

Original Article



Elevated MRGPRX2 Levels Related to Disease Severity in Patients With Chronic Spontaneous Urticaria

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Disclosure

There are no financial or other issues that might lead to conflict of interest.

ABSTRACT

Mas-related G-protein coupled receptor-X2 (MRGPRX2), a receptor on mast cells, basophils, and eosinophils associated with immunoglobulin E (IgE)-independent degranulation, has been reported to be highly expressed on cutaneous mast cells in patients with severe chronic spontaneous urticaria (CSU). We sought to investigate whether MRGPRX2 levels in the sera from CSU patients differ from those in healthy control subjects and to evaluate the clinical utility of MRGPRX2 levels in CSU patients. Severe CSU was defined as urticaria activity score over 7 days (UAS7) ≥ 28 . Serum samples from 116 (73 severe and 43 non-severe) CSU patients and 50 healthy subjects were screened for MRGPRX2 using enzyme-linked immunosorbent assay. Serum MRGPRX2 levels were significantly higher in patients with severe CSU (median [interquartile range], 16.5 [10.8–24.8]) than in healthy controls (11.7 [6.5–21.2], $P = 0.036$) and in non-severe CSU patients (8.7 [4.5–18.8], $P = 0.002$), although they did not differ between healthy subjects and non-severe CSU patients. Serum MRGPRX2 levels in CSU patients showed positive correlations with UAS7 and specific IgE against *Dermatophagoideis farinae* in CSU subjects, whereas no correlations were observed for age, sex, urticaria duration, atopy, combined angioedema, autologous serum skin test positivity, or total IgE levels. Logistic regression analysis identified serum MRGPRX2 ≥ 12 ng/mL (odds ratio, 6.421; $P = 0.002$) as an independent risk factor for severe CSU, along with increased serum total IgE levels, peripheral basophil percentage, and angioedema. In conclusion, we suggest that serum MRGPRX2 could help indicate severe CSU.

Keywords: Chronic urticaria; disease severity; MRGPRX2

INTRODUCTION

Chronic spontaneous urticaria (CSU) is a common mast cell-driven skin disease characterized by the repeated appearance of hives and/or angioedema for at least 6 weeks in the absence of identifiable triggers.¹ Recently, 2 emerging endotypes of autoimmune hypersensitivity have been proposed in CSU: type I autoimmunity showing autoallergy against endogenous allergens and type IIb autoimmunity with mast cell-activating autoantibodies most commonly directed against high-affinity receptors for immunoglobulin E (IgE).²

In the pathogenesis of urticaria, the degranulation of skin mast cell-releasing histamine, cytokines, and proteases as well as the subsequent production of arachidonic acid metabolites and platelet-activating factor result in vasodilation and sensory nerve stimulation.³ Mas-related G-protein coupled receptor-X2 (MRGPRX2) is a well-known protein that mediates IgE-independent activation of mast cells, basophils, and eosinophils.³ Research has shown that MRGPRX2 acts as a receptor for basic neuropeptides, such as substance P (SP), vasoactive intestinal peptide, and host defense peptides, to promote host defense, thereby contributing to the pathogenesis of allergic and inflammatory diseases.⁴⁻⁶ In patients with severe chronic urticaria (CU), MRGPRX2 has been reported to be highly expressed on skin mast cells compared to healthy controls.⁷

Recently, increasing evidence has suggested that mast cells can communicate with other immune cells via the secretion of membrane-enclosed vesicles detectable in biological fluids.⁸ Interestingly, MRGPRX2 has been found to be present in both plasma membranes and at intracellular sites of tryptase- and chymase-expressing mast cells in the dermis.⁷ Therefore, MRGPRX2 itself and MRGPRX2-containing vesicles released by exocytosis and direct budding from plasma membranes have been detected in the sera of patients with allergic diseases.^{4,9} To date, however, there has been no study as to whether serum samples from CU patients contain MRGPRX2 or not. Therefore, we sought to compare serum MRGPRX2 levels in patients with CSU in relation to clinical characteristics of CSU.

MATERIALS AND METHODS

Study subjects

In this study, 116 patients with CSU and 50 normal healthy controls (NCs) were enrolled in this study. NCs were confirmed by using Biobank as not having any previous history of inflammatory or allergic skin disease. Serum samples from the patients were collected after they stopped taking antihistamines for at least 5 days and were stored at -70°C until used (required, needed, use, subsequent experiments). Atopy was deemed present when there is a positive result to at least 1 allergen in skin prick test with common inhalant allergens (pollens of alder, birch, oak, grass mixture, mugwort, and ragweed, cat and dog allergens, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Aspergillus niger*, and *Alternaria spp.*) (Lofarma, Milan, Italy). Patients with CSU underwent autologous serum skin test (ASST) and were assigned a urticaria activity score over 7 days (UAS7) in reflection of disease activity. CSU with a UAS7 score ≥ 28 at the sampling visit were classified as having severe CSU; those with a UAS7 score < 28 were classified as having non-severe CSU. Written informed consent was obtained from all study subjects. The study was approved by our Institutional Review Board (AJIRB-BMR-SMP-18-74).

