10 (0.99-1.21)	0.97 (0.79-1.19)
Mastocytosis	12.79 (5.98-27.02)	11.69 (3.19-42.74)
Laboratory tests		
Absolute eosinophil count ≥500 (K/mL)	0.66 (0.60-0.73)	0.68 (0.56-0.83)
Total IgE ≥100 (IU/mL)	1.07 (0.85-1.36)	1.24 (0.81-1.90)
Tryptase ≥11.4 (ng/mL)	1.40 (0.66-2.97)	1.57 (0.38-6.49)

MRGPRX2 Mutations

Residues responsible for ligand binding (red)

G protein coupling (green)

Phosphorylation (blue)



4 naturally occurring MRGPRX2 missense variants (G165E, D184H, W243R, and H259Y) that failed to respond to SP and HK-1

Alkanfari I, et al. J Immunol. 2018;201(2):343–9

Potentially G protein-coupling-deficient variants with loss-of-function phenotype (V123F, R138C, R141C, and V282M)

Chompunud Na Ayudhya C, et al. Int J Mol Sci. 2019;20(21):5247.

Phosphorylation-defective variants with gain-of-function phenotype (S325L and L329Q) for SP-induced mast cell activation and degranulation

Chompunud Na Ayudhya C, et al. Int J Mol Sci. 2019;20(21):5247.

Mutations could alter specific ligand binding properties, making the receptor more or less responsive to some but not all MRGPRX2 agonists

Curr Allergy Asthma Rep (2021) 21: 3

Biological screening of a unique drug library targeting MRGPRX2

Fan Yang[1,2†], Nathachit Limjunyawong[3†], Qi Peng[3], John T. Schroeder[1], Sarbjit Saini[1], Donald MacGlashan Jr[1], Xinzhong Dong[3] and Li Gao[3*]

Front. Immunol. 13:997389.
doi: 10.3389/fimmu.2022.997389

84/3456 drugs MRGPRX2-selective active hit compounds representing diverse classes according to primary drug indications

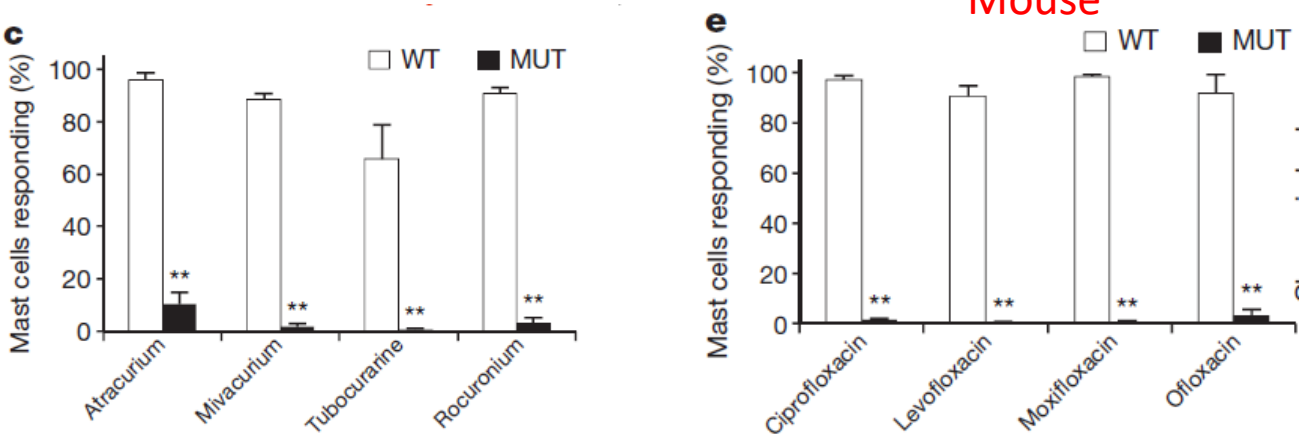
- Fluoroquinolone antibiotics (n=5, 15%);
- Nonfluoroquinolone antibiotics (n=3, 9%);
- Antidepressive/antipsychotic agents (n=8, 23%);
- Anti-allergic agents, first generation H1-antihistamine (n=13, 38%)
- Antineoplastic agents (n=5, 15%).

Identification of a mast-cell-specific receptor crucial for pseudo-allergic drug reactions

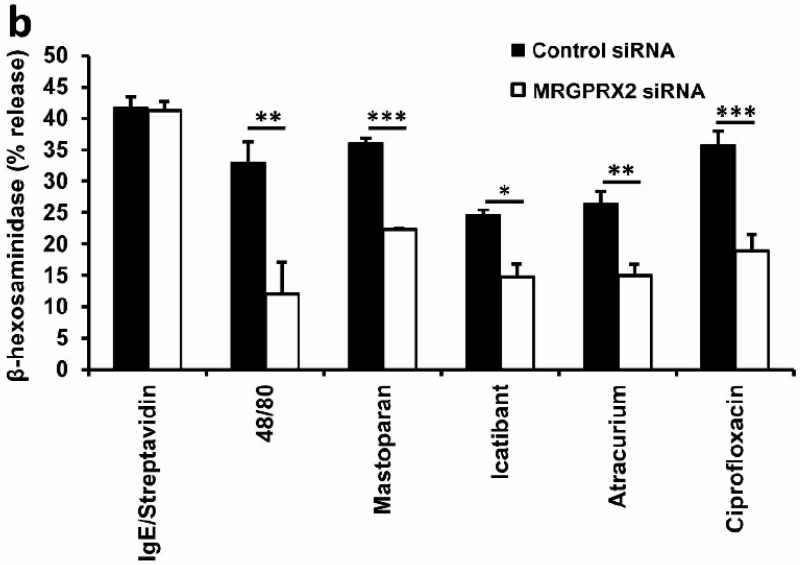
RESEARCH LETTER

McNeil BD, et al. Nature. 2015 Mar 12;519(7542):237-41.

Mouse



Human LAD2 mast cells



MRGPRX2 and Adverse Drug Reactions

Frontiers in Immunology

August 2021 | Volume 12 | Article 676354

*Benjamin D. McNeil**

Fluoroquinolones

An unusual property of many fluoroquinolones is that they accumulate in specific tissues at concentrations well above peak plasma concentrations

Due to lipophilicity of the molecule

this unusual tissue distribution pattern of fluoroquinolones may result in intense mast cell activation that is both delayed and restricted to certain tissues.

Activation of MRGPRX2

Allergenic and Mas-Related G Protein-Coupled Receptor X2-Activating Properties of Drugs: Resolving the Two

Vito Sabato, MD, PhD[a,b,*], Didier G. Ebo, MD, PhD[a,b,*], Marie-Line M. Van Der Poorten, MD[a,c], Alessandro Toscano, MD[a], Athina L. Van Gasse, MD, PhD[a,c], Christel Mertens, MLT[a], Michel Van Houdt, MLT[a], Michiel Beyens, MD[a], and Jessy Elst, MSc, PhD[a] *Antwerp and Ghent, Belgium*

Clinical differences and similarities

Clinics	IgE	MRGPRX2
	Entrire IDHR spectrum Slower resolution of cutaneous symptoms	Entire IDHR spectrum Cutaneous symptoms invariantly present Faster resolution of cutaneous symptoms
Prior exposure	Required *	Not required
Affinity (EC50)	High	Low
Dose	Low	High
Comorbidity/cofactor	Not essential	Mandatory?
Genetics	Not essential	MRGPRX2 polymorphisms
Biomarkers	Tryptase	Tryptase
Cross-reactivity	Structure related	THIQ and beyond
Diagnostics/ Mechanistic studies	Skin tests, sIgE, BAT, pMAT**, TAT	Skin tests, cBAT, MAT
Desensitization	Possible	Questionable
Drug challenges	Hypothetically positive at lower doses	Hypothetically positive at higher doses

