

Review article

Mas-related G protein-coupled receptor X2 and its activators in dermatologic allergies

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The Mas-related G protein-coupled receptor X2 (MRGPRX2) is a multiligand receptor responding to various exogenous and endogenous stimuli. Being highly expressed on skin mast cells, MRGPRX2 triggers their degranulation and release of proinflammatory mediators, and it promotes multicellular signaling cascades, such as itch induction and transmission in sensory neurons. The expression of MRGPRX2 by skin mast cells and the levels of the MRGPRX2 agonists (eg, substance P, major basic protein, eosinophil peroxidase) are upregulated in the serum and/or skin of patients with inflammatory and pruritic skin diseases, such as chronic spontaneous urticaria or atopic dermatitis. Therefore, MRGPRX2 and its agonists might be potential biomarkers for the progression of cutaneous inflammatory diseases and the response to treatment. In addition, they may represent promising targets for prevention and treatment of signs and symptoms in patients with skin diseases or drug reactions. To assess this possibility, this review explores the role and relevance of MRGPRX2 and its activators in cutaneous inflammatory disorders and chronic pruritus. (J Allergy Clin Immunol 2020;■■■:■■■-■■■.)

Key words: MRGPRX2, MRGX2, agonists, chronic spontaneous urticaria, atopic dermatitis, substance P, neuropeptides, eosinophil granule proteins, antimicrobial peptides, pruritus, itch

First described in 2001, the family of Mas-related G protein-coupled receptors (MRGPRs [formerly MRGs]) comprises approximately 50 members in mice¹ and 8 members in humans.²

Abbreviations used

AD: Atopic dermatitis
 AMP: Antimicrobial peptide
 BAM22: Bovine adrenal medulla 22
 C48/80: Compound 48/80
 CSU: Chronic spontaneous urticaria
 ECP: Eosinophil cationic protein
 EDN: Eosinophil-derived neurotoxin
 EPO: Eosinophil peroxidase
 FcεRI: High-affinity IgE receptor
 GPCR: G protein-coupled receptor
 hBD-2: Human β-defensin-2
 hBD-3: Human β-defensin-3
 HC: Healthy control
 MBP-1: Major basic protein-1
 MBP-2: Major basic protein-2
 MC: Mast cell
 MC_T: Mast cell expressing tryptase but not chymase
 MC_{TC}: Mast cell expressing tryptase and chymase
 MRGPR: Mas-related G protein-coupled receptor
 NMBA: Neuromuscular blocking agent
 PAF: Platelet-activating factor
 PAMP: Proadrenomedullin N-terminal peptide
 PTx: Pertussis toxin
 SCF: Stem cell factor
 SP: Substance P
 VIP: Vasoactive intestinal peptide

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It is a family of 7-transmembrane domain receptors that regulate cell proliferation, development, metabolism, survival, and neuronal signal transmission.³ On the basis of sequence homology, the family of MRGPRs can be divided into several subfamilies, namely, MRGPRA to MRGPRG and MRGPRX. Of these, the subfamilies MRGPR-A, MRGPR-B, and MRGPR-C are uniquely conserved in rodents, whereas the subfamily MRGPRX is characteristic among primates and humans. In humans, 4 MRGPRX genes, *MRGPRX1*, *MRGPRX2*, *MRGPRX3*, and *MRGPRX4*, have been described.¹⁻³

MRGPRX2 encodes for MRGPRX2 (also known as MRGX2), a 37 kDa G protein–coupled receptor (GPCR) that consists of 330 amino acids.^{2,4} Mrgprb2 and Mrgprb3 are mouse and rat orthologues of MRGPRX2, respectively.⁵ As MRGPRX2, these orthologues are expressed on mast cells (MCs). On the neuronal level, expression of MRGPRX2 overlaps with expression of murine Mrgpra1 and Mrgpra4, as well as with expression of 1 rat Mrgpr.^{5,6} However, MRGPRX2 shares only 45% to 65% amino acid sequence identity with the mouse and rat MRGPR.⁷

Interestingly, the human MRGPRX2 is activated by various exogenous and endogenous ligands with diverse chemical structures. It has been suggested to modulate both inflammatory responses and pseudoallergic drug reactions.

EXPRESSION OF MRGPRX2 ON MCs

A comprehensive single-molecule cDNA sequencing study that included cap analysis of gene expression across 975 human samples was performed. It revealed a major expression of MRGPRX2 on MCs, suggesting MRGPRX2 as a marker for skin-derived human MCs.⁸ In confirmation, quantitative mass spectrometry–based proteome analysis showed a unique proteome signature of MCs among immune lineages, in which MRGPRX2 was highly expressed by human skin MCs.⁹

MCs are key effector cells of the innate and adaptive immune system that regulate host defense mechanisms. In tissues exposed to the environment, MCs are present in high quantity. In skin, MCs account for up to 10% of all dermal immune cells.¹⁰ Most MCs found in human skin are connective tissue MCs expressing tryptase and chymase (MC_{TC}s), whereas most lung and gut MCs express tryptase but not chymase (MC_{TS}s).^{3,11} Interestingly, the copy number of *MRGPRX2* transcripts in connective tissue–like human cord blood–derived MCs was remarkably higher than in mucosal-like MCs.⁷ In line with this, skin MC_{TC}s but not lung MC_{TS} degranulated in response to basic compounds, such as compound 48/80 (C48/80) and substance P (SP) via MRGPRX2.^{7,11,12} Furthermore, PCR and microarray data showed that MRGPRX2 is expressed at high levels in human skin and synovial MC_{TC}s but at low levels in lung MC_{TS}.¹³ Notably, expression of MRGPRX2 decreased in primary human skin MCs with time of cultivation, decreasing approximately 10-fold within 4 to 5 weeks in culture.⁸ In addition, maintenance of human skin MCs in culture with stem cell factor (SCF) and IL-4 downregulated the expression of MRGPRX2 as well as MRGPRX2-triggered degranulation.¹⁴ LAD2 MCs and CD34⁺ cell–derived primary human MCs are known to express functional MRGPRX2 but not the immature human MC line HMC-1.¹⁵

In addition to occurring in MCs of the connective tissue, expression of MRGPRX2 has been described in small-diameter neurons of the dorsal root ganglion,¹⁶ keratinocytes,¹⁷ thymocytes,

and T-lymphocytes,¹⁸ as well as in blood basophils and eosinophils.¹⁹

HOW IS MRGPRX2 ACTIVATED?

Characteristically, MRGPRX2 is activated by ligands with cationic properties²⁰ that are endogenous and exogenous compounds with diverse chemical features ranging from peptides^{21,22} and proteins²³ to cysteine proteases²⁴ and opioids²⁵ (eg, antimicrobial host defense peptides, neuropeptides, eosinophil granule proteins, and many US Food and Drug Administration–approved peptidergic drugs). Tatemoto et al screened peptide and chemical libraries by using a reporter gene assay in PC12 cells transiently transfected with cDNA encoding MRGPRX2. They found that all of the known basic secretagogues tested activate MRGPRX2, a finding that has been confirmed in other studies.^{7,26-28} Thus, in contrast to the activation pattern of other GPCRs, MRGPRX2 is activated by a broad range of ligands.^{29,30}

Unlike other GPCRs (eg, C3aR), MRGPRX2 may be resistant to phosphorylation, internalization, and desensitization following activation by certain, albeit not all, agonists.^{15,31} The latter fact may explain the responsiveness of MRGPRX2 to multiple ligands.³ A single amino acid of the receptor was identified to be critical for binding and activation by its ligands, including pruritogens such as SP.⁶ Recently, naturally occurring loss-of-function missense variants of MRGPRX2 were described. These showed impaired responses to SP, hemokinin-1, human β -defensin-3 (hBD-3), and icatibant.⁴ Furthermore, Ayudhya et al have reported gain-of-function variants of MRGPRX2.³²

Activation of MRGPRX2 triggers G protein–coupled signaling cascades (Fig 1). The synthetic polymer C48/80, the basic secretagogue mastoparan, and antimicrobial proteins (AMPs) were able to activate MRGPRX2 and elicit the pertussis toxin (PTx)-sensitive G_{ai} pathway, specifically G_{ai2} and G_{ai3}.^{15,27,33} This induced phospholipase C signaling,³⁴ calcium release, and degranulation in MCs.^{15,27,33} In addition, AMPs were shown to induce phosphorylation of the mitogen-activated protein kinases p38, extracellular signal-regulated protein kinase (ERK)1/2, and c-Jun N-terminal kinases,^{21,35,36} which are required for AMP-induced IL-31 release.²¹ Codeine induced MRGPRX2 activation in LAD2 cells triggered by phosphorylation of ERK and JNK, but not p38.³⁷

Inhibitory studies were performed using the ion La³⁺, which blocks calcium release–activated calcium channels on the endoplasmic reticulum and plasma membrane. Their results suggest that MRGPRX2 additionally signals via the PTx-insensitive G_{aq} protein.^{15,27} Indeed, calcium mobilization in human MCs or MRGPRX2-transfected RBL-2H3 cells was not attenuated by PTx, but it required direct inhibition of calcium release–activated calcium channels. Furthermore, codeine-triggered degranulation could also be suppressed by a phosphoinositide 3-kinase inhibitor, whereas it was only partially dependent on extracellular calcium.³⁷

Besides activation of G_{ai} protein– and G_{aq} protein–coupled signaling, recent studies further suggest a MRGPRX2-mediated activation of β -arrestin.³¹ Interestingly, the MRGPRX2 ligand C48/80 induced robust β -arrestin activation and receptor internalization, whereas the MRGPRX2 ligands icatibant and the angiogenic host defense protein Ag-30/5C did not, indicating ligand-dependent internalization mechanisms potentially mediated via a differential activation of β -arrestin.³¹

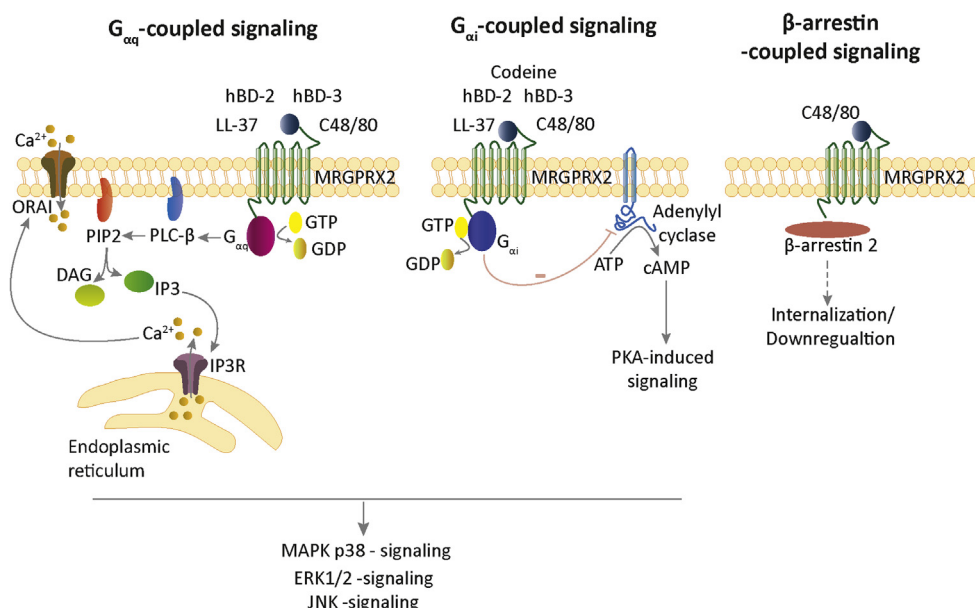


FIG 1. Signaling pathways triggered after MRGPRX2 activation.

REGULATION OF MRGPRX2 BY EXTRACELLULAR CUES

MCs are highly plastic cells, and their phenotype and functional programs are subject to constant adaptation by stimuli from the immediate micromilieu. Microenvironmental factors thereby constitute another level by which responsiveness to MRGPRX2 ligands can be regulated (as a result of altered MRGPRX2 expression, signaling, or both). Several mediators have been demonstrated over recent decades to regulate responsiveness to high-affinity IgE receptor (FcεRI) aggregation,^{38,39} whereas little is known about the regulation of MRGPRX2 expression.