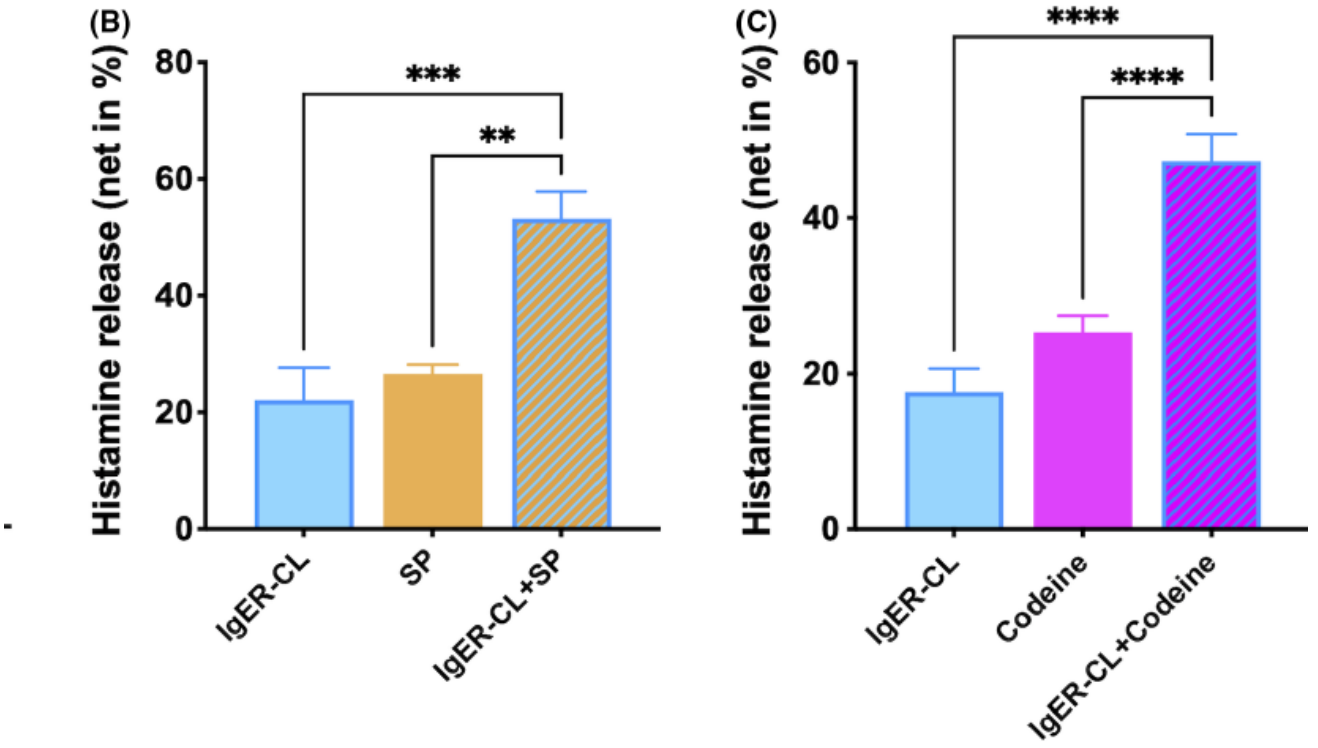
J Allergy Clin Immunol Pract 2023;11:395-404

FcεRI- and MRGPRX2-evoked acute degranulation responses are fully additive in human skin mast cells

Allergy - 2022

Babina M et al.

IgE-dependent and IgE- independent pathways unite to boost exocytosis of human skin MCs.

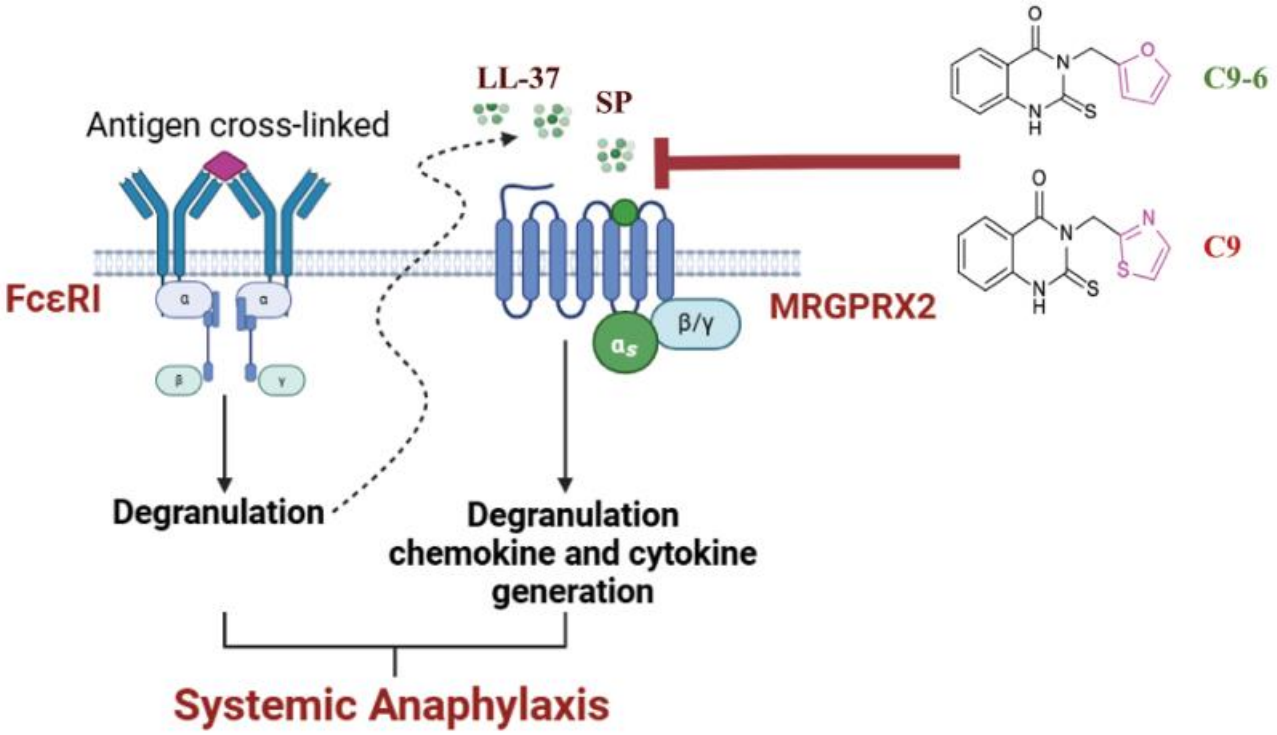


The same degree of mediator release and clinical response may thus be achieved by strong activation of FcεRI or MRGPRX2 individually or by weaker activation of the two simultaneously, the latter likely the common scenario in vivo.