Notwithstanding, SCF was shown to attenuate MC degranulation elicited via MRGPRX2.⁴⁰ The negative effect of SCF was also maintained after chronic exposure, probably as a result of receptor downregulation. The typical T_H2 cytokine IL-4 also interfered with MRGPRX2 expression and function while promoting allergic degranulation.¹⁴ Additionally, retinoic acid attenuated the pseudoallergic route during prolonged treatment, a response likewise associated with downregulation of MRGPRX2 expression.^{41,42}

Although the signaling route triggered by activation of the FcεRI more typically shows an inverse pattern of regulation *vis-à-vis* the MRGPRX2-initiated pathway, this is not a strict rule. With IL-33, for instance, both routes were attenuated, albeit to different degrees, with the effects on allergic degranulation being rather subtle,⁴³ whereas MRGPRX2 function was basically eradicated, as was receptor expression in IL-33-cultured cells.⁴²

Because MC-supportive factors are typically increased in MC-associated diseases such as atopic dermatitis (AD) and urticaria, the clinical significance of MRGPRX2 modulation by extracellular cues is uncertain at present but raises the interesting possibility that MRGPRX2 may act as a sensor of MC-supportive surroundings. Both SCF and IL-33 are enhanced in cutaneous inflammatory diseases,^{44,45} and they reduce MRGPRX2 function. Therefore, MCs may be equipped with a

mechanism to protect themselves and the surrounding tissue from overt stimulation in inflammatory disorders by dampening MRGPRX2, possibly as part of a negative feedback.

CELLULAR EFFECTS OF MRGPRX2 ACTIVATION AND ITS ROLE IN HEALTH AND DISEASE

Activation of MCs via MRGPRX2 has been described to trigger degranulation, chemotaxis, and cytokine release.³ For example, SP and other endogenous MRGPRX2 ligands are able to degranulate MCs (Fig 2, Table I⁴⁶⁻⁶²). Furthermore, AMPs, which are released following activation of epithelial cells, cause MC chemotaxis and degranulation via MRGPRX2,^{3,15} which modulates immune responses, orchestrates microbial clearance, and promotes wound healing.^{7,15}

Notably, there is a large interindividual variability in the responsiveness to MRGPRX2 ligands, as shown in skin MCs *ex vivo*.⁴⁰ Degranulation is largely equipotent between FcεRI aggregation and MRGPRX2 triggering on a population (yet not individual) basis.⁴⁰ However, FcεRI-driven allergic secretion did not correlate with the MRGPRX2-driven process.⁴⁰

Activation of MCs via MRGPRX2 may contribute to neurogenic inflammation, pain, itch, and pruritic skin diseases, including chronic spontaneous urticaria (CSU) and AD.³ For example, increased numbers of MCs and marked degranulation have been found in chronic lesional skin of patients with AD.⁶³ Furthermore, the release of histamine and other inflammatory mediators by skin MCs promotes acute and chronic urticaria and may contribute to allergic contact dermatitis.⁶⁴ Increased numbers of MRGPRX2-positive cells have been reported in the skin of patients with CSU.¹² In complementary studies, patients with CSU responded with augmented wheal reactions to intradermal application of the MRGPRX2 agonists SP and vasoactive intestinal peptide (VIP).^{65,66} Moreover, activation of MRGPRX2 has been suggested to lead to G_{αi}-dependent production of the

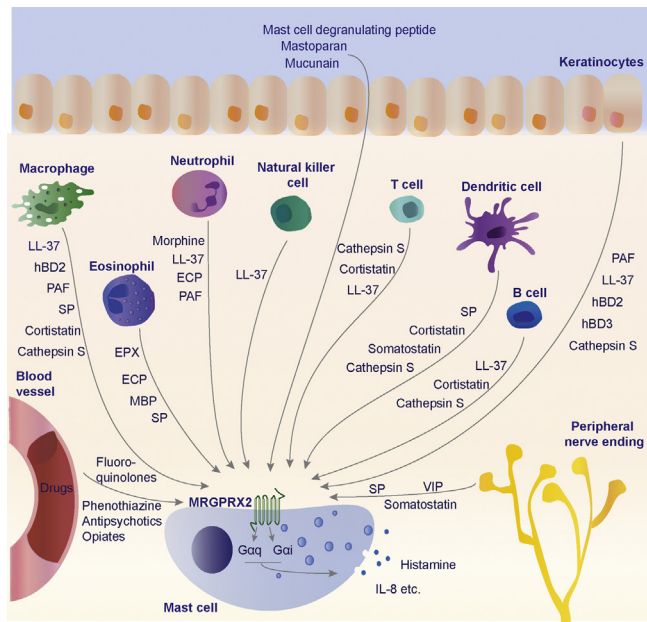


FIG 2. MRGPRX2-mediated MC activation in the human skin. Secretion of MRGPRX2-agonists from various dermal and immune cells and MRGPRX2-mediated MC degranulation. References are to be found in [Table I](#).

pruritogenic cytokine IL-31.³ Finally, MRGPRX2-associated MC activation may result in IgE-independent pseudoallergic reactions including, injection site reactions to opioids and other cationic drugs.²⁶

Interestingly, recent studies suggested a substantial MRGPRX2/Mrgprb2-dependent interaction of MCs and nociceptive sensory neurons modulating the development of allergic skin inflammation⁵⁹ and contributing to neurogenic inflammation and pain.⁶⁷ Thereby, the neuronal release of SP activates MRGPRX2 and its murine homolog Mrgprb2 on MCs, triggering the secretion of proinflammatory cytokines, whereas MCs can address sensory neurons by the release of distinct signaling molecules ([Fig 2](#), [Table I](#)), possibly promoting allergic contact dermatitis.^{59,67,68} Deletion of *Mrgprb2* or *Tac1*, which encodes the precursor of SP, reduced the clinical scores of house dust mite-triggered allergic reactions in mice.⁵⁹ Also, Mrgprb2 critically influenced immune cell recruitment and inflammation-induced hypersensitivity in a murine model of postoperative inflammatory pain.⁶⁷ Together with the notion that Mrgprb2-dependent activation of MCs causes a distinct degranulation pattern with granule morphology and released secretagogues that is different from IgE-dependent degranulation,^{68,69} these studies underline the mechanistic role of the murine homolog of MRGPRX2 in the development of MC-mediated skin diseases.

In summary, the notion that MRGPRX2 contributes to the pathogenesis of pruritic skin diseases is supported by (1) the functional expression of MRGPRX2 on skin MCs, (2) its responsiveness to multiple agonists, (3) the role of the murine homolog of MRGPRX2 in allergic conditions in mice, and (4) the upregulation of MRGPRX2 in the skin of patients with skin diseases (eg, CSU). This review explores this possibility by presenting and discussing the most important and interesting ligands of MRGPRX2 and evaluation of their relevance in pruritic skin conditions.

THE ROLE OF MRGPRX2 LIGANDS IN CHRONIC PRURITIC SKIN DISEASES

Neuropeptides and MRGPRX2

Neuropeptides such as SP are critically involved in the crosstalk of nerves and MCs, which is a key element of the bidirectional interaction between the immune and the nervous system. This crosstalk contributes to neurogenic inflammation, pain, and pruritus, which are important clinical features of many skin diseases.⁷⁰ After activation and degranulation, MCs release inflammatory, algogenic and pruritogenic mediators, which bind to specific nociceptors on sensory nerve fibers and activate them. Activated nerve fibers, in turn, secrete SP and other neuropeptides with inflammatory and vasoactive properties, which again activate MCs. Activated MCs can also produce neuropeptides, which may trigger autocrine signaling, modulating MC activation.⁷¹

SP and many other neuropeptides, including cortistatin, somatostatin, VIP, and the proadrenomedullin N-terminal peptides (PAMPs) PAMP-20 and PAMP-12, are MRGPRX2 agonists. They increase, via MRGPRX2 activation, the intracellular calcium concentration in MCs and degranulate them in a dose-dependent manner ([Table II](#)⁷²⁻¹⁰²).

The MRGPRX2-agonist SP. SP, a member of the tachykinin family, exerts its effects via MRGPRX2 and its conventional receptor, the tachykinin receptor 1 (NK-1).^{12,103} SP is produced by neuronal and nonneuronal cells, including immune cells. Importantly, it contributes to dermal antimicrobial host defense, neurogenic inflammation, itch, and pain.¹⁰⁴

Does SP play a role in chronic inflammatory skin diseases? Blood levels of SP in patients with CSU were increased in 3 of 4 studies.¹⁰⁵⁻¹⁰⁸ Also, SP levels were reported to correlate with CSU activity¹⁰⁵ and to be linked to absolute blood basophil counts.¹⁰⁶ SP induced wheal and flare reactions after intradermal injection or skin prick testing. These reactions are suppressed by antihistamines, indicating that SP activates MCs in the skin to release histamine.⁸² Interestingly, intradermal injections of SP resulted in significantly larger and longer-lasting wheal responses in patients with CSU than in healthy controls (HCs).⁶⁵

Blood SP levels are also increased in patients with AD, in whom they correlate with disease activity and pruritus intensity. In addition, Kim et al reported that SP increases the production of TNF- α , a proinflammatory cytokine, in PBMCs of patients with AD.¹⁰⁹ SP gene expression and immunoreactivity are increased in the lesional skin of patients with AD.¹¹⁰⁻¹¹² In contrast, many studies reported reduced wheal and/or flare responses to SP after intradermal injection and/or skin prick testing in patients with AD.^{81,113} This may be because of tachyphylaxis of MCs and blood vessels or their abnormal sensitivity to SP activation.¹¹³

Furthermore, blood levels and skin expression of SP were found to be increased in patients with cold urticaria, psoriasis, and chronic prurigo.^{105,114,115} In bullous pemphigoid, itch severity was described to correlate with SP expression as assessed by immunofluorescence staining.¹¹⁶ The clinical data are summarized in [Table III](#).¹¹⁷⁻¹³⁷

Supplementary to the strongly indicative data in humans, recent murine studies have reinforced the mechanistic role of Mrgprb2 and SP in the development of cutaneous inflammatory conditions. In a murine model of allergic inflammation triggered by house dust mite and *Staphylococcus aureus*, primary sensory neurons were shown to release SP, which provoked MC

TABLE I. Expression of MRGPRX2 agonists by cell types present in the human skin

Agonist	Expressing cells in the skin	References
hBD-2	Keratinocytes, macrophages	46
hBD-3	Keratinocytes	46
Cathepsin S	Keratinocytes, dendritic cells, B cells, T cells	47-49
ECP	Eosinophils, neutrophils	50
EPO	Eosinophils	51
LL-37	Neutrophils, macrophages, keratinocytes, natural killer cells, T cells, B cells	52
MBP	Eosinophils	53
Morphine	Neutrophils	54
PAF	Keratinocytes, macrophages, neutrophils	55,56
Somatostatin	Dendritic cells, nerve fibers	57,58
SP	Nerve fibers, macrophages, eosinophils	59-61
VIP	Nerve fibers	62

degranulation via Mrgprb2 and drove disease progression.⁵⁹ The activation of murine MCs by SP was furthermore shown to regulate recruitment of innate immune cells. These results may be transferable to humans, as SP-induced activation of LAD2 equally triggered the release of proinflammatory and immunomodulatory chemokines and cytokines.⁶⁷

Neuropeptide activation of MRGPRX2 beyond SP. MRGPRX2 binding and activation is a common feature of neuropeptides (Table II), which are thought to contribute to skin diseases. These include VIP, which in addition to MRGPRX2, exerts its effects via the VIP receptors type 1 and 2 (VPAC-1 and VPAC-2), chemoattractant receptor-homologous molecule expressed on T_H2 cells (CRTH2), and pituitary adenylate cyclase-activating polypeptide receptor 1 (PAC1) expressed on MCs, eosinophils, and other immune cells.¹³⁸

Patients with CSU exhibit increased wheal reactions to intradermal injections of VIP,⁶⁶ as well as moderately, albeit not significantly, increased blood levels of VIP.¹⁰⁷ Blood levels of VIP are also increased in patients with AD, and they correlate with itch intensity in 1 study but not in others.^{114,126,127} Similar to SP, responses to VIP skin prick testing and intradermal injections are reduced in patients with AD^{81,113,127} (summarized in Table III).