MRGPRX2 facilitates IgE-mediated systemic anaphylaxis in a newly established knock-in mouse model

Maram Bawazir, BDS,[a,b] Sangita Sutradhar, PhD,[a] Saptarshi Roy, PhD,[a]* and Hydar Ali, PhD[a] *Philadelphia, Pa, and Jeddah, Saudi Arabia*

mice expressing human MRGPRX2



J Allergy Clin Immunol 2025;155:974-87

Glatiramer acetate induces mast cell degranulation via MRGPRX2, implications for local and systemic adverse reactions

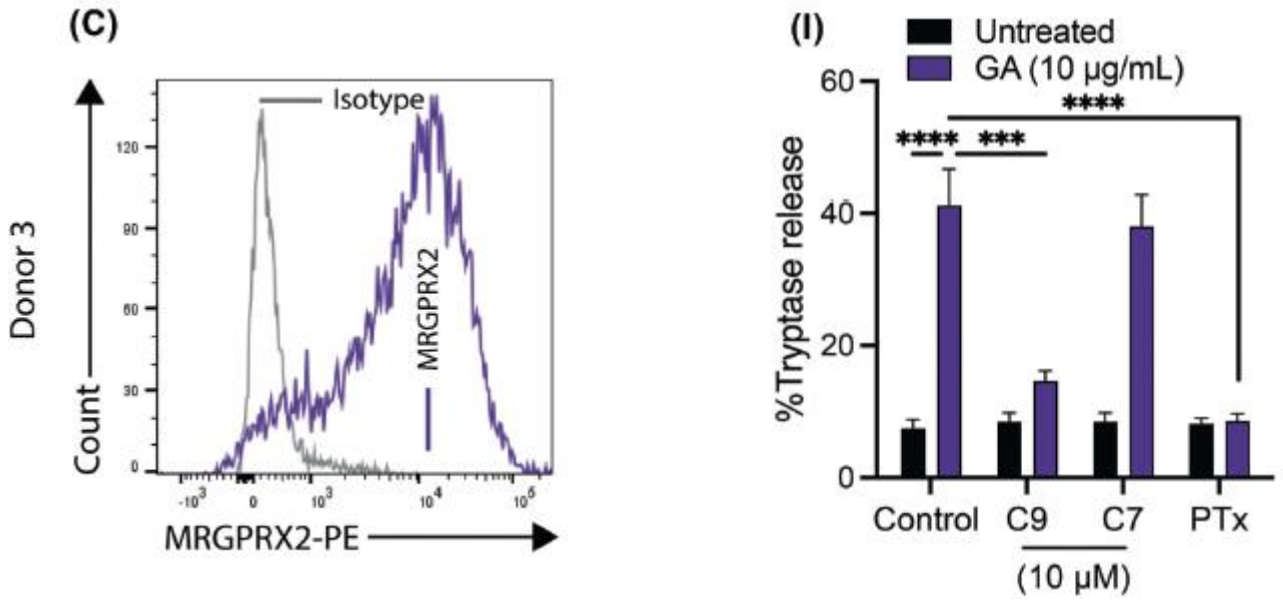
Chaki S, et al. Allergy. 2024 Mar;79(3):758-761

Glatiramer acetate (GA) is an immunomodulatory drug: a mixture of cationic peptides

It causes injection site reactions (ISRs) such as erythema and pruritus in most patients

It causes immediate postinjection systemic reactions (IPISR) in ~10% of the patients and may lead to the discontinuation of its use

Primary human healthy donors skin-derived MCs



C9 is an inverse agonist of MEGPRX2, C7 is an inactive analog

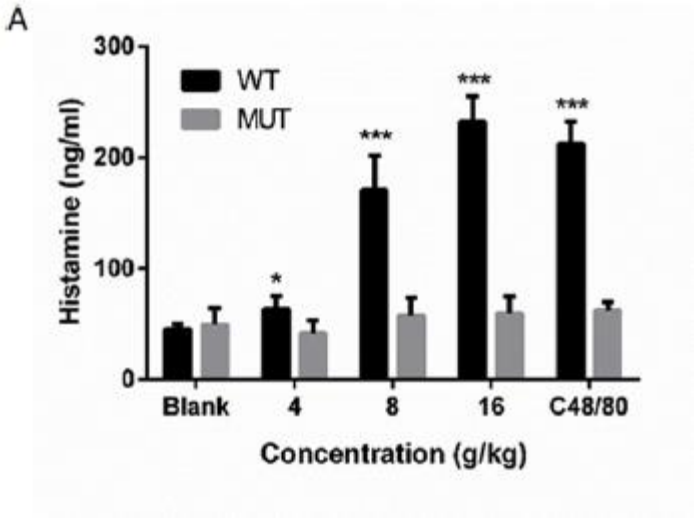
MRGPRX2 mediates immediate-type pseudo-allergic reactions induced by iodine-containing iohexol

Feng Yuan [a,1], Chi Zhang [a,1], Maji Sun [a], Dongying Wu [a], Lin Cheng [a], Bin Pan [a], Ting Li [b], Delu Che [c,d,*]

Biomedicine & Pharmacotherapy 137 (2021) 111323

OMNIPAQUE

Iohexol was shown to induce immediate-type pseudo-allergic reactions via MRGPRX2, which was dependent on the presence of iodine



Intravenous iron reactions: Insights from an allergy and immunology perspective

Check for updates

Jordon Jaggers, MD,[a] Cosby Stone, Jr, MD, MPH,[a] Matthew Krantz, MD,[a] and Elizabeth Phillips, MD[a,b] *Nashville, Tenn, and Murdoch, Australia*

J Allergy Clin Immunol Global 2025;4:100543.

Retrospective cohort study of patients with reactions to intravenous iron who

51 patients presenting for adverse reactions to intravenous iron, 48 had skin testing performed. The skin testing results were deemed negative in all 48 cases (100%).

Many patients (56%) were dermatographic, and their drug alert labels included opioids (31%), fluoroquinolones (14%), and radiocontrast dye (8%)

Key messages

- **Intravenous iron reactions have different clinical manifestations but are largely non–IgE-mediated and nonallergic, suggesting that they are not absolute contraindications for future administration.**
- **Most subjects tolerate future administration of intravenous iron with antihistamine pretreatment and reduced infusion rates, thus enhancing safety and feasibility.**
- **A high frequency of dermatographism and common co-labeling of drug alerts to MRGPRX2-activating drugs suggests a possible shared pathophysiologic mechanism for intravenous iron reactions.**

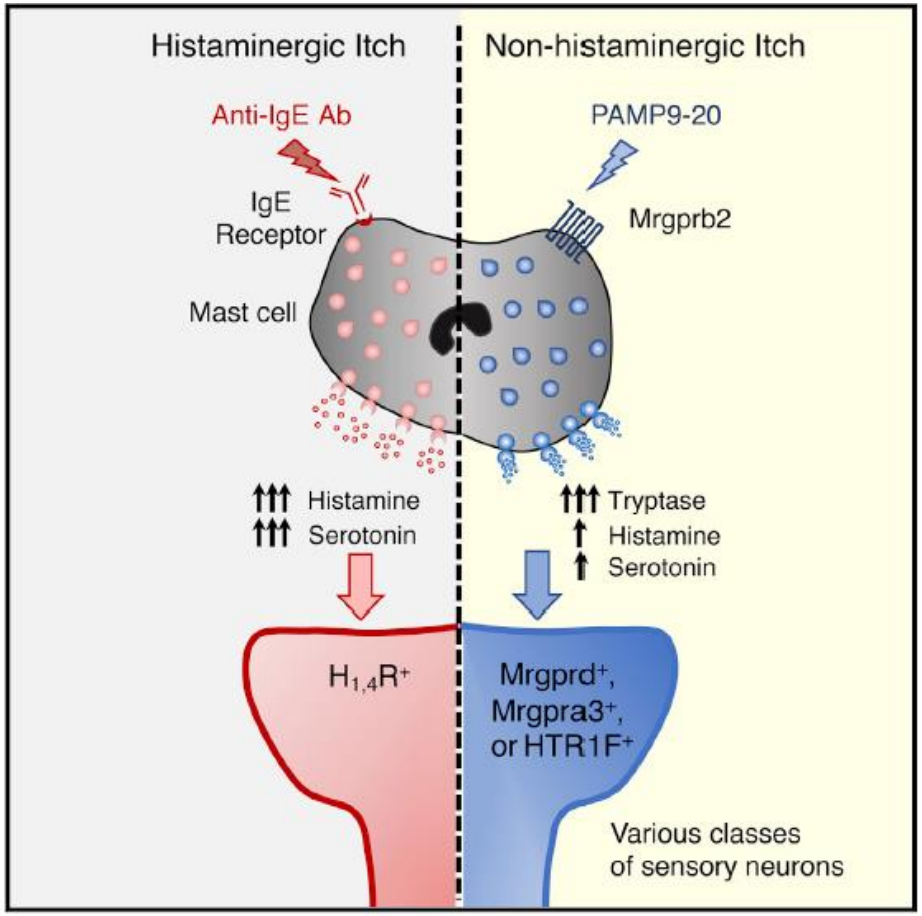
Symptoms frequently associated with activation of MEGPRX2