The role and relevance of other neuropeptide MRGPRX2 agonists, such as cortistatin, somatostatin, bovine adrenal medulla 22 docosaepptide (BAM22), neuropeptide FF, and PAMP-20, in the pathogenesis of chronic inflammatory skin diseases are less well characterized.²² Cortistatin and somatostatin are products of different genes but have structural similarity. Apart from MRGPRX2, they both activate somatostatin receptors (SSTR1 to SSTR5).¹³⁹ In 1 study, cortistatin blood levels and skin expression were lower in patients with psoriasis than in healthy subjects.¹⁴⁰ Furthermore, cortistatin can suppress keratinocyte growth *in vitro* in a dose-dependent manner. Somatostatin expression was detected in the skin of patients with eczema, urticaria pigmentosa, and psoriasis, as well as in HCs.¹¹² A recent murine study described the PAMP-triggered release of tryptase from murine MCs, whereas there was only a minor release of histamine. This possibly has implications on cutaneous pruritic diseases resistant to treatment with antihistamines, especially as dermal PAMP level was upregulated in patients with allergic contact dermatitis.⁶⁸

Several questions need to be addressed by future research. How important is the activation of MRGPRX2 by neuropeptides in skin diseases compared with that of other neuropeptide receptors (eg, NK-1 for SP)? Are BAM22, neuropeptide FF, and PAMP present in the skin and blood of patients with skin diseases? Can they

induce wheal and flare responses after intradermal injection or skin prick testing? How relevant are they for the development of cutaneous disorders?

Activation of MRGPRX2 by eosinophil granule proteins

Peptides and proteins that activate MRGPRX2 are rich in the amino acids proline, phenylalanine, tryptophan, and arginine/lysine, which are responsible for their hydrophobic and cationic properties.⁸⁴ Granules of human eosinophils contain various cationic proteins, including major basic protein-1 (MBP-1) and major basic protein-2 (MBP-2), eosinophil peroxidase (EPO [also referred to as EPX]), and the ribonuclease A superfamily members eosinophil cationic protein (ECP) and the eosinophil-derived neurotoxin (EDN).¹⁴¹ Because of their biochemical properties, these peptides are highly cytotoxic, and they kill parasites as well as bacteria by disrupting the integrity of their lipid bilayer.¹⁴¹⁻¹⁴³ In addition, these peptides are involved in viral defense and activation of effector cells. For example, MBP promotes superoxide anion production and IL-8 release from neutrophils,¹⁴⁴ EPO promotes the generation of reactive oxidants and radicals,¹⁴⁵ and ECP and EDN possess a potent antiviral activity.¹⁴⁶

MBP, EPO, and ECP have been described to induce histamine release from human MCs (Table II).^{23,85} However, their target receptor remained undefined until the discovery of MRGPRX2 on human MCs.⁷ Recent studies have provided direct evidence that binding of MBP, EPO, and ECP to MRGPRX2 leads to MC activation (ie, calcium influx and histamine release) in the absence of antigens.^{12,23} In contrast, EDN was unable to induce MRGPRX2-dependent calcium influx or histamine release from MCs.¹² This can be attributed to its lower content of basic amino acids as compared with MBP, EPO, or ECP,¹² as strong cationic proteins more likely trigger MRGPRX2 activation.¹²

Eosinophil infiltration in the skin is a key feature of several chronic pruritic skin diseases such as CSU.^{92,147} For example, MBP and EPO levels are increased in lesions of patients with chronic urticaria,^{12,147} and ECP serum levels and disease activity are linked in this disease.¹³⁰ Patients with AD exhibit elevated serum levels of MBP, EDN, and ECP, which correlate with disease activity (Table III). Interestingly, these proteins are detected in the skin of patients with AD even in the absence of eosinophils.¹⁴⁸ Eosinophil granule proteins persist in the skin and may drive sustained and disease-promoting activation of MRGPRX2.

TABLE II. Characteristic of endogenous and exogenous agonists of MRGPRX2

		Activation of MRGPRX2/ Mrgprb2-expressing cells		Cytokine release and/or degranulation of MCs		Wheal and flare reaction	
Class	Agonist	Human	Murine	Human	Murine/rat	Intradermal injection	Skin prick testing
Endogenous agonists							
Neuropeptides	PAMP-20 and PAMP-12	Yes ²²	Yes ²⁶	Yes (LAD2 ⁷²)	Yes (peritoneal ⁷³)	Yes ⁷⁴	—
	SP	Yes ^{7,75}	Yes ²⁶	Yes (skin, ^{12,13,76} synovial, ⁷⁵ cord, ⁷ LAD2 ⁷⁷); no (lung, gut, adenoids, heart ⁷⁸)	Yes (bone marrow ⁷⁹ , peritoneal ⁸⁰)	Yes ^{66,80,81}	Yes ^{81,82}
	VIP	Yes ⁷	—	Yes (cord ⁷ , skin ⁷⁶ , LAD2 ⁷⁷)	Yes (peritoneal ⁸⁰)	Yes ^{66,80,81}	Yes ⁸¹
	Cortistatin	Yes ⁸³	Yes ²⁶	Yes (cord ⁷ , LAD2 ⁸⁴)	Yes (peritoneal ²⁶)	—	—
	Somatostatin	Yes ⁷	—	Cord ⁷ , skin ⁷⁶	Yes (peritoneal ⁸⁰)	Yes ⁸⁰	—
	Neuropeptide FF	Yes ⁸³	—	—	—	—	—
	BAM22	Yes ⁸³	—*	—	—	—	—
Eosinophil granule proteins	ECP	Yes ²³	—	Yes (heart ⁸⁵ , cord blood–derived ²³)	—	—	—
	EPO	Yes ¹²	—	Yes (skin MC ¹²)	—	—	—
	EDN	No ¹²	—	No ^{12,85}	—	—	—
	MBP	Yes ²³	—	Yes (heart ⁸⁵ , cord blood–derived ^{23,86} , lung ⁸⁶ , skin ¹²)	—	—	—
AMPs	LL-37	Yes ¹⁵	—	Yes (LAD2, CD34 ⁺ -derived ⁸⁷)	—	—	—
	hBD-2	Yes ²⁷	—	Yes (LAD2, CD34 ⁺ -derived ²⁷)	No (BMMCs ²⁷)	—	—
	hBD-3	Yes ²⁷	—	Yes (LAD2, CD34 ⁺ -derived ²⁷)	No (BMMCs, peritoneal ²⁷)	—	—
Proteases	Cathepsin S	—	—†	Yes (LAD2 ²⁴)	—	—	—
Opioids	Morphine	Yes ²⁵	—	Yes (LAD2 ²⁵ , skin ⁷⁸); no (lung, gut, adenoids, heart ⁷⁸)	—	Yes ⁸⁸	Yes ⁸⁹
	Codeine	Yes ²⁵	—	Yes (LAD2, CD34 ⁺ -derived ³⁷)	—	Yes ⁸⁸	Yes ⁹⁰
Phospholipid	PAF	—	—	Yes (lung, peripheral blood–derived ⁹¹); no (skin ⁹¹)	—	—	—
Protein fragment	Chaperonin-10	Yes ²⁹	—	Yes (CD34 ⁺ -derived ²⁹)	—	—	—
Exogenous agonists							
Protease	Mucinain	Yes ²⁴	—	Yes (LAD2 ²⁴)	—	—	No ⁹²
Synthetic Polymer	C48/80	Yes ²⁸	Yes ^{26,‡}	Yes (LAD2, CD34 ⁺ -derived, skin ^{28,78}); no (HMC-1, lung, gut, adenoids, heart ^{28,78})	Yes (peritoneal ⁹³)	Yes ⁶⁶	Yes ⁹⁴
Drugs	Fluoroquinolone antibiotics	Yes ²⁶	Yes ²⁶	Yes (LAD2 ²⁶)	Yes (peritoneal ²⁶)	Yes ⁹⁵	Yes ⁹⁵
	Phenothiazine antipsychotics	Yes ⁹⁶	Yes ⁹⁶	Yes (LAD2 ⁹⁶)	—	—	—
	NMBAs	Yes ^{26,97}	Yes ^{26,97,98}	Yes (LAD2 ^{26,97,98})	Yes (peritoneal ^{26,97,98})	Yes ⁹⁹	Yes ¹⁰⁰
	Hormone and kinin receptor (ant)agonists	Yes ^{4,26}	Yes ²⁶	Yes (LAD2 ^{26,31})	Yes (peritoneal ²⁶)	Yes ¹⁰¹	—
	Natural remedies	Yes ^{25,93}	Yes ⁹³	Yes (LAD2 ^{93,102})	Yes (peritoneal ⁹³)	—	—

Em dash indicates no data.

*BAM22 is able to activate rat MrgprC.

†Cathepsin S was shown to activate murine Mrgprc11.

‡Mrgprb2-KO MCs show deficient activation.^{26,93}**AMPs as MRGPRX2 ligands**

Antimicrobial peptides (AMPs) are key innate defense molecules that strengthen epithelial barrier function,¹⁴⁹ modulate immune responses¹⁵⁰ and interact with bacteria inducing membrane damage.¹⁵¹ The human AMPs LL-37 and β -defensins are secreted by various cell types and tissues, including neutrophils,

keratinocytes, and MCs.¹⁵¹ LL-37, as well as human β -defensin (hBD)-2 (DEFB4) and hBD-3 (DEFB103), activate and degranulate MCs at low micromolar concentrations, with subsequent release of proinflammatory IL-8 and pruritogenic IL-31.²¹ This effect of AMPs on MCs is mediated via MRGPRX2, as has been shown in LAD2 and CD34⁺ cell-derived primary MCs by

TABLE III. Evidence for a possible role of MRGPRX2 agonists in selected skin diseases

Parameter	CSU	AD	Other skin diseases
SP			
Serum levels	↑ ¹⁰⁵⁻¹⁰⁷ or no difference ¹⁰⁸ ; correlation with disease activity ¹⁰⁵	↑ ^{114,117,118} ; correlation with intensity of pruritus ^{114,118} and disease activity ¹¹⁷	↑ in patients with cold urticaria, ¹⁰⁵ psoriasis, ¹¹⁴ and prurigo ¹¹⁵
Wheal and flare reaction after i.d. injections and/or skin prick testing	↑ In patients with CSU vs in HCs ⁶⁵ ; no difference ⁶⁶	↓ in patients with AD vs in HCs ^{81,113} ; ↑ in patients with AD vs in HCs ¹¹⁹	No difference between patients with psoriasis and HCs ¹²⁰
Presence/expresson in the skin	—	↑ ¹¹⁰⁻¹¹² or ↓ ¹²¹	↑ in patients with cold urticaria (vs in HCs), ¹²² psoriasis, ¹¹⁰ lesional nummular eczema skin, ¹¹¹ prurigo nodularis, ^{115,123,124} bullous pemphigoid, ¹¹⁶ and chronic itch; no difference in patients with eczema, psoriasis, or axillary hyperhidrosis vs in controls ¹²⁵ ; no correlation with itch in patients with prurigo nodularis ¹²³
PAMP			
Presence/expresson in the skin	—	—	↑ in patients with allergic contact dermatitis ⁶⁸
VIP			
Serum levels	No difference ¹⁰⁷	↑ ¹¹⁴ ; correlation with itch intensity ¹¹⁴ or no correlation with itch ¹²⁶ or severity ¹²⁷	↓ in patients with acute urticaria ¹²⁸ ; ↑ in patients with dermatographism and cold urticaria (vs in HCs) ¹²²
Wheal and flare reaction after i.d. injections and/or skin prick testing	↑ in patients with CSU vs in HCs ⁶⁶	↓ in patients with AD vs in HCs ^{81,113,127}	—
Presence/expresson in the skin	—	↑ in patients with lesional vs nonlesional skin ¹²⁹ or no difference ¹¹¹ ; no difference vs HCs ¹¹²	↑ in patients with cold urticaria, eczema, and psoriasis (vs in HCs) ^{122,125} ; no difference in lesional vs nonlesional skin of patients with nummular eczema and prurigo nodularis (vs in HCs) ^{111,124}
Somatostatin			
Serum levels	—	—	↑ in acute urticaria (vs HCs) ¹²⁸
Presence/expresson in the skin	—	No difference in patients with AD vs in HCs ¹¹²	No difference in patients with eczema, psoriasis, or axillary hyperhidrosis vs in controls ¹²⁵ ; ↑ in lesional skin of patients with urticaria pigmentosa ⁵⁸ ; ↑ in patients with urticaria pigmentosa (vs in HCs) ⁵⁸
ECP			
Serum levels	↑ ¹³⁰	↑ ^{131,132} ; correlation with disease activity ^{131,132}	—
hBD-2			
Serum levels	—	↑ ¹³³ ; correlation with disease activity ¹³³	—
Presence/expresson in the skin	—	↑ ¹³⁴	—
LL-37			
Serum levels	—	Correlation with disease activity ¹³⁵	—
Presence/expresson in the skin	—	↑ ¹³⁶	—
Cathepsin S			
Presence/expresson in the skin	—	—	↑ in patients with seborrheic dermatitis, ¹³⁷ psoriasis, and actinic keratosis ⁴⁹

i.d., Intradermal.