- Itch,
- Pain,
- Neurogenic inflammation,
- Chronic inflammatory diseases,
- Anaphylactoid reactions

Itch

Activation of Mast-Cell-Expressed Mas-Related G-Protein-Coupled Receptors Drives Non-histaminergic Itch

Meixiong et al., 2019, Immunity *50*, 1163–1171



Proadrenomedullin peptide 9-20 (PAMP 9-20)

Tryptases cleave proteinase-activated receptors (PARs) present on itch sensory neurons

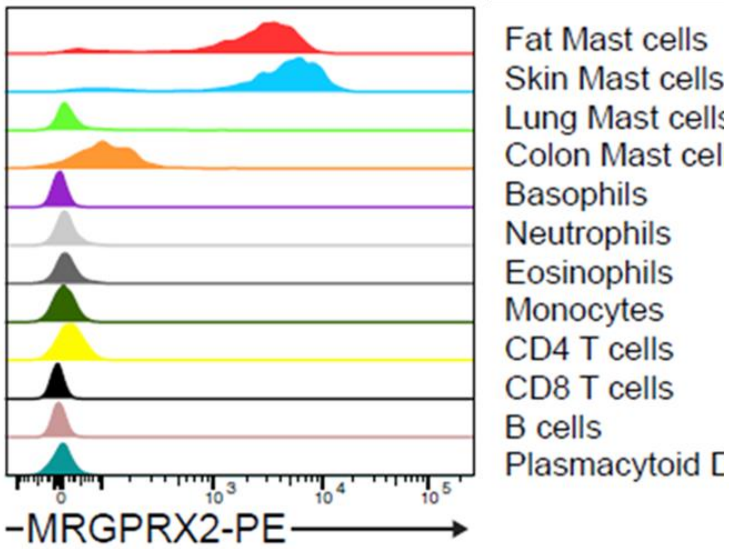
pruriceptive sensory nerves

Mrgprb2 deficiency decreased itch in multiple preclinical models of allergic contact dermatitis (ACD)

Can we in vitro evaluate MRGPRX2?

Directly on mast cells or conditioned basophils

Flow cytometry

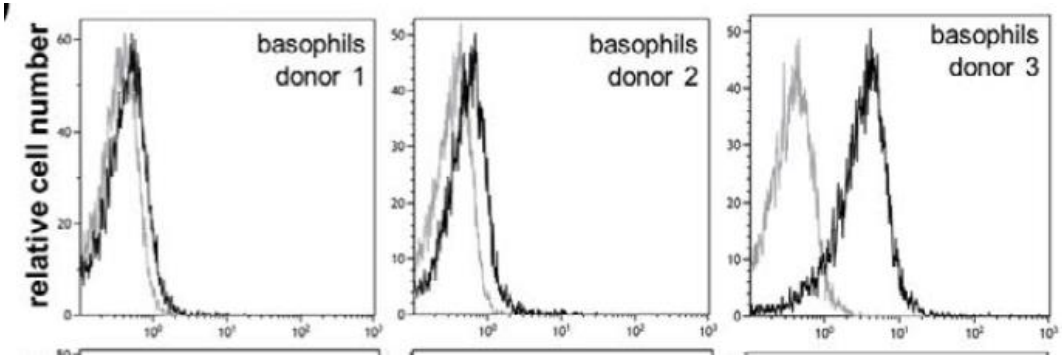


The pseudoallergen receptor MRGPRX2 on peripheral blood basophils and eosinophils: Expression and function

Allergy. 2020;75:2229–2242

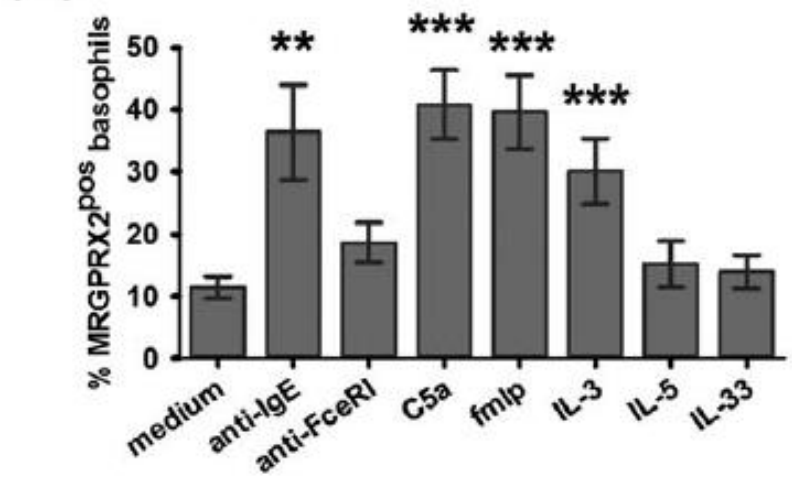
Bettina Wedi | Manuela Gehring | Alexander Kapp

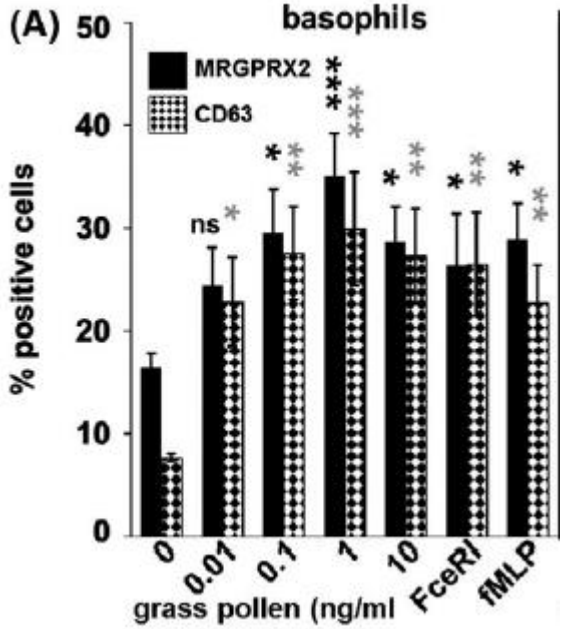
atopic and nonatopic results (n = 19-27), there was no statistically significant difference between basophils and eosinophils regarding %MRGPRX2$_{pos}$ cells