Upward arrow indicates upregulated or increased levels or larger reaction; downward arrow indicates downregulated or decreased levels or reduced reaction, and em dash indicates no data.

using knockdown approaches^{15,27} (Table II). After binding to MRGPRX2, LL-37 is internalized, where it is colocalized with MRGPRX2 in the perinuclear region.⁸⁷

AMPs are thought to be involved in the pathogenesis of AD, CSU, and other cutaneous allergic and inflammatory diseases by promoting inflammatory responses via the

activation of MCs (Table III). AMP protein levels, but not mRNA, are increased in the lesional skin of patients with AD.^{136,152-155} Furthermore, the expression of LL-37 and hBD-2 has been reported to moderately correlate with bacterial colony numbers¹⁵² and disease severity in patients with AD quantified with use of the Scoring Atopic

Dermatitis (SCORAD) index.^{133,135,153} As a result, LL-37 and hBD-2 have been proposed to serve as biomarkers for AD severity, although AMP expression in the skin of patients with AD does not appear to be linked directly to the degree of skin inflammation as evaluated by dermal infiltrates and epidermal CD3⁺ T cells.¹³⁶

Several synthetic and humanized AMPs showed antimicrobial activity and induced MRGPRX2-dependent degranulation of LAD2 cells.^{31,156} Interestingly, mutated hBD-3 in which all cysteine residues were replaced by serine residues and a shortened LL-37 (comprising residues 17-29) induced MRGPRX2-dependent degranulation of LAD2 cells and lacked the lipopolysaccharide-induced blockage of degranulation.¹⁵⁶ Thus, compared with naturally occurring AMPs, these synthetic AMPs potentially exhibit augmented antimicrobial efficiency.

Activation of MRGPRX2 by proteases

The cysteine endopeptidase cathepsin S activates MRGPRX2, possibly via hydrolysis of its N-terminus,¹⁵⁷ and it degranulates MCs²⁴ (Table II). Cathepsin S also hydrolyses the N-terminus of the protease-activated receptors (PARs) PAR 2 and PAR 4, which unmasks the peptide sequence SLIGKV, activates PAR2, and evokes itch.^{158,159} Mouse studies suggest that both, MRGPRX2 and PAR2-mediated effects contribute to cathepsin S-induced itch.^{157,160} Cathepsin S has been suggested to contribute to the pathogenesis of chronic inflammatory and pruritic skin diseases. First, its overexpression induces a spontaneous AD-like disorder in mice.¹⁶¹ Second, cathepsin S expression is strongly increased in the dermis of patients with AD, actinic keratosis, and psoriasis.⁴⁹ Moreover, increased expression of cathepsin S correlates with pruritus in dandruff/seborrheic dermatitis¹³⁷ (Table III).

MRGPRX2 is further activated by mucunain, a cysteine protease of the tropical legume *mucuna pruriens*.¹⁶² Mucunain also activates PAR2 and PAR4. When mucunain is applied intradermally with use of cowhage spicules, it induces strong itch but does not cause a wheal,^{163,164} although it has been reported to degranulate LAD2 MCs *in vitro*.^{6,24,162} In patients with AD, mucunain causes increased itch sensations, stronger hyperkinesia,¹⁶⁵ and prolonged itch duration compared with in HCs.¹⁶⁶ Taken together with an enhanced wheal formation in response to histamine, the latter predicts AD with a sensitivity of 91% and a specificity of 94%.¹⁶⁶

Opioids, opiates, and MRGPRX2

Opioids (ie, natural and synthetic substances that act on opioid receptors) and opiates (ie, drugs derived from opium) both degranulate MCs, especially rat peritoneal and human skin MCs.^{88,167} Opiates are used as positive controls in skin prick testing and intradermal tests,^{89,90} and opiate therapy can cause urticaria and pruritus.¹⁶⁸ Opioids and opiates have long been known to degranulate MCs^{37,89} and recent overexpression and knock-down strategies hinted at MRGPRX2 as the preferential receptor^{25,169} (Table II). A recent study demonstrated that human skin MCs degranulate in response to morphine and SP whereas lung or heart MCs do not,¹³ corroborating the preferential expression of MRGPRX2 on skin MCs.¹²

Whether endogenous opioids contribute to chronic inflammatory skin diseases, such as AD or urticaria, is currently unknown. A positive correlation between β -endorphin levels and AD

severity has been reported in several studies.^{170,171} κ -Opioid receptor agonists as well as μ -opioid receptor antagonists are potential therapeutics in the treatment of AD itch,¹⁷² and this action is likely unrelated to MRGPRX2 but is mediated by the targeted opioid receptors alone. Because some deregulation of the opioid system (including the canonic opioid receptors) is apparently involved in AD pathology, a proper interpretation of the relative contribution of MRGPRX2 versus opioid receptors to the connections between endogenous opioids and AD will require more definitive evidence (eg, from Mrgprb2-deficient versus opioid receptor-deficient mice).

In contrast to in AD, β -endorphin levels were found to be decreased in acute spontaneous urticaria.¹²⁸

Drugs that activate MRGPRX2

Several classes of commonly used drugs are known to activate MRGPRX2 (Table II). These include fluoroquinolone antibiotics, phenothiazines, neuromuscular blocking agents (NMBAs), hormone receptor (ant)agonists, and natural remedies.

Fluoroquinolone antibiotics. The fluoroquinolones ciprofloxacin, moxifloxacin, norfloxacin, and lomefloxacin are broad-spectrum antibiotics that inhibit prokaryotic gyrase enzymes. In 2015, McNeil et al demonstrated activation of MRGPRX2 by these fluoroquinolones *in vitro*, as measured by calcium mobilization assays with use of a MRGPRX2-overexpressing cell culture model and by β -hexosaminidase release of human LAD2.²⁶ Han et al confirmed these findings,¹⁰² whereas Lansu et al questioned them.²⁵ Hypersensitivity reactions (such as urticaria or anaphylaxis) after fluoroquinolone medication are common,^{95,173,174} as is pruritus.^{175,176} However, a few studies reported no pruritus as an adverse event in fluoroquinolone treatment.^{177,178} This might be due to differences in dose, application type, or study design.

Phenothiazines. Phenothiazines, in particular the world's first antipsychotic, chlorpromazine, have a wide field of action (eg, antipsychotic, sedative, anesthetic); a broad range of molecular interactions (eg, anticholinergic, antihistaminergic, antiadrenergic); and thus, a vast variety of adverse effects such as extrapyramidal symptoms,¹⁷⁹ hepatotoxicity,¹⁸⁰ and weight gain.¹⁸¹ The phenothiazine family members chlorpromazine, thioridazine, and trifluoperazine activate MRGPRX2, as shown by calcium mobilization and degranulation assays.⁹⁶ They also interact with various receptors, such as dopamine receptors.¹⁸²

NMBAs. The NMBA atracurium and its isomer cisatracurium induce intracellular calcium influx and MC degranulation via MRGPRX2 *in vitro*.^{26,98} Other NMBAs such as tubocurarine and mivacurium also likely activate MRGPRX2, as they have been shown to be agonists for the murine MRGPRX2 homolog Mrgprb2.²⁶ All 3 substances provoke wheal and flare responses following intradermal injection in humans.⁹⁹ Additionally, atracurium, which is used as a paralytic NMBA, is associated with perioperative hypersensitivity reactions.¹⁸³ In 2018, Navinés-Ferrer et al. demonstrated a direct link between NMBAs, MRGPRX2, and hypersensitivity reactions by using sera from patients who experienced anaphylactic reactions during anesthesia. These sera, but not control sera, induced MC degranulation, which was reduced following MRGPRX2 knockdown.¹⁶⁹

Hormone and kinin receptor (ant)agonists. Several hormone or kinin receptor agonists and antagonists bind to MRGPRX2. These include cetrorelix (a gonadotropin-releasing hormone receptor antagonist), icatibant (a bradykinin B₂ receptor

antagonist), the gonadotropin-releasing hormone receptor agonist leuporelin (also known as leuprolide), and sermorelin (a growth hormone–releasing hormone receptor agonist). All of them induced intracellular calcium influx in MRGPRX2-overexpressing cells.²⁶ For icatibant, Alkanfari et al additionally provided evidence for MRGPRX2-dependent MC degranulation in stably transfected RBL cells.⁴ Subcutaneous injections of icatibant, which is used for the treatment of swelling attacks by patients with hereditary angioedema, induced MC-mediated urticarial injection site reactions¹⁸⁴ that were attenuated by H1 antihistamines.¹⁰¹

Natural remedies. Many natural remedies, especially those used in traditional Chinese medicine, are ligands of MRGPRX2 and are therefore associated with anaphylactic or pseudoallergic reactions. These include praeruptorin A, schizandrin A, baicalin, the alkaloid sinomenine, the flavone glucuronides apigenin and luteolin, and salvianolic acid and its isomers.^{25,93,102,185} On the other hand, natural remedies are proposed to also act as MRGPRX2 antagonists (eg, quercetin¹⁸⁶ and saikosaponin A¹⁸⁷).

Additional MRGPRX2 activators

PAF. The role of platelet-activating factor (PAF) in the pathogenesis of anaphylaxis is well described in both murine models and human subjects.¹⁸⁸ It is a phospholipid (1-O-alkyl-2-acetyl-sn-glycero-3-phosphorylcholine) released by various human cells, such as platelets, MCs, macrophages, keratinocytes, and basophils,^{55,56,189} and it is involved in the regulation of immune responses. PAF can directly induce human MC degranulation (Table II), and it potentiates IgE-dependent mediator release. Marked heterogeneity of human MCs in the lung and skin has been observed with regard to mRNA expression of both the PAF receptor and MRGPRX2 receptors.⁹¹ This finding indicates a role of MRGPRX2 in PAF-mediated MC activation. Recently, serum PAF levels were found to be increased and linked to disease activity in patients with CSU.¹⁹⁰

Chaperonin-10. Certain endogenous protein and enzyme fragments, including chaperonin-10 fragment, have been shown to act as bioactive peptides and MRGPRX2-activating ligands, inducing degranulation of MCs (Table II). Chaperonin-10 is the N-terminal fragment of proteins found abundantly in the body. In a study utilizing porcine brain and intestinal extracts, chaperonin-10 demonstrated potent degranulating effects in human MCs that are comparable to those of other better-known basic secretagogues (eg, SP). Identification of these MC-activating protein fragments provides future opportunities in study of the role of MRGPRX2 in various inflammatory disorders.^{29,191}

C48/80. C48/80, which is a synthetic polymer produced by condensation of N-methyl-p-methoxy phenylethylamine with formaldehyde, has been known to activate human and rodent MCs since the early 1970s.¹⁹² When applied dermally, C48/80 induces MC degranulation and histamine release, triggering acute pruritus, protein extravasation, wheal and flare reactions, and increased heat radiation.⁹⁴ In 2011, Kashem et al showed that C48/80 degranulates differentiated, mature MCs as well as LAD2 and CD34⁺ cell–derived MCs but not cells of the immature MC line HMC-1.²⁸ As the latter lacks MRGPRX2 expression, MRGPRX2 was suggested to be a receptor for C48/80 (Table II). Indeed, C48/80 triggers transient calcium influx and degranulation in MRGPRX2-overexpressing RBL-1 cells.²⁸

Studies of anaphylactic reactions during cutaneous diseases showed that patients with CSU exhibited responses to C48/80 treatment similar to those exhibited by HCs.⁶⁶ Compared with the reaction of patients with CSU toward C48/80, the reaction of patients with AD to C48/80 treatment has aroused more controversy. Although several studies have suggested no aberrant C48/80 reaction in patients with AD,^{193,194} others have observed a reduced extravasation response in patients with AD.¹⁹⁵ Interestingly, C48/80-induced pruritus was abolished in HCs by application of the histamine receptor H1 inhibitor cetirizine, whereas H1 blockage did not affect pruritus in patients with AD.¹⁹⁵ These observations suggest that further MC mediators are involved in C48/80-induced pruritus.