Grass pollen-allergic donors

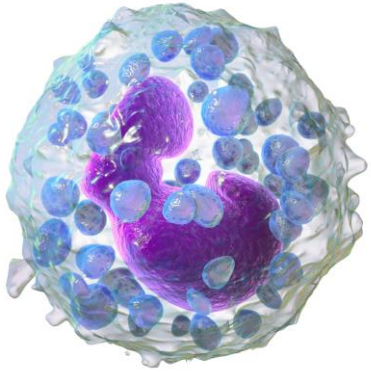
after 24 hours of incubation at 37°C



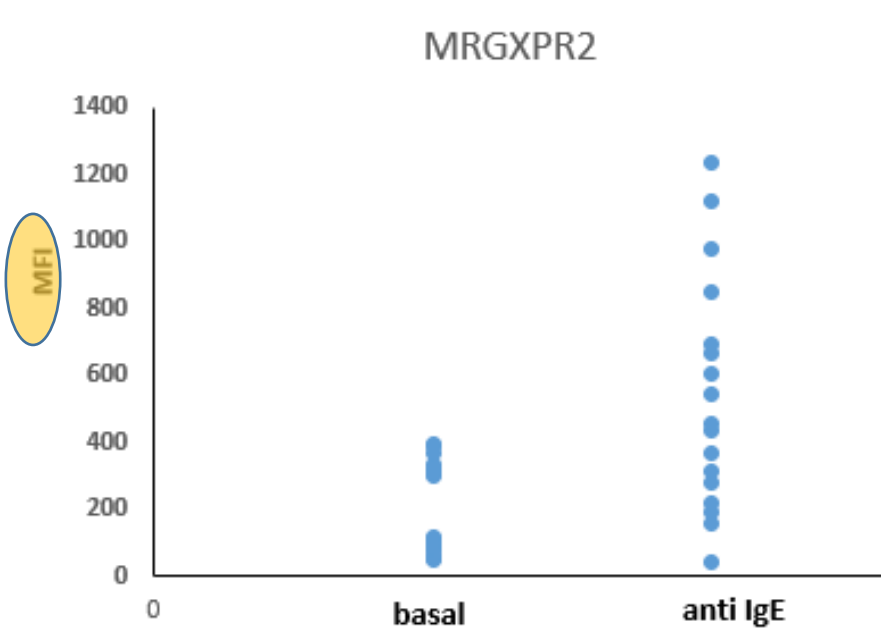


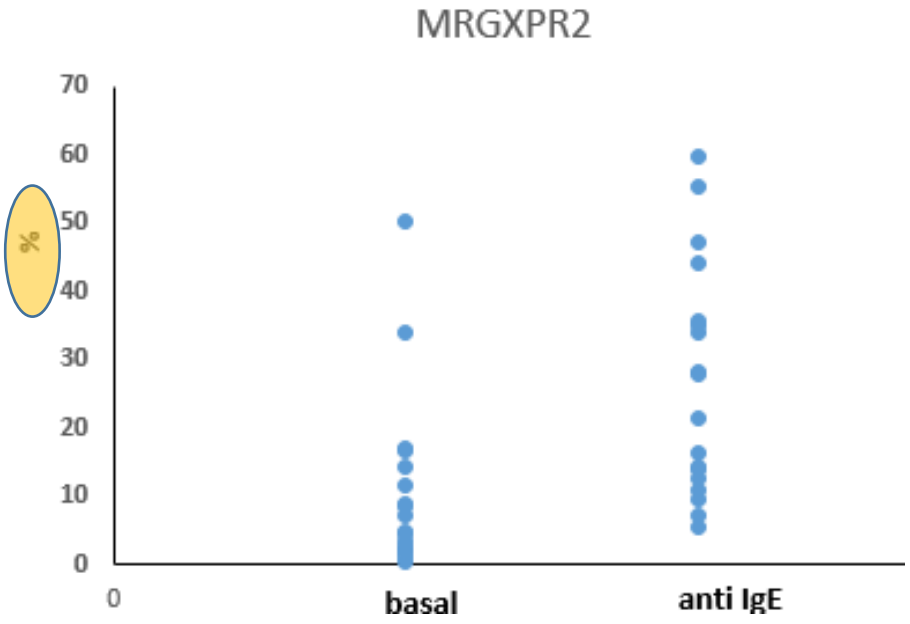
Expression of MRCPRX2 on basophils

Resting basophils barely express MRGPRX2 on their surface, but this expression can be quickly upregulated after stimulation, making 'conditioned' cells responsive to MRGPRX2 occupation



Our experience





Different patients with both long Covid syndrome or suspected mast cells disregulation

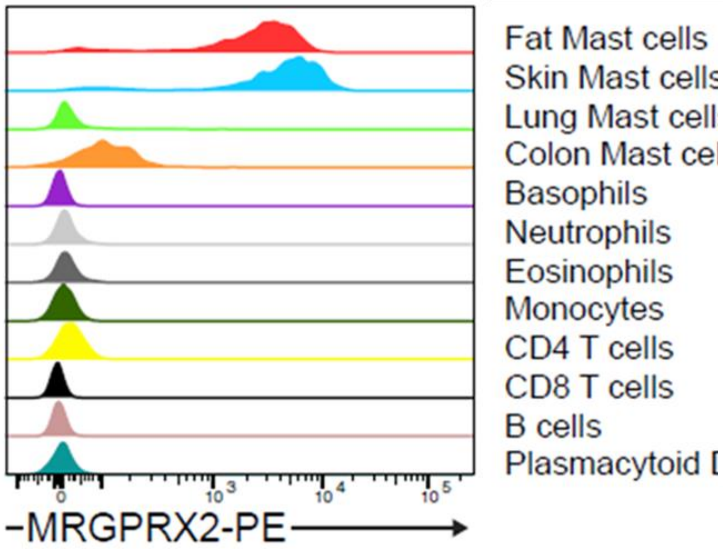
Can we in vitro evaluate MRGPRX2?

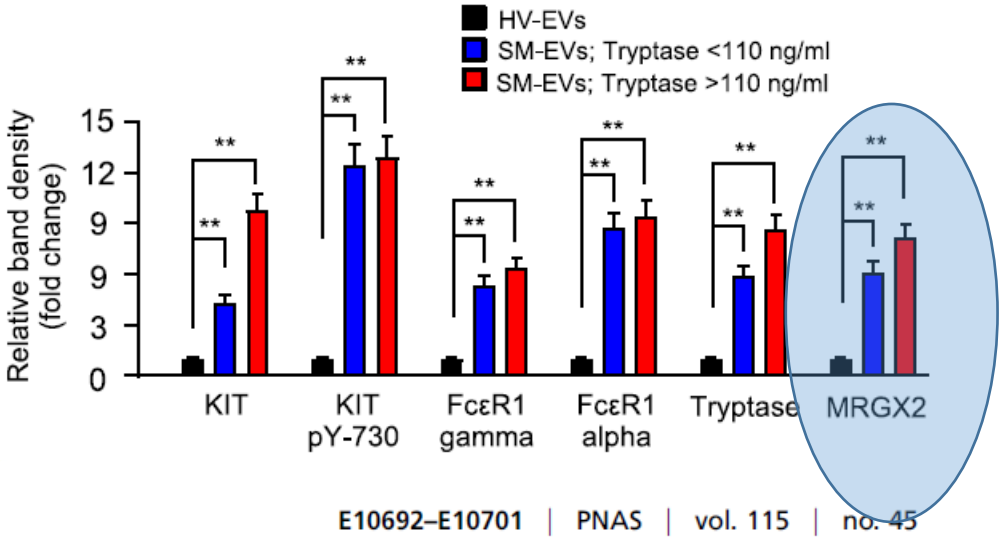
Directly on mast cells or conditioned basophils

In plasma/serum as soluble receptor or extracellular vescicles

Flow cytometry

ELISA, Western blotting





Clinical & Experimental Allergy, 2024; 0:1–3

Mas-Related G-Protein Coupled Receptor-X2 and Chemokine (C–C Motif) Ligand 2 Correlate With Disease Activity Among Treatment-Naïve Chinese Patients With Chronic Spontaneous Urticaria

Kristie Lao[1] | Hugo W. F. Mak[1] | Valerie Chiang[2] | Mukesh Kumar[3] | Billy K. C. Chow[3] | Philip H. Li[1]