Because of the potent histamine-releasing effects of C48/80, the substance has been widely used to study anaphylaxis in humans and mouse models. Besides having well-studied effects on human MCs via MRGPRX2, C48/80 also activates the human MRGPRX1,²⁸ potentially triggering further MRGPRX2-independent signaling cascades.

LIMITATIONS, CONTROVERSIES, AND PERSPECTIVES

In summary, the current state of research implies an important role of MRGPRX2 agonists in pruritic skin diseases. MRGPRX2 and several of the endogenous MRGPRX2 agonists, including SP, MBP, EP, LL-37, and hBD-2, are upregulated in serum and/or skin of patients with pruritic skin diseases such as AD and CSU. This suggests a link between MRGPRX2 signaling and the progression of cutaneous diseases.

Although the summarized data provide strong evidence for the contribution of MRGPRX2 and its agonists in dermatologic diseases, the exact role of MRGPRX2 in development of these diseases remains to be clarified. Elevated levels of MRGPRX2 agonists in the skin of patients with cutaneous allergies suggest that MCs are activated via MRGPRX2; as of now, however, possible negative feedback mechanisms have not been taken into account. MRGPRX2 internalization may affect availability of the receptor in skin diseases. Whether MRGPRX2 activation triggers receptor internalization is still controversially discussed, since C48/80 provoked β -arrestin-mediated receptor internalization, but LL-37 and AG-30/5C did not.^{15,31} In this context, it is likely that the variety of MRGPRX2 agonists have different effects on MRGPRX2 availability, MC activation, released signaling molecules, and immune cell recruitment. Until now, the role of MRGPRX2 agonists in skin diseases has been analyzed only individually; however, it is very likely that allergic conditions are provoked by a “cocktail” of MRGPRX2 agonists that may complement or reverse each other’s effects.

Importantly, MRGPRX2 and its agonists might be potential biomarkers for cutaneous diseases and/or responses to treatment, as has recently been proposed for allergic asthma.¹⁹⁶ Moreover, MRGPRX2 might represent a promising target for the treatment of symptoms in patients with CSU, AD, and other skin diseases or drug reactions. For example, MRGPRX2 antagonists have recently been identified and have been able to completely inhibit the degranulation of human cord blood–derived MCs¹⁹⁷ and lessen C48/80-induced local allergic inflammation in mice.¹⁹⁸

Targeting of MRGPRX2 further enables aimed MC depletion as recently described⁹ and may thus introduce new treatment options for patients with dermatologic allergies.

REFERENCES

- Dong X, Han S, Zylka MJ, Simon MI, Anderson DJ. A diverse family of GPCRs expressed in specific subsets of nociceptive sensory neurons. *Cell* 2001;106:619-32.
- Lembo PMC, Grazzini E, Groblewski T, O'Donnell D, Roy M-O, Zhang J, et al. Proenkephalin A gene products activate a new family of sensory neuron-specific GPCRs. *Nat Neurosci* 2002;5:201-9.
- Subramanian H, Gupta K, Ali H. Roles of Mas-related G protein-coupled receptor X2 on mast cell-mediated host defense, pseudoallergic drug reactions, and chronic inflammatory diseases. *J Allergy Clin Immunol* 2016;138:700-10.
- Alkanfari I, Gupta K, Jahan T, Ali H. Naturally occurring missense MRGPRX2 variants display loss of function phenotype for mast cell degranulation in response to substance P, hemokinin-1, human β -defensin-3, and icatibant. *J Immunol* 2018;201:343-9.
- Bader M, Alenina N, Andrade-Navarro MA, Santos RA. Mas and its related G Protein-coupled receptors, mrgprs. *Pharmacol Rev* 2014;66:1080-105.
- Reddy VB, Graham TA, Azimi E. A single amino acid in MRGPRX2 necessary for binding and activation by pruritogens. *J Allergy Clin Immunol* 2017;140:1726-8.
- Tatemoto K, Nozaki Y, Tsuda R, Konno S, Tomura K, Furuno M, et al. Immunoglobulin E-independent activation of mast cell is mediated by Mrg receptors. *Biochem Biophys Res Commun* 2006;349:1322-8.
- Motakis E, Guhl S, Ishizu Y, Itoh M, Kawaji H, De Hoon M, et al. Redefinition of the human mast cell transcriptome by deep-CAGE sequencing. *Blood* 2014;123:58-67.
- Plum T, Wang X, Rettel M, Krijgsvelde J, Feyerabend TB, Rodewald HR. Human mast cell proteome reveals unique lineage, putative functions, and structural basis for cell ablation. *Immunity* 2020;52:404-16.e5.
- Dwyer DF, Barrett NA, Austen KF, Kim EY, Brenner MB, Shaw L, et al. Expression profiling of constitutive mast cells reveals a unique identity within the immune system. *Nat Immunol* 2016;17:878-87.
- Oskeritzian CA, Zhao W, Min H-K, Xia H-Z, Pozez A, Kiev J, et al. Surface CD88 functionally distinguishes the MCTC from the MCT type of human lung mast cell. *J Allergy Clin Immunol* 2005;115:1162-8.
- Fujisawa D, Kashiwakura J-II, Kita H, Kikukawa Y, Fujitani Y, Sasaki-Sakamoto T, et al. Expression of Mas-related gene X2 on mast cells is upregulated in the skin of patients with severe chronic urticaria. *J Allergy Clin Immunol* 2014;134:622-33.e9.
- Varricchi G, Pecoraro A, Loffredo S, Poto R, Rivellese F, Genovese A, et al. Heterogeneity of human mast cells with respect to MRGPRX2 receptor expression and function. *Front Cell Neurosci* 2019;13:299.
- Babina M, Wang Z, Artuc M, Guhl S, Zuberbier T. MRGPRX2 is negatively targeted by SCF and IL-4 to diminish pseudo-allergic stimulation of skin mast cells in culture. *Exp Dermatol* 2018;27:1298-303.
- Subramanian H, Gupta K, Guo Q, Price R, Ali H. Mas-related gene X2 (MrgX2) is a novel G protein-coupled receptor for the antimicrobial peptide LL-37 in human mast cells: resistance to receptor phosphorylation, desensitization, and internalization. *J Biol Chem* 2011;286:44739-49.
- Zhang L, Taylor N, Xie Y, Ford R, Johnson J, Paulsen JE, et al. Cloning and expression of MRG receptors in macaque, mouse, and human. *Mol Brain Res* 2005;133:187-97.
- Kiatsurayanon C, Niyonsaba F, Chieosilapatham P, Okumura K, Ikeda S, Ogawa H. Angiogenic peptide (AG)-30/5C activates human keratinocytes to produce cytokines/chemokines and to migrate and proliferate via MrgX receptors. *J Dermatol Sci* 2017;83:190-9.
- van Hagen PM, Dalm VA, Staal F, Hofland LJ. The role of cortistatin in the human immune system. *Mol Cell Endocrinol* 2008;286:141-7.
- Wedi B, Gehring M, Kapp A. The pseudoallergen receptor MRGPRX2 on peripheral blood basophils and eosinophils: expression and function. *Allergy* 2020;1-39.
- Ferry X, Brehin S, Kamel R, Landry Y. G protein-dependent activation of mast cell by peptides and basic secretagogues. *Peptides* 2002;23:1507-15.
- Niyonsaba F, Ushio H, Hara M, Yokoi H, Tominaga M, Takamori K, et al. Antimicrobial peptides human β -defensins and cathelicidin LL-37 induce the secretion of a pruritogenic cytokine IL-31 by human mast cells. *J Immunol* 2010;184:3526-34.
- Kamohara M, Matsuo A, Takasaki J, Kohda M, Matsumoto M, Matsumoto SI, et al. Identification of MrgX2 as a human G-protein-coupled receptor for proadrenomedullin N-terminal peptides. *Biochem Biophys Res Commun* 2005;330:1146-52.
- Ogasawara H, Furuno M, Edamura K, Noguchi M. Peptides of major basic protein and eosinophil cationic protein activate human mast cells. *Biochem Biophys Reports* 2020;21:100719.
- Reddy VB, Azimi E, Chu L, Lerner EA. Mas-related G-protein coupled receptors and cowhage-induced Itch. *J Invest Dermatol* 2018;138:461-4.
- Lansu K, Karpiak J, Liu J, Huang X-PP, McCorvy JD, Kroeze WK, et al. In silico design of novel probes for the atypical opioid receptor MRGPRX2. *Nat Chem Biol* 2017;13:529-36.
- McNeil BD, Pundir P, Meeker S, Han L, Undem BJ, Kulka M, et al. Identification of a mast-cell-specific receptor crucial for pseudo-allergic drug reactions. *Nature* 2015;519:237-41.
- Subramanian H, Gupta K, Lee D, Bayir AK, Ahn H, Ali H. β -Defensins activate human mast cells via Mas-related gene X2. *J Immunol* 2013;191:345-52.
- Kashem SW, Subramanian H, Collington SJ, Magotti P, Lambris JD, Ali H. G protein coupled receptor specificity for C3a and compound 48/80-induced degranulation in human mast cells: roles of Mas-related genes MrgX1 and MrgX2. *Eur J Pharmacol* 2011;668:299-304.
- Tatemoto K, Nozaki Y, Tsuda R, Kaneko S, Tomura K, Furuno M, et al. Endogenous protein and enzyme fragments induce immunoglobulin E-independent activation of mast cells via a G protein-coupled receptor, MRGPRX2. *Scand J Immunol* 2018;87:e12655.
- Grimes J, Desai S, Charter NW, Lodge J, Moita Santos R, Isidro-Llobet A, et al. MrgX2 is a promiscuous receptor for basic peptides causing mast cell pseudo-degranulation and anaphylactoid reactions. *Pharmacol Res Perspect* 2019;7:1-12.
- Roy S, Ganguly A, Haque M, Ali H. Angiogenic host defense peptide AG-30/5C and bradykinin B2 receptor antagonist icatibant are G protein biased agonists for MRGPRX2 in mast cells. *J Immunol* 2019;202:1229-38.
- Ayudhya CCN, Roy S, Alkanfari I, Ganguly A, Ali H. Identification of gain and loss of function missense variants in MRGPRX2's transmembrane and intracellular domains for mast cell activation by substance P. *Int J Mol Sci* 2019;20.
- Ferry X, Eichwald V, Daeflter L, Landry Y. Activation of $\beta\gamma$ subunits of Gi2 and G13 proteins by basic secretagogues induces exocytosis through phospholipase C β and arachidonate release through phospholipase C γ in mast cells. *J Immunol* 2001;167:4805-13.
- Niyonsaba F, Iwabuchi K, Someya A, Hirata M, Matsuda H, Ogawa H, et al. A cathelicidin family of human antibacterial peptide LL-37 induces mast cell chemotaxis. *Immunology* 2002;106:20-6.
- Chen X, Niyonsaba F, Ushio H, Nagaoka I, Ikeda S, Okumura K, et al. Human cathelicidin LL-37 increases vascular permeability in the skin via mast cell activation, and phosphorylates MAP kinases p38 and ERK in mast cells. *J Dermatol Sci* 2006;43:63-6.
- Yu Y, Huang Z, Mao Z, Zhang Y, Jin M, Chen W, et al. Go is required for the release of IL-8 and TNF- α , but not degranulation in human mast cells. *Eur J Pharmacol* 2016;780:115-21.
- Sheen CH, Schleimer RP, Kulka M. Codeine induces human mast cell chemokine and cytokine production: involvement of G-protein activation. *Allergy* 2007;62:532-8.
- Gilfillan AM, Tkaczuk C. Integrated signalling pathways for mast-cell activation. *Nat Rev Immunol* 2006;6:218-30.
- Huber M, Cato ACB, Ainooson GK, Freichel M, Tsvilovsky V, Jessberger R, et al. Regulation of the pleiotropic effects of tissue-resident mast cells. *J Allergy Clin Immunol* 2019;1-15.
- Babina M, Guhl S, Artuc M, Zuberbier T. Allergic Fc ϵ RI- and pseudo-allergic MRGPRX2-triggered mast cell activation routes are independent and inversely regulated by SCF. *Allergy* 2018;73:256-60.
- Babina M, Artuc M, Guhl S, Zuberbier T. Retinoic acid negatively impacts proliferation and MCTC specific attributes of human skin derived mast cells, but reinforces allergic stimulatory. *Int J Mol Sci* 2017;18.
- Wang Z, Guhl S, Franke K, Artuc M, Zuberbier T, Babina M. IL-33 and MRGPRX2-triggered activation of human skin mast cells-elimination of receptor expression on chronic exposure, but reinforced degranulation on acute priming. *Cells* 2019;8:341.
- Babina M, Wang Z, Franke K, Guhl S, Artuc M, Zuberbier T. Yin-Yang of IL-33 in human skin mast cells: reduced degranulation, but augmented histamine synthesis through p38 activation. *J Invest Dermatol* 2019;139:1516-25.e3.
- Kanbe T, Soma Y, Kawa Y, Kashima M, Mizoguchi M. Serum levels of soluble stem cell factor and soluble KIT are elevated in patients with atopic dermatitis and correlate with the disease severity. *Br J Dermatol* 2001;144:1148-53.
- Salimi M, Barlow JL, Saunders SP, Xue L, Gutowska-Owsiak D, Wang X, et al. A role for IL-25 and IL-33-driven type-2 innate lymphoid cells in atopic dermatitis. *J Exp Med* 2013;210:2939-50.
- Chieosilapatham P, Ogawa H, Niyonsaba F. Current insights into the role of human β -defensins in atopic dermatitis. *Clin Exp Immunol* 2017;190:155-66.
- Sage J, De Quérat D, Leblanc-Noblesse E, Kurfurst R, Schnebert S, Perrier E, et al. Differential expression of cathepsins K, S and V between young and aged Caucasian women skin epidermis. *Matrix Biol* 2014;33:41-6.