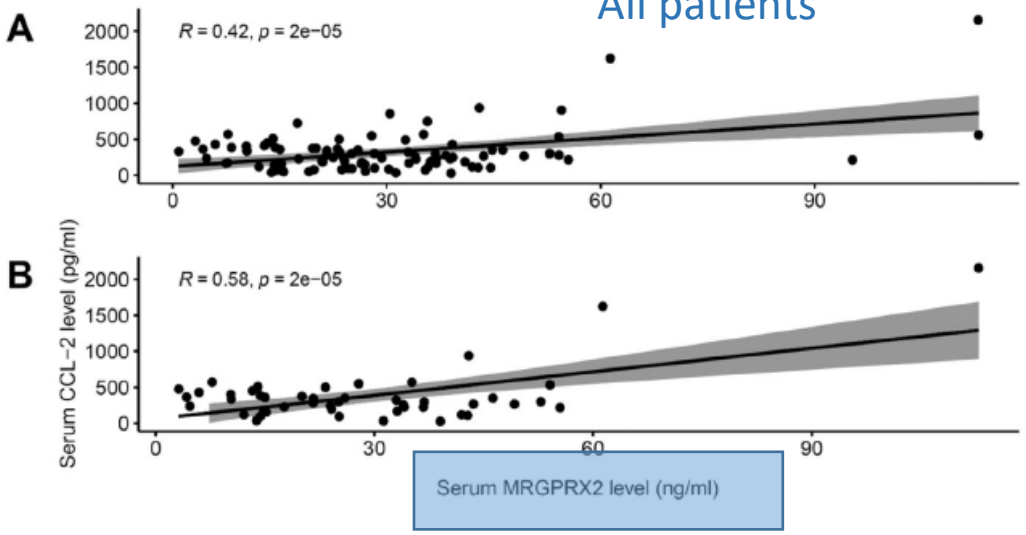
95 newly diagnosed CSU patients were recruited, of which 47 (49%) were treatment-naïve (79%) and 20 (21%) of patients had severe and nonsevere disease at recruitment

UAS7 ≥28 and <28 patients were classified as 'severe' and 'nonsevere', respectively

Patients with severe disease had higher **serum MRGPRX2** levels (severe: 41.06 ± 26.98; nonsevere: 26.64 ± 15.91 p = 0.011).

Subgroup analysis demonstrated that MRGPRX2 was only significantly higher among **treatment-naïve patients**

All patients



Treatment naive patients

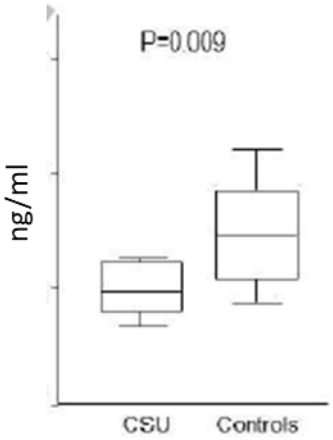
Severe chronic spontaneous urticaria responding and not responding to omalizumab: analysis of the prognostic value of known and novel in-vitro variables

Asero R et al

32 patients with chronic spontaneous urticaria at baseline and one week after Omalizumab

baseline

Soluble MRGPRX2



No differences after one week of treatment

Final response to Omalizumab may be predicted by IgE anti FCεRI at one week

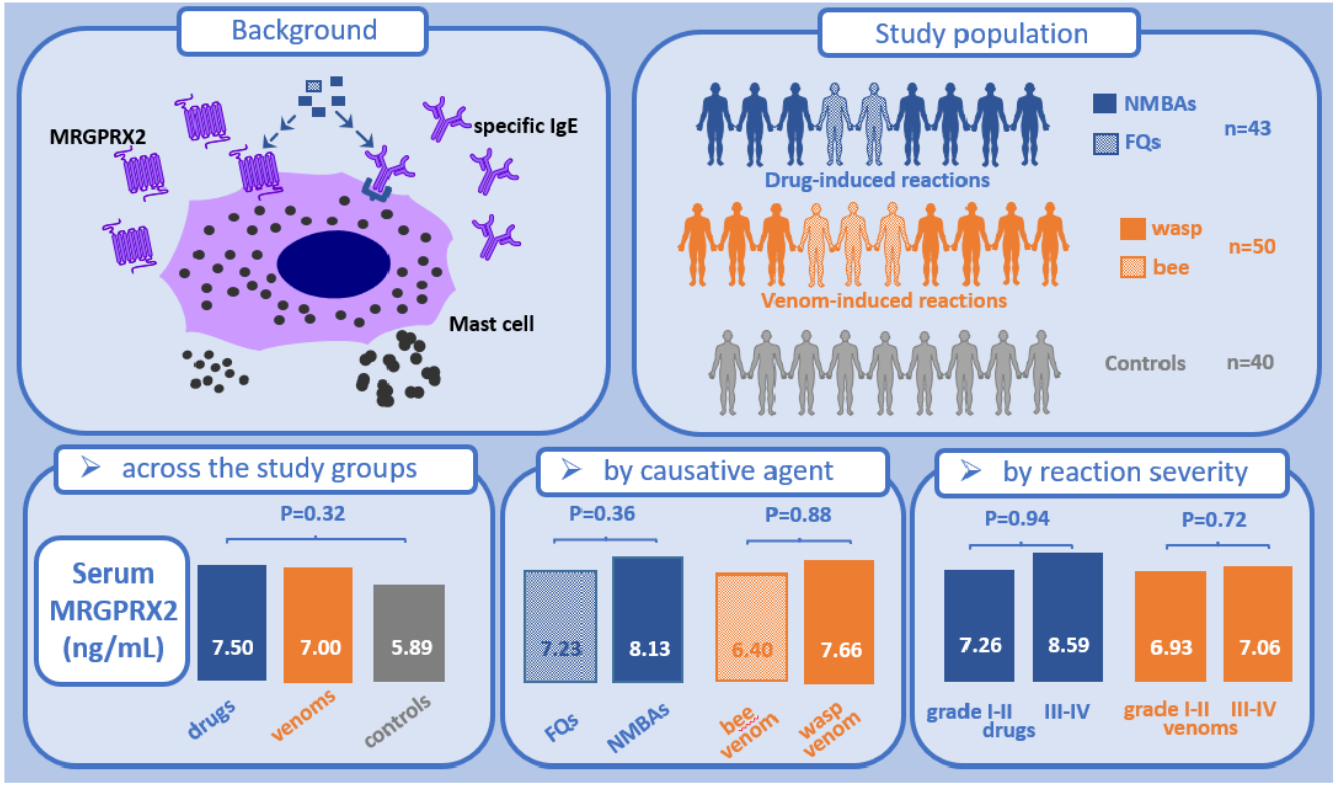
Eur Ann Allergy Clin Immunol. 2024 Nov 15

POLISH ARCHIVES OF
Internal Medicine

2026

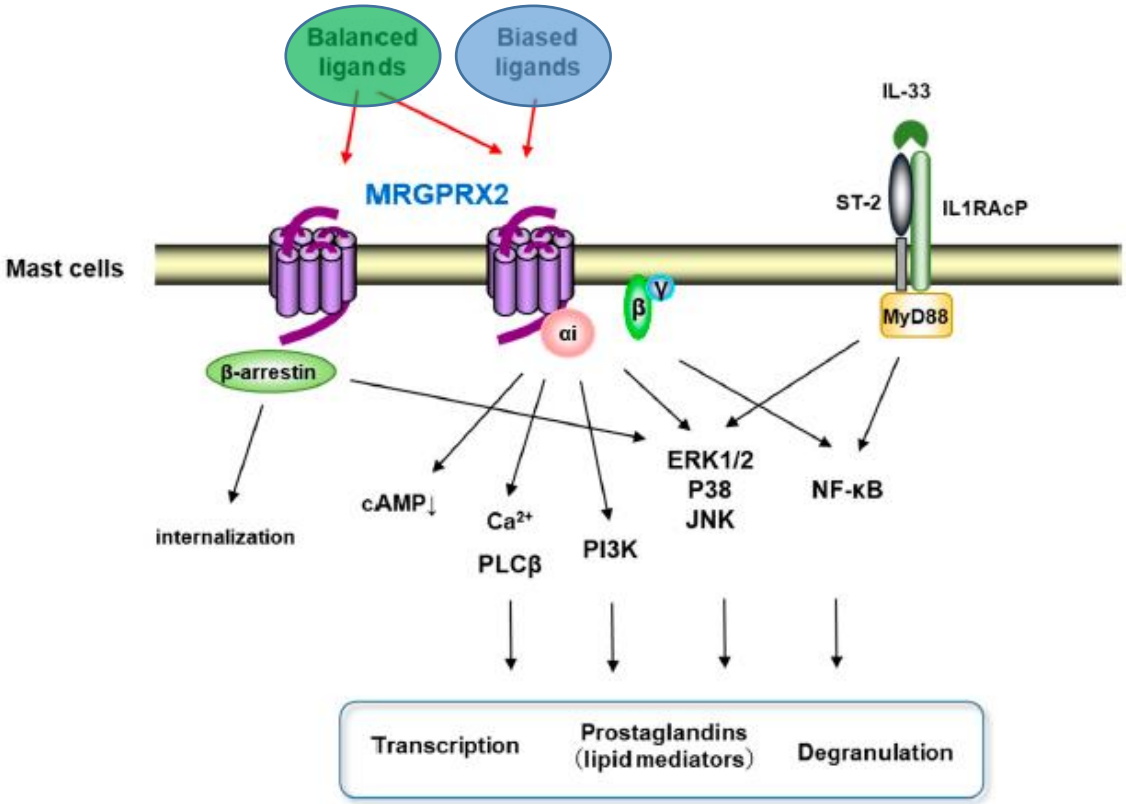
Serum Mas-related G protein-coupled receptor X2 concentration in drug-induced immediate hypersensitivity reactions

Alicja Dziadowiec[1], Mateusz Kwitniewski[2], Peter Kopac[3,4], Lenka Sedlackova[5], Adriana Srotova[6], Ana Koren[3], Joanna Gluck[7], Grzegorz Porebski[1]



Serum MRGPRX2 does not predict risk or severity of NMBA- or fluoroquinolone-induced immediate hypersensitivity reactions, challenging its role as a clinical biomarker.

SOLUBE RECEPTOR: BALANCED AND BIASED LIGANDS OF MRGPRX2



C48/80 or SP recruits β-arrestin 1 via MRGPRX2 to internalize and desensitize MRGPRX2 at the plasma membran

Babina, Met al. J. Investig. Dermatol. 2021, 141, 1286–1296

Icatibant was at least as potent as SP in eliciting MRGPRX2 internalization and (cross) desensitization in skin MCs.

Li Z, et al. Biomolecules. 2025 Aug 25;15(9):1224

Host defense peptides (HDPs) such as LL-37 — reported to activate G-protein signaling without significant β-arrestin recruitment

Chompunud Na Ayudhya C, et al. Int J Mol Sci. 2021 May 18;22(10):5318.

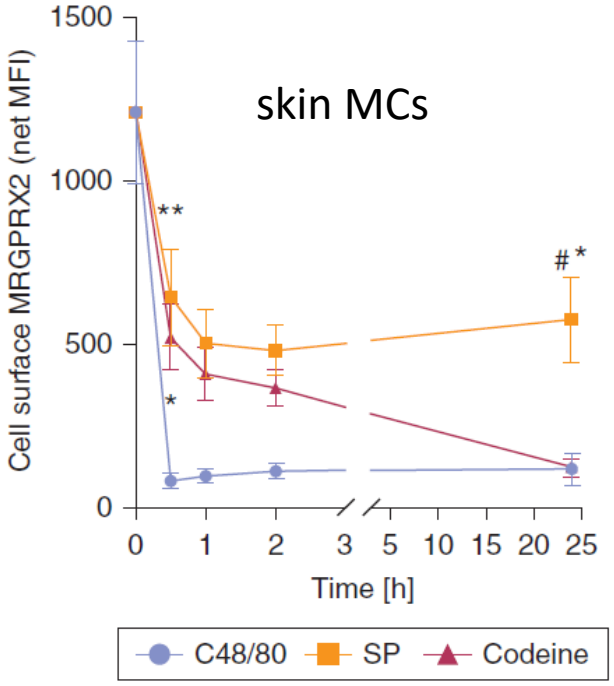
MRGPRX2 Is the Codeine Receptor of Human Skin Mast Cells: Desensitization through β-Arrestin and Lack of Correlation with the FcεRI Pathway

Magda Babina[1], Zhao Wang[1], Saptarshi Roy[2], Sven Guhl[1], Kristin Franke[1], Metin Artuc[1], Hydar Ali[2,3] and Torsten Zuberbier[1,3]

MRGPRX2 likely serves as the sole opiate receptor on skin MCs

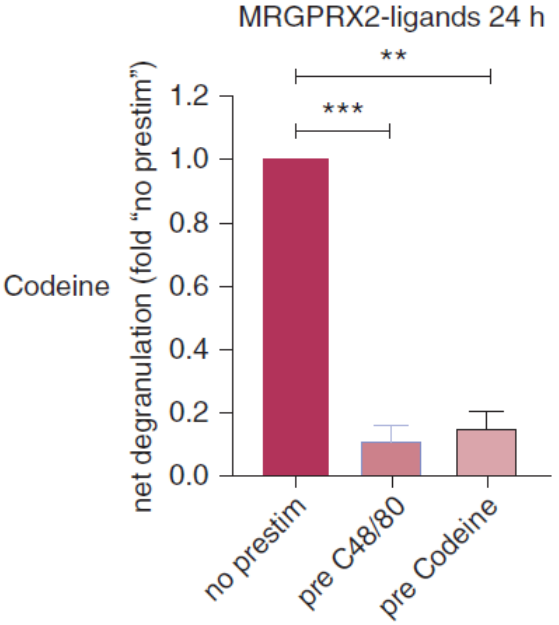
Codeine alone was able to achieve maximum MRGPRX2 activation.

MRGPRX2 was rapidly internalized on codeine binding in a b-arrestin-1–dependent manner.



Prestimulation with MRGPRX2 agonists (but not C3a or FceRI aggregation) resulted in refractoriness to further stimulation by the same or another MRGPRX2 ligand (cross desensitization).