48. Villadangos JA, Bryant RAR, Deussing J, Driessen C, Lennon-Duménil AM, Riese RJ, et al. Proteases involved in MHC class II antigen presentation. *Immunol Rev* 1999;172:109-20.
49. Schönefuß A, Wendt W, Schattling B, Schulten R, Hoffmann K, Stuecker M, et al. Upregulation of cathepsin S in psoriatic keratinocytes. *Exp Dermatol* 2010;19:e80-8.
50. Ribas D, Fernández-Carranco MC, Hajji N, Bobadilla P, Monteseirín J. Eosinophil cationic protein and histamine production by neutrophils from patients with periodontitis. *J Periodontol* 2018;89:228-34.
51. Nair P, Ochkur SI, Protheroe C, Radford K, Efthimiadis A, Lee NA, et al. Eosinophil peroxidase in sputum represents a unique biomarker of airway eosinophilia. *Allergy* 2013;68:1177-84.
52. Bals R, Wilson JM. Cathelicidins—a family of multifunctional antimicrobial peptides. *Cell Mol Life Sci* 2003;60:711-20.
53. Furuta GT, Ackerman SJ, Varga J, Spiess AM, Wang MY, Wershil BK. Eosinophil granule-derived major basic protein induces IL-8 expression in human intestinal myofibroblasts. *Clin Exp Immunol* 2000;122:35-40.
54. Glattard E, Welters ID, Lavaux T, Muller AH, Laux A, Zhang D, et al. Endogenous morphine levels are increased in sepsis: A partial implication of neutrophils. *PLoS One* 2010;5.
55. Travers JB, Huff JC, Rola-Pleszczynski M, Gelfand EW, Morelli JG, Murphy RC. Identification of functional platelet-activating factor receptors on human keratinocytes. *J Invest Dermatol* 1995;105:816-23.
56. Kenneth M, Weaver C. Janeway's immunobiology. 9th ed. New York: Garland Science; 2017. p. 613-5.
57. Johansson O, Vaalasti A. Immunohistochemical evidence for the presence of somatostatin-containing sensory nerve fibres in the human skin. *Neurosci Lett* 1987;73:225-30.
58. Johansson O, Nordlind K. Immunohistochemical localization of somatostatin-like immunoreactivity in skin lesions from patients with urticaria pigmentosa. *Virchows Arch B Cell Pathol Incl Mol Pathol* 1984;46:155-64.
59. Serhan N, Basso L, Sibilano R, Petitfils C, Meixiong J, Bonnart C, et al. House dust mites activate nociceptor-mast cell clusters to drive type 2 skin inflammation. *Nat Immunol* 2019;20:1435-43.
60. Bost KL, Breeding SAL, Pascual DW. Modulation of the mRNAs encoding substance P and its receptor in rat macrophages by LPS. *Reg Immunol* 1992;4:105-12.
61. Weinstock JV, Blum AM. Release of substance P by granuloma eosinophils in response to secretagogues in murine schistosomiasis mansonii. *Cell Immunol* 1990;125:380-5.
62. Schulze E, Witt M, Fink T, Hofer A, Funk RHW. Immunohistochemical detection of human skin nerve fibers. *Acta Histochem* 1997;99:301-9.
63. Irani A-MA, Sampson HA, Schwartz LB. Mast cells in atopic dermatitis. *Allergy* 1989;44:31-4.
64. Voisin T, Chiu IM. Mast cells get on your nerves in itch. *Immunity* 2019;50:1117-9.
65. Borici-Mazi R, Kouridakis S, Kontou-Fili K. Cutaneous responses to substance P and calcitonin gene-related peptide in chronic urticaria: the effect of cetirizine and dimethindene. *Allergy* 1999;54:46-56.
66. Smith CH, Atkinson B, Morris RW, Hayes N, Foreman JC, Lee TH. Cutaneous responses to vasoactive intestinal polypeptide in chronic idiopathic urticaria. *Lancet* 1992;339:91-3.
67. Green DP, Limjunyawong N, Gour N, Pundir P, Dong X. A mast-cell-specific receptor mediates neurogenic inflammation and pain. *Neuron* 2019;101:412-20.e3.
68. Meixiong J, Anderson M, Limjunyawong N, Sabbagh MF, Hu E, Mack MR, et al. Activation of mast-cell-expressed Mas-related G-protein-coupled receptors drives non-histaminergic itch. *Immunity* 2019;50:1163-71.e5.
69. Gaudenzio N, Sibilano R, Marichal T, Starkl P, Reber LL, Cenac N, et al. Different activation signals induce distinct mast cell degranulation strategies. *J Clin Invest* 2016;126:3981-98.
70. Gupta K, Harvima IT. Mast cell-neural interactions contribute to pain and itch. *Immunol Rev* 2018;282:168-87.
71. Tore F, Tuncel N. Mast cells: target and source of neuropeptides. *Curr Pharm Des* 2009;15:3433-45.
72. Lu L, Parmar MB, Kulka M, Kwan P, Unsworth LD. Self-assembling peptide nanoscaffold that activates human mast cells. *acs appl mater interfaces* 2018;10:6107-17.
73. Yoshida M, Yoshida H, Kitaichi K, Hiramatsu K, Kimura T, Ito Y, et al. Adrenomedullin and proadrenomedullin N-terminal 20 peptide induce histamine release from rat peritoneal mast cell. *Regul Pept* 2001;101:163-8.
74. Hasbak P, Eskesen K, Lind H, Holst J, Edvinsson L. The vasorelaxant effect of adrenomedullin, proadrenomedullin N-terminal 20 peptide and amylin in human skin. *Basic Clin Pharmacol Toxicol* 2006;99:162-7.
75. Okamura Y, Mishima S, Kashiwakura J-I, Sasaki-Sakamoto T, Toyoshima S, Kuroda K, et al. The dual regulation of substance P-mediated inflammation via human synovial mast cells in rheumatoid arthritis. *Allergol Int* 2017;66S: S9-20.
76. Church MK, El-Lati S, Caulfield JP. Neuropeptide-induced secretion from human skin mast cells. *Int Arch Allergy Appl Immunol* 1991;94:310-8.
77. Kulka M, Sheen CH, Tancowny BP, Grammer LC, Schleimer RP. Neuropeptides activate human mast cell degranulation and chemokine production. *Immunology* 2008;123:398-410.
78. Lowman MA, Rees PH, Benyon RC, Church MK. Human mast cell heterogeneity: Histamine release from mast cells dispersed from skin, lung, adenoids, tonsils, and colon in response to IgE-dependent and nonimmunologic stimuli. *J Allergy Clin Immunol* 1988;81:574-9.
79. Cheng FL, Li QT, Zhao CQ, An YF, Qi XP. [Experimental study on substance P in the regulation of degranulation of cultured murine mast cells]. *Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi* 2016;51:675-80.
80. Piotrowski W, Foreman JC. On the actions of substance P, somatostatin, and vasoactive intestinal polypeptide on rat peritoneal mast cells and in human skin. *Nahrungsmittelchemie Arch Pharmacol* 1985;331:364-8.
81. Zirbs M, Reindel U, Hermann K, Church MK, Behrendt H, Ring J, et al. Reduced skin reactivity to vasoconstrictor and vasodilator substances in atopic eczema. *Eur J Dermatol* 2013;23:812-9.
82. Van Neste D, Coussement C, Ghys L, Rihoux JP. Agonist-antagonist interactions in the skin: comparison of effects of loratadine and cetirizine on skin vascular responses to prick tests with histamine and substance P. *J Dermatol Sci* 1992;4:172-9.
83. Robas N, Mead E, Fidock M. MrgX2 is a high potency cortistatin receptor expressed in dorsal root ganglion. *J Biol Chem* 2003;278:44400-4.
84. Subramanian H, Kashem SW, Collington SJ, Qu H, Lambris JD, Ali H. PMX-53 as a dual CD88 antagonist and an agonist for Mas-related gene 2 (MrgX2) in human mast cells. *Mol Pharmacol* 2011;79:1005-13.
85. Patella V, de Crescenzo G, Marinò I, Genovese A, Adt M, Gleich GJ, et al. Eosinophil granule proteins activate human heart mast cells. *J Immunol* 1996;157:1219-25.
86. Piliponsky AM, Gleich GJ, Nagler A, Bar I, Levi-Schaffer F. Non-IgE-dependent activation of human lung- and cord blood-derived mast cells is induced by eosinophil major basic protein and modulated by the membrane form of stem cell factor. *Blood* 2003;101:1898-904.
87. Murakami T, Suzuki K, Niyonsaba F, Tada H, Reich J, Tamura H, et al. MrgX2-mediated internalization of LL-37 and degranulation of human LAD2 mast cells. *Mol Med Rep* 2018;18:4951-9.
88. Casale TB, Bowman S, Kaliner M. Induction of human cutaneous mast cell degranulation by opiates and endogenous opioid peptides: evidence for opiate and nonopiate receptor participation. *J Allergy Clin Immunol* 1984;73:775-81.
89. Nasser SM, Ewan PW. Opiate-sensitivity: clinical characteristics and the role of skin prick testing. *Clin Exp Allergy* 2001;31:1014-20.
90. Lin RY, Erlich ER, Don PC. Skin prick test responses to codeine, histamine, and ragweed utilizing the Multitest(TM) device. *Ann Allergy* 1990;65:222-6.
91. Kajiwara N, Sasaki T, Bradding P, Cruse G, Sagara H, Ohmori K, et al. Activation of human mast cells through the platelet-activating factor receptor. *J Allergy Clin Immunol* 2010;125:1137-45.e6.
92. Kolkhir P, Church MK, Altrichter S, Skov PS, Hawro T, Frischbutter S, et al. Eosinopenia, in chronic spontaneous urticaria, is associated with high disease activity, autoimmunity, and poor response to treatment. *J Allergy Clin Immunol Pract* 2020;8:318-25.
93. Liu R, Che D, Zhao T, Pundir P, Cao J, Lv Y, et al. MRGPRX2 is essential for sinomenine hydrochloride induced anaphylactoid reactions. *Biochem Pharmacol* 2017;146:214-23.
94. Stüttgen G, Flesch U, Witt H, Wendt H. Thermographic analysis of skin test reaction using AGA thermovision. *Arch Dermatol Res* 1980;268:113-28.
95. Demir S, Gelincik A, Akdeniz N, Aktas-Cetin E, Olgac M, Unal D, et al. Usefulness of in vivo and in vitro diagnostic tests in the diagnosis of hypersensitivity reactions to quinolones and in the evaluation of cross-reactivity: a comprehensive study including the latest quinolone gemifloxacin. *Allergy, Asthma Immunol Res* 2017;9:347-59.
96. Hou Y, Che D, Wei D, Wang C, Xie Y, Zhang K, et al. Phenothiazine antipsychotics exhibit dual properties in pseudo-allergic reactions: activating MRGPRX2 and inhibiting the H1 receptor. *Mol Immunol* 2019;111:118-27.
97. Che D, Wang J, Ding Y, Liu R, Cao J, Zhang Y, et al. Mivacurium induce mast cell activation and pseudo-allergic reactions via MAS-related G protein coupled receptor-X2. *Cell Immunol* 2018;332:121-8.
98. Che D, Rui L, Cao J, Wang J, Zhang Y, Ding Y, et al. Cisatracurium induces mast cell activation and pseudo-allergic reactions via MRGPRX2. *Int Immunopharmacol* 2018;62:244-50.