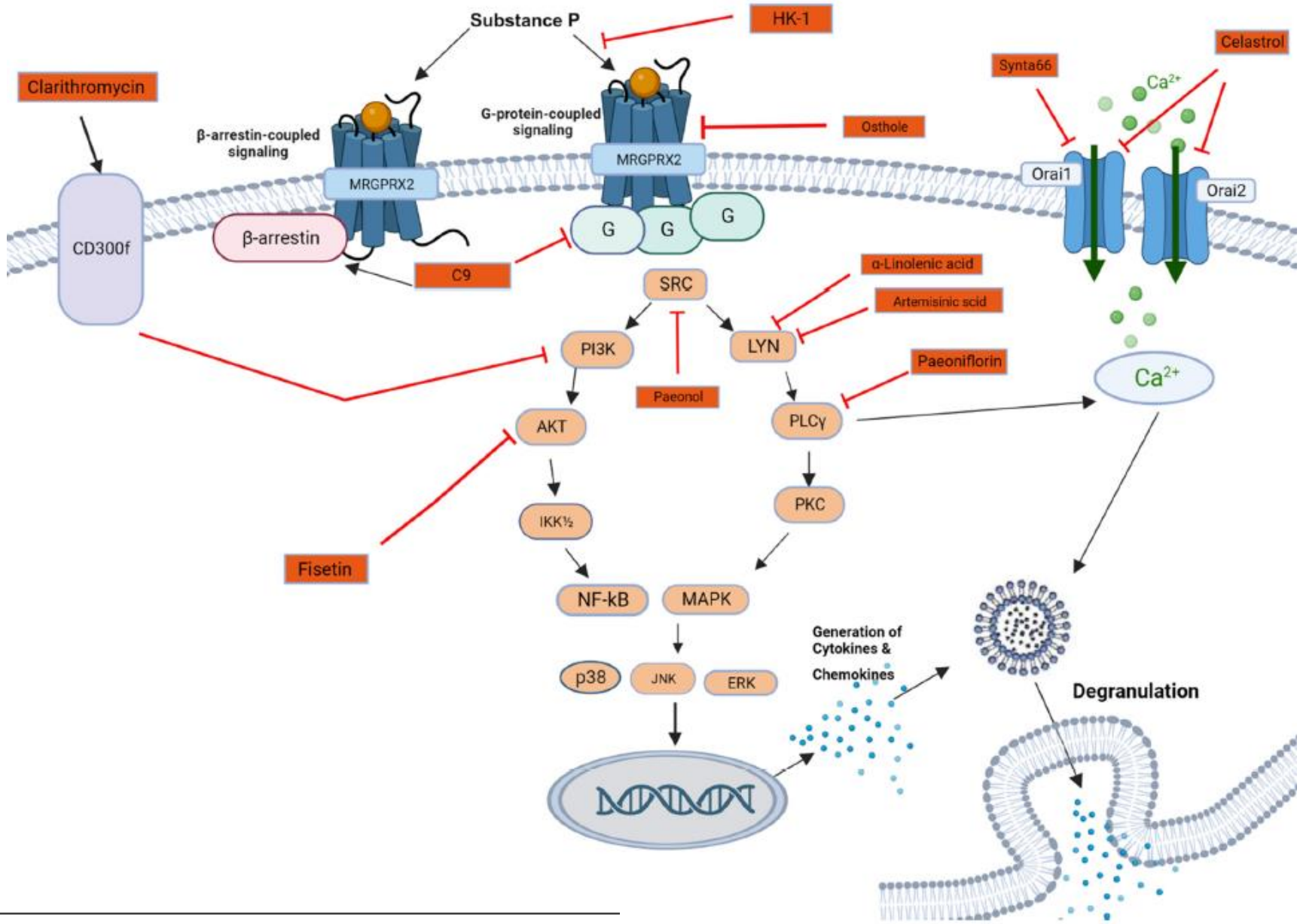
It has yet to be determined whether codeine-induced refractoriness could be exploited to desensitize MRGPRX2 to prevent severe pseudoallergic reactions.



Journal of Investigative Dermatology (2021) **141**, 1286–1296; doi:10.1016/j.jid.2020.09.017

MRGPRX2 a potential therapeutic target

β-arrestins have been reported to be negative regulators of MRGPRX2



Cells **2024**, *13*, 220. https://doi.org/10.3390/cells13030220

SMALL MOLECULES REGULATOR OF MRGPRX2 ACTIVATION

EP262, the first selective oral MRGPRX2 antagonist to enter Phase II clinical trials, inhibited MC degranulation in preclinical models in a dose dependent manner →chronic inducible urticaria

Wollam J, et al. J Allergy Clin Immunol. (2024) 154:1033–43.

GE1111, a small molecule identified through structure-based optimization, selectively inhibited MRGPRX2-mediated degranulation and attenuated the release of MCP-1 and PGD2

Kumar M, et al. J Allergy Clin Immunol. (2023) 151:1110–22.

C9, the first reported MRGPRX2-selective inverse agonist, effectively blocked both basal and ligand-induced G protein/b-arrestin-dependent signaling and inhibited degranulation induced by SP, PAMP-12, and rocuronium in primary human MCs and LAD2 cells

Bawazir M, et al. Front Immunol. (2022) 13:1033794

Compound B (GSK) inhibited SP-induced tryptase and histamine release in human skin MCs and demonstrated antipruritic efficacy in a humanized MRGPRX2 mouse model

Macphee CH, et al. Front Immunol. (2024) 15:1433982.

PSB-172656 potential to be developed for the treatment of so far difficult-to-treat mast cell-dependent diseases

Al Hamwi G, et al. Signal Transduct Target Ther. 2025 Apr 21;10(1):128

Prospective: Can we identify patients more susceptible to MRGPRX2 activation?

Clearly identify drugs able to activate MRGPRX2: injectable but not only… responsible of reactions mainly cutaneous but not only……

Selection of a panel of ligands:

- Rocuronium,
- Atracurium,
- Ciprofloxacine,
- Codeine,
- Vancomicin

Possible future use of cutaneous inhibitors of Bruton tyrosin kinase to inhibit IgE reactions

In vivo tests: SPTs

In vitro tests: BAT

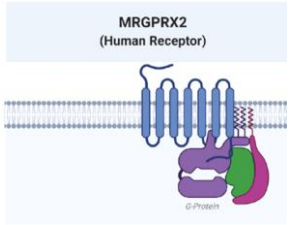
Concentration of drugs

Peak plasma levels used for IgE mediated reactions

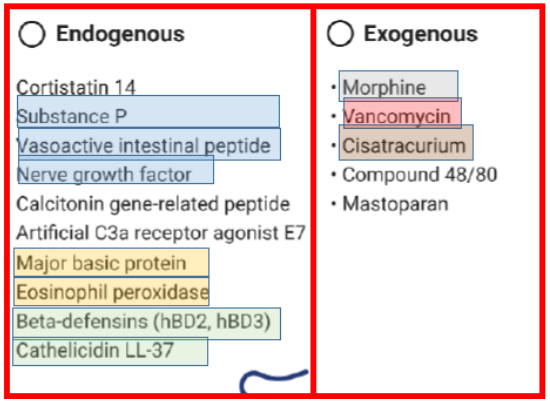
Higer concentrations to evaluate MRGPRX2 activation

Summary

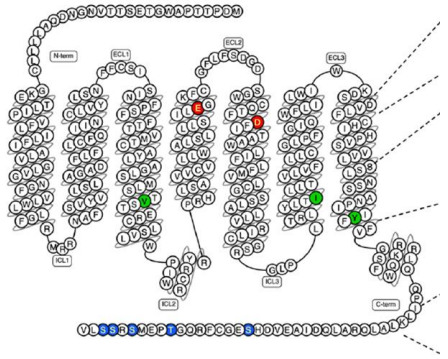
MRGPRX2 is a mast cell activator receptor, mainly present on Mast cells M_{TC}



Ligands are endogenous and exogenous peptides with cationic charge, many drugs included

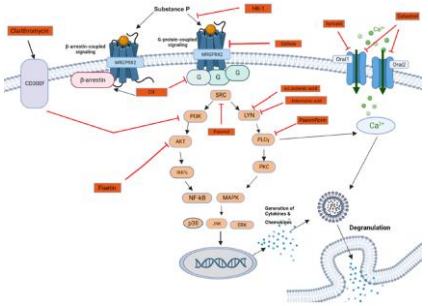


High drug concentrations activate MRGPRX2 receptor without a sensitization phase



Gene mutations can increase or decrease receptor activation

Symptoms of drug reactions mediated by MRGPRX2 activation are predominantly cutaneous and typically short-lived, although anaphylactoid reactions may also occur



Therapeutic strategies are focused on directly blocking or desensitizing the receptor or the transduced signals

Grazie per l’attenzione!