99. Levy JH, Gottge M, Szlam F, Zaffer R, McCall C. Weal and flare responses to intradermal rocuronium and cisatracurium in humans. *Br J Anaesth* 2000;85: 844-9.
100. Dhonneur G, Combes X, Chassard D, Merle JC. Skin sensitivity to rocuronium and vecuronium: a randomized controlled prick-testing study in healthy volunteers. *Anesth Analg* 2004;98:986-9.
101. Lumry WR, Li HH, Levy RJ, Potter PC, Farkas H, Moldovan D, et al. Randomized placebo-controlled trial of the bradykinin B₂ receptor antagonist icatibant for the treatment of acute attacks of hereditary angioedema: the FAST-3 trial. *Ann Allergy Asthma Immunol* 2011;107:529-37.
102. Han S, Lv Y, Kong L, Che D, Liu R, Fu J, et al. Use of the relative release index for histamine in LAD2 cells to evaluate the potential anaphylactoid effects of drugs. *Sci Rep* 2017;7:1-9.
103. Azimi E, Reddy VB, Pereira PJS, Talbot S, Woolf CJ, Lerner EA. Substance P activates Mas-related G protein-coupled receptors to induce itch. *J Allergy Clin Immunol* 2017;140:447-53.e3.
104. O'Connor TM, O'Connell J, O'Brien DI, Goode T, Bredin CP, Shanahan F. The role of substance P in inflammatory disease. *J Cell Physiol* 2004;201:167-80.
105. Metz M, Krull C, Hawro T, Saluja R, Groffik A, Stanger C, et al. Substance P is upregulated in the serum of patients with chronic spontaneous urticaria. *J Invest Dermatol* 2014;134:2833-6.
106. Zheng W, Wang J, Zhu W, Xu C, He S. Upregulated expression of substance P in basophils of the patients with chronic spontaneous urticaria: induction of histamine release and basophil accumulation by substance P. *Cell Biol Toxicol* 2016;32:217-28.
107. Basak PY, Erturan I, Yuksel O, Kazanoglu OO, Vural H. Evaluation of serum neuropeptide levels in patients with chronic urticaria. *Indian J Dermatol Venereol Leprol* 80:483.
108. Tedeschi A, Lorini M, Asero R. No evidence of increased serum substance P levels in chronic urticaria patients with and without demonstrable circulating vasoactive factors. *Clin Exp Dermatol* 2005;30:171-5.
109. Kim KH, Park KC, Chung JH, Choi HR. The effect of substance P on peripheral blood mononuclear cells in patients with atopic dermatitis. *J Dermatol Sci* 2003; 32:115-24.
110. Nattkemper LA, Tey HL, Valdes-Rodriguez R, Lee H, Mollanazar NK, Albornoz C, et al. The genetics of chronic itch: gene expression in the skin of patients with atopic dermatitis and psoriasis with severe itch. *J Invest Dermatol* 2018;138: 1311-7.
111. Järvikallio A, Harvima IT, Naukkarinen A. Mast cells, nerves and neuropeptides in atopic dermatitis and nummular eczema. *Arch Dermatol Res* 2003;295:2-7.
112. Pincelli C, Fantini F, Massimi P, Girolomoni G, Seidenari S, Giannetti A. Neuropeptides in skin from patients with atopic dermatitis: an immunohistochemical study. *Br J Dermatol* 1990;122:745-50.
113. Giannetti A, Girolomoni G. Skin reactivity to neuropeptides in atopic dermatitis. *Br J Dermatol* 1989;121:681-8.
114. Teresiak-Mikolajczak E, Czarniecka-Operacz M, Jenerowicz D, Silny W. Neurogenic markers of the inflammatory process in atopic dermatitis: relation to the severity and pruritus. *Postep dermatologii i Alergol* 2013;30:286-92.
115. Ohanyan T, Schoepke N, Eirefelt S, Hoey G, Koopmann W, Hawro T, et al. Role of substance P and its receptor neurokinin 1 in chronic prurigo: a randomized, proof-of-concept, controlled trial with topical aprepitant. *Acta Derm Venereol* 2018;98:26-31.
116. Hashimoto T, Kursewicz CD, Fayne RA, Nanda S, Shah SM, Nattkemper L, et al. Pathophysiologic mechanisms of itch in bullous pemphigoid. *J Am Acad Dermatol* 2020;83:53-62.
117. Toyoda M, Nakamura M, Makino T, Hino T, Kagoura M, Morohashi M. Nerve growth factor and substance P are useful plasma markers of disease activity in atopic dermatitis. *Br J Dermatol* 2002;147:71-9.
118. Panahi Y, Taherzadeh ES, Davoudi SM, Sahebkar A, Ranjbar R. Investigation of serum substance P status in patients with chronic pruritic skin lesions due to sulfur mustard: a cross-sectional study. *Cutan Ocul Toxicol* 2013;32:4-8.
119. Coulson IH, Holden CA. Cutaneous reactions to substance P and histamine in atopic dermatitis. *Br J Dermatol* 1990;122:343-9.
120. Amatya B, Nordlind K, Wahlgren C-F. Responses to intradermal injections of substance P in psoriasis patients with pruritus. *Skin Pharmacol Physiol* 2010; 23:133-8.
121. Fantini F, Pincelli C, Romualdi P, Donatini A, Giannetti A. Substance P levels are decreased in lesional skin of atopic dermatitis. *Exp Dermatol* 1992;1:127-8.
122. Wallengren J, Möller H, Ekman R. Occurrence of substance P, vasoactive intestinal peptide, and calcitonin gene-related peptide in dermatographism and cold urticaria. *Arch Dermatol Res* 1987;279:512-5.
123. Haas S, Capellino S, Phan NQ, Böhm M, Luger TA, Straub RH, et al. Low density of sympathetic nerve fibers relative to substance P-positive nerve fibers in lesional skin of chronic pruritus and prurigo nodularis. *J Dermatol Sci* 2010; 58:193-7.
124. Abadia Molina F, Burrows NP, Jones RR, Terenghi G, Polak JM. Increased sensory neuropeptides in nodular prurigo: a quantitative immunohistochemical analysis. *Br J Dermatol* 1992;10/01 1992;127:344-51.
125. Anand P, Springall DR, Blank MA, Sellu D, Polak JM, Bloom SR. Neuropeptides in skin disease: increased VIP in eczema and psoriasis but not axillary hyperhidrosis. *Br J Dermatol* 1991;124:547-9.
126. Lee C-H, Chuang H-Y, Shih C-C, Jong S-B, Chang C-H, Yu H-S. Transepidermal water loss, serum IgE and beta-endorphin as important and independent biological markers for development of itch intensity in atopic dermatitis. *Br J Dermatol* 2006;154:1100-7.
127. Umemoto N, Kakurai M, Okazaki H, Kiyosawa T, Demitsu T, Nakagawa H. Serum levels of vasoactive intestinal peptide are elevated in patients with atopic dermatitis. *J Dermatol Sci* 2003;31:161-4.
128. Hernanz A, Muelas G, Borbujo J. Plasma neuropeptide pattern in acute idiopathic urticaria. *Int Arch Allergy Appl Immunol* 1989;90:198-200.
129. Pincelli C, Fantini F, Romualdi P, Lesa G, Giannetti A. Skin levels of vasoactive intestinal polypeptide in atopic dermatitis. *Arch Dermatol Res* 1991;283: 230-2.
130. Lorenzo GD, Mansueto P, Melluso M, Candore G, Cigna D, Pellitteri ME, et al. Blood eosinophils and serum eosinophil cationic protein in patients with acute and chronic urticaria. *Mediators Inflamm* 1996;5:113-5.
131. Angelova-Fischer I, Hipler UC, Bauer A, Fluhr JW, Tsankov N, Fischer TW, et al. Significance of interleukin-16, macrophage-derived chemokine, eosinophil cationic protein and soluble E-selectin in reflecting disease activity of atopic dermatitis—from laboratory parameters to clinical scores. *Br J Dermatol* 2006; 154:1112-7.
132. Wu K-G, Li T-H, Chen C-J, Cheng H-I, Wang T-Y. Correlations of serum interleukin-16, total IgE, eosinophil cationic protein and total eosinophil counts with disease activity in children with atopic dermatitis. *Int J Immunopathol Pharmacol* 2011;24:15-23.
133. Kanda N, Watanabe S. Increased serum human β -defensin-2 levels in atopic dermatitis: relationship to IL-22 and oncostatin M. *Immunobiology* 2012;217: 436-45.
134. Clausen ML, Slotved HC, Krogfelt KA, Agner T. Measurements of AMPs in stratum corneum of atopic dermatitis and healthy skin-tape stripping technique. *Sci Rep* 2018.
135. Leung TF, Ching KW, Kong AP, Wong GW, Chan JC, Hon KL. Circulating LL-37 is a biomarker for eczema severity in children. *J Eur Acad Dermatol Venereol* 2012;26:518-22.
136. Ballardini N, Johansson C, Lilja G, Lindh M, Linde Y, Scheynius A, et al. Enhanced expression of the antimicrobial peptide LL-37 in lesional skin of adults with atopic eczema. *Br J Dermatol* 2009;161:40-7.
137. Viodé C, Lejeune O, Turlier V, Rouquier A, Casas C, Mègeaud V, et al. Cathepsin S, a new pruritus biomarker in clinical dandruff/seborrheic dermatitis evaluation. *Exp Dermatol* 2014;23:274-5.
138. Verma AK, Manohar M, Upparahalli Venkateshaiah S, Mishra A. Neuroendocrine cells derived chemokine vasoactive intestinal polypeptide (VIP) in allergic diseases. *Cytokine Growth Factor Rev* 2017;38:37-48.
139. Siehler S, Nunn C, Hannon J, Feuerbach D, Hoyer D. Pharmacological profile of somatostatin and cortistatin receptors. *Mol Cell Endocrinol* 2008;286:26-34.
140. Li D, He H-L, Yao M-Z, Chen M-L, Chen X. Cortistatin is dysregulated in skin tissue of patients with psoriasis vulgaris and suppresses keratinocyte proliferation in vitro. *Int J Dermatol* 2015;54:e309-14.
141. De Oliveira PC, De Oliveira Lopes D, Do Vale Coelho IE, Pereira MC. Cytotoxic activities of eosinophil cationic protein and eosinophil-derived neurotoxin: in silico analysis. *cancer genomics. Proteomics* 2015;12:397-402.
142. Abu-Ghazaleh RI, Gleich GJ, Prendergast FG. Interaction of eosinophil granule major basic protein with synthetic lipid bilayers: a mechanism for toxicity. *J Membr Biol* 1992;128:153-64.
143. O'Connell AE, Hess JA, Santiago GA, Nolan TJ, Lok JB, Lee JJ, et al. Major basic protein from eosinophils and myeloperoxidase from neutrophils are required for protective immunity to *Strongyloides stercoralis* in mice. *Infect Immun* 2011; 79:2770-8.
144. Plager DA, Loegering DA, Weiler DA, Checkel JL, Wagner JM, Clarke NJ, et al. A novel and highly divergent homolog of human eosinophil granule major basic protein. *J Biol Chem* 1999;274:14464-73.
145. Wu W, Samoszuk MK, Comhair SA, Thomassen MJ, Farver CF, Dweik RA, et al. Eosinophils generate brominating oxidants in allergen-induced asthma. *J Clin Invest* 2000;105:1455-63.
146. Domachowski JB, Dyer KD, Adams AG, Leto TL, Rosenberg HF. Eosinophil cationic protein/RNase 3 is another RNase A-family ribonuclease with direct antiviral activity. *Nucleic Acids Res* 1998;26:3358-63.

147. Kay AB, Ying S, Ardelean E, Mlynek A, Kita H, Clark P, et al. Elevations in vascular markers and eosinophils in chronic spontaneous urticarial weals with low-level persistence in uninvolved skin. *Br J Dermatol* 2014;171:505-11.
148. Ott NL, Gleich GJ, Peterson EA, Fujisawa T, Sur S, Leiferman KM. Assessment of eosinophil and neutrophil participation in atopic dermatitis: comparison with the IgE-mediated late-phase reaction. *J Allergy Clin Immunol* 1994;94:120-8.
149. Kiatsurayanon C, Niyonsaba F, Smithrithee R, Akiyama T, Ushio H, Hara M, et al. Host defense (antimicrobial) peptide, human β -defensin-3, improves the function of the epithelial tight-junction barrier in human keratinocytes. *J Invest Dermatol* 2014;134:2163-73.
150. Otvos L. Immunomodulatory effects of anti-microbial peptides. *Acta Microbiol Immunol Hung* 2016;63:257-77.
151. Nakatsuji T, Chen TH, Narala S, Chun KA, Two AM, Yun T, et al. Antimicrobials from human skin commensal bacteria protect against. *Sci Transl Med* 2017;9:eaah4680.
152. Asano S, Ichikawa Y, Kumagai T, Kawashima M, Imokawa G. Microanalysis of an antimicrobial peptide, β -defensin-2, in the stratum corneum from patients with atopic dermatitis. *Br J Dermatol* 2008;159:97-104.
153. Clausen ML, Jungersted JM, Andersen PS, Slotved HC, Krogfelt KA, Agner T. Human β -defensin-2 as a marker for disease severity and skin barrier properties in atopic dermatitis. *Br J Dermatol* 2013;169:587-93.
154. Howell MD, Boguniewicz M, Pastore S, Novak N, Bieber T, Girolomoni G, et al. Mechanism of HBD-3 deficiency in atopic dermatitis. *Clin Immunol* 2006;121:332-8.
155. Gambichler T, Skrygan M, Tomi NS, Othlinghaus N, Brockmeyer NH, Altmeyer P, et al. Differential mRNA expression of antimicrobial peptides and proteins in atopic dermatitis as compared to psoriasis vulgaris and healthy skin. *Int Arch Allergy Immunol* 2008;147:17-24.
156. Gupta K, Subramanian H, Ali H. Modulation of host defense peptide-mediated human mast cell activation by LPS. *Innate Immun* 2016;22:21-30.
157. Reddy VB, Sun S, Azimi E, Elmariam SB, Dong X, Lerner EA. Redefining the concept of protease-activated receptors: cathepsin S evokes itch via activation of Mrgpr. *Nat Commun* 2015;6:1-10.
158. Reddy VB, Shimada SG, Sikand P, Lamotte RH, Lerner EA. Cathepsin S elicits itch and signals via protease-activated receptors. *J Invest Dermatol* 2010;130:1468-70.
159. Steinhoff M, Neisius U, Ikoma A, Fartasch M, Heyer G, Skov PS, et al. Proteinase-activated receptor-2 mediates itch: a novel pathway for pruritus in human skin. *J Neurosci* 2003;23:6176-80.
160. Chung K, Pitcher T, Grant AD, Hewitt E, Lindstrom E, Malcangio M. Cathepsin S acts via protease-activated receptor 2 to activate sensory neurons and induce itch-like behaviour. *Neurobiol Pain* 2019;6:100032.
161. Kim N, Bae KB, Kim MO, Yu DH, Kim HJ, Yuh HS, et al. Overexpression of cathepsin S induces chronic atopic dermatitis in mice. *J Invest Dermatol* 2012;132:1169-76.
162. Reddy VB, Iuga AO, Shimada SG, LaMotte RH, Lerner EA. Cowhage-evoked itch is mediated by a novel cysteine protease: a ligand of protease-activated receptors. *J Neurosci* 2008;28:4331-5.
163. Kosteletzky F, Namer B, Forster C, Handwerker HO. Impact of scratching on itch and sympathetic reflexes induced by cowhage (*Mucuna pruriens*) and histamine. *Acta Derm Venereol* 2009;89:271-7.
164. Hawro T, Lehmann S, Deuring E, Weller K, Altrichter S, Church MK, et al. Comparison of pruritus and sensory qualities induced by capsaicin, histamine and cowhage. *J Eur Acad Dermatol Venereol* 2019;33:1755-61.
165. Andersen HH, Elberling J, Sølvsten H, Yosipovitch G, Arendt-Nielsen L. Nonhistaminergic and mechanical itch sensitization in atopic dermatitis. *Pain* 2018;158:1780-91.
166. Hawro T, Lehmann S, Altrichter S, Fluhr JW, Zuberbier T, Church MK, et al. Skin provocation tests may help to diagnose atopic dermatitis. *Allergy* 2016;71:1745-52.
167. Shanahan F, Lee TDG, Bienenstock J, Befus AD. The influence of endorphins on peritoneal and mucosal mast cell secretion. *J Allergy Clin Immunol* 1984;74:499-504.
168. Duthie DJR, Nimmo WS. Adverse effects of opioid analgesic drugs. *Br J Anaesth* 1987;59:61-77.
169. Navinés-Ferrer A, Serrano-Candelas E, Lafuente A, Muñoz-Cano R, Martín M, Gastaminza G. MRGRPX2-mediated mast cell response to drugs used in perioperative procedures and anaesthesia. *Sci Rep* 2018;8:11628.
170. Lee C-H, Hong C-H, Yu W-T, Chuang H-Y, Huang S-K, Chen G-S, et al. Mechanistic correlations between two itch biomarkers, cytokine interleukin-31 and neuropeptide β -endorphin, via STAT3/calcium axis in atopic dermatitis. *Br J Dermatol* 2012;167:794-803.
171. Georgala S, Schulps KH, Papaconstantinou ED, Stratigos J. Raised beta-endorphin serum levels in children with atopic dermatitis and pruritus. *J Dermatol Sci* 1994;8:125-8.
172. Leslie TA, Greaves MW, Yosipovitch G. Current topical and systemic therapies for itch. *Handb Exp Pharmacol* 2015;226:337-56.
173. Lee SW, Cheong SH, Byun JY, Choi YW, Choi HY. Occupational hand eczema among nursing staffs in Korea: self-reported hand eczema and contact sensitization of hospital nursing staffs. *J Dermatol* 2013;40:182-7.
174. Giavina-Bianchi P, Gonçalves DG, Zanandrea A, Borges de Castro R, Garro LS, Kalil J, et al. Anaphylaxis to quinolones in mastocytosis: hypothesis on the mechanism. *J Allergy Clin Immunol Pract* 2019;7:2089-90.
175. Rahman A, Rizwan S, Waycaster C, Wall GM. Pooled analysis of two clinical trials comparing the clinical outcomes of topical ciprofloxacin/dexamethasone otic suspension and polymyxin B/neomycin/hydrocortisone otic suspension for the treatment of acute otitis externa in adults and children. *Clin Ther* 2007;29:1950-6.
176. Oreagba IA, Oshikoya KA, Ogar C, Adefurin AO, Ibrahim A, Awodele O, et al. Adverse reactions to fluoroquinolones in the Nigerian population: an audit of reports submitted to the National Pharmacovigilance Centre from 2004 to 2016. *Pharmacol Res Perspect* 2017;5:e00297.
177. Ohnishi K, Kimura K, Masuda G, Tsunoda T, Obana M, Yoshida H, et al. Oral administration of fluoroquinolones in the treatment of typhoid fever and paratyphoid fever in Japan. *Intern Med* 2000;39:1044-8.
178. Shichmanter R, Miller EB, Landau Z. Adverse drug reactions due to prolonged antibiotic therapy for malignant external otitis. *Eur J Intern Med* 2004;15:441-5.
179. Güzey C, Scordo MG, Spina E, Landsem VM, Spigset O. Antipsychotic-induced extrapyramidal symptoms in patients with schizophrenia: associations with dopamine and serotonin receptor and transporter polymorphisms. *Eur J Clin Pharmacol* 2007;63:233-41.
180. Selim K, Kaplowitz N. Hepatotoxicity of psychotropic drugs. *Hepatology* 1999;29:1347-51.
181. Leucht S, Cipriani A, Spineli L, Mavridis D, Örey D, Richter F, et al. Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: a multiple-treatments meta-analysis. *Lancet* 2013;382:951-62.
182. Farde L, Wiesel FA, Halldin C, Sedvall G. Central D2-dopamine receptor occupancy in schizophrenic patients treated with antipsychotic drugs. *Arch Gen Psychiatry* 1988;45:71-6.
183. Berroa F, Lafuente A, Javaloyes G, Cabrera-Freitag P, De La Borbolla JM, Moncada R, et al. The incidence of perioperative hypersensitivity reactions: a single-center, prospective, cohort study. *Anesth Analg* 2015;121:117-23.
184. Maurer M, Church MK. Inflammatory skin responses induced by icatibant injection are mast cell mediated and attenuated by H1-antihistamines. *Exp Dermatol* 2012;21:154-5.
185. Lin Y, Lv Y, Fu J, Jia Q, Han S. A high expression Mas-related G protein coupled receptor X2 cell membrane chromatography coupled with liquid chromatography and mass spectrometry method for screening potential anaphylactoid components in kudiezi injection. *J Pharm Biomed Anal* 2018;159:483-9.
186. Ding Y, Che D, Li C, Cao J, Wang J, Ma P, et al. Quercetin inhibits Mrgprx2-induced pseudo-allergic reaction via PLC γ -IP3R related Ca²⁺ fluctuations. *Int Immunopharmacol* 2019;66:185-97.
187. Wang N, Che D, Zhang T, Liu R, Cao J, Wang J, et al. Saikosaponin A inhibits compound 48/80-induced pseudo-allergy via the Mrgprx2 pathway in vitro and in vivo. *Biochem Pharmacol* 2018;148:147-54.
188. Vadas P, Gold M, Perelman B, Liss GM, Lack G, Blyth T, et al. Platelet-activating factor, PAF acetylhydrolase, and severe anaphylaxis. *N Engl J Med* 2008;358:28-35.
189. Triggiani M, Schleimer RP, Warner JA, Chilton FH. Differential synthesis of 1-acetyl-2-acetyl-sn-glycero-3-phosphocholine and platelet-activating factor by human inflammatory cells. *J Immunol* 1991;147:660-6.
190. Ulambayar B, Yang E-M, Cha H-Y, Shin Y-S, Park H-S, Ye Y-M. Increased platelet activating factor levels in chronic spontaneous urticaria predicts refractoriness to antihistamine treatment: an observational study. *Clin Transl Allergy* 2019;9:33.
191. Karhu T, Akiyama K, Vuolteenaho O, Bergmann U, Naito T, Tatemoto K, et al. Mast cell degranulation via MRGRPX2 by isolated human albumin fragments. *Biochim Biophys Acta Gen Subj* 2017;1861:2530-4.
192. Rothschild AM. Mechanisms of histamine release by compound 48-80. *Br J Pharmacol* 1970;38:253-62.
193. Ruzicka T, Glück S. Cutaneous histamine levels and histamine releasability from the skin in atopic dermatitis and hyper-IgE-syndrome. *Arch Dermatol Res* 1983;275:41-4.
194. Wahlgren CF, Hägermark O, Bergström R. Patients' perception of itch induced by histamine, compound 48/80 and wool fibres in atopic dermatitis. *Acta Derm Venereol* 1991;71:488-94.

195. Rukwied R, Lischetzki G, McGlone F, Heyer G, Schmelz M. Mast cell mediators other than histamine induce pruritus in atopic dermatitis patients: a dermal microdialysis study. *Br J Dermatol* 2000;142:1114-20.
196. An J, Lee JH, Won HK, Kang Y, Song WJ, Kwon HS, et al. Clinical significance of serum MRGPRX2 as a new biomarker in allergic asthma. *Allergy Eur J Allergy Clin Immunol* 2019;1-4.
197. Ogasawara H, Furuno M, Edamura K, Noguchi M. Novel MRGPRX2 antagonists inhibit IgE-independent activation of human umbilical cord blood-derived mast cells. *J Leukoc Biol* 2019;1-9.
198. Wang J, Zhang Y, Wang J, Liu R, Zhang G, Dong K, et al. Paeoniflorin inhibits MRGPRX2-mediated pseudo-allergic reaction via calcium signaling pathway. *Phyther Res* 2019;401-8.