Measurement of serum MRGPRX2, total IgE, and specific IgE to *D. farinae*

Serum MRGPRX2 levels were measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (MyBioSource, Inc., San Diego, CA, USA) according to the manufacturer's instructions. The serum samples were diluted in the detection range of MRGPRX2 (3.12–100 ng/mL). ImmunoCAP was employed to measure total and specific IgE levels to *D. farinae* (D2) (Thermo Fisher Scientific, Waltham, MA, USA).

Statistical analysis

Categorical variables were analyzed using χ^2 test with Bonferroni multiple comparisons. Continuous variables were analyzed by *t* test or Mann-Whitney *U* test. Spearman's correlation

was used to assess correlations among MRGPRX2 levels and other clinical/laboratory parameters. One-way analysis of variance with Tukey's test or Kruskal-Wallis test with Dunn's corrections were used for multiple comparisons. Receiver-operating characteristic (ROC) curves were drawn to determine the optimal cutoff value of serum MRGPRX2 level in order to distinguish severe CSU. The area under the curve (AUC) with 95% confidence intervals (CI) was estimated therefrom. To identify risk factors for severe CSU, a logistic regression analysis was applied. Statistical analyses were performed using IBM SPSS, version 22 for Windows (SPSS Inc., Chicago, IL, USA) and GraphPad Prism, version 8.4.3 (GraphPad Software Inc., San Diego, CA, USA). Significance levels for all analyses were set at $P < 0.05$.

RESULTS

Clinical characteristics of the study subjects

The demographic characteristics of the study subjects are summarized in **Table 1**. The number of atopic subjects was significantly larger in CSU patients than in NCs (56% vs. 28%, $P = 0.001$). The mean urticaria duration and UAS7 were 23.8 and 30.2 months, respectively. Positivity rates to ASST were 50%, and the prevalence of angioedema was 39% in CSU subjects. Mean log-transformed total IgE levels were higher in CSU patients than in NCs (2.1 ± 0.5 vs. 1.7 ± 0.5 , $P < 0.001$).

According to a cutoff score for UAS7 of 28, CSU patients were stratified into the non-severe and severe disease groups. There were no differences in age or sex between the 2 groups (**Table 1**). Atopy was more common in the severe CSU group than in NCs (60.3% vs. 28.0%, $P = 0.003$), but did not differ between the severe and non-severe CSU groups. Mean log-transformed total IgE levels were significantly higher in the severe CSU group than in the non-severe CSU group (2.2 ± 0.4 vs. 1.9 ± 0.5 , $P < 0.001$) and NCs (vs. 1.8 ± 0.5 , $P < 0.001$). There were no differences in ASST positivity rates or mean urticaria duration between the non-severe and severe CSU groups. In comparison to the non-severe CSU group, a history of angioedema was more prevalent (49.3% vs. 22.5%, $P = 0.006$) and the percentage of peripheral basophils was significantly lower in the severe CSU group than in the non-severe group (0.5 ± 0.2 vs. 0.6 ± 0.2 , $P < 0.001$).

Table 1. Clinical characteristics of the study subjects

Variables	Severe CSU (n = 73)	Non-severe CSU (n = 43)	CSU (n = 116)	NC (n = 50)	P value* CSU vs. NC	P value†		
						Severe vs. Non-severe	Severe vs. NC	Non-severe vs. NC
Age (yr)	38.7 ± 8.7	40.2 ± 9.7	39.3 ± 9.1	40.7 ± 9.8	0.357	0.698	0.478	0.959
Female	44 (60.3)	29 (67.4)	73 (62.9)	30 (60.0)	0.721	0.726	1.000	1.000
Atopy	44 (60.3)	21 (48.8)	65 (56.0)	14 (28.0)	0.001	0.693	0.003	0.117
Urticaria duration (mon)	20.1 ± 28.4	30.1 ± 59.0	23.8 ± 42.4	ND	NA	0.218	NA	NA
UAS7 (0–42)	35.6 ± 5.0	20.4 ± 5.9	30.0 ± 9.1	ND	NA	< 0.001	NA	NA
Allergic rhinitis	28 (38.4)	12 (28.6)	0	14 (28.0)	< 0.001	0.599		
Log (total IgE [kU/L])	2.2 ± 0.4	1.8 ± 0.5	2.1 ± 0.5	1.7 ± 0.5	< 0.001	< 0.001	< 0.001	0.147
D2 > 0.35kU/L	34 (47.2)	15 (34.1)	49 (42.2)	15 (30.0)	0.165	0.218	0.195	1.000
ASST	36 (49.3)	22 (51.2)	58 (50.0)	ND	NA	0.978	NA	NA
Angioedema	34/69 (49.3)	9/40 (22.5)	43/109 (39.4)	ND	NA	0.006	NA	NA
Peripheral basophil (%)	0.5 ± 0.2	0.6 ± 0.2	0.5 ± 0.3	ND	NA	< 0.001	NA	NA

Data are shown as mean ± standard deviation or number (%).

CSU, chronic spontaneous urticaria; NC, normal healthy control; ND, not detected; NA, not available; UAS7, urticaria activity score over 7 days; IgE, immunoglobulin E; D2, specific IgE level to *Dermatophagoides farinae*; ASST, autologous serum skin test.

* χ^2 test and t -test were applied for categorical and continuous variables, respectively; †Analysis of variance with Tukey's multiple comparison tests of numerous variables and χ^2 test with Bonferroni corrections for categorical variables were used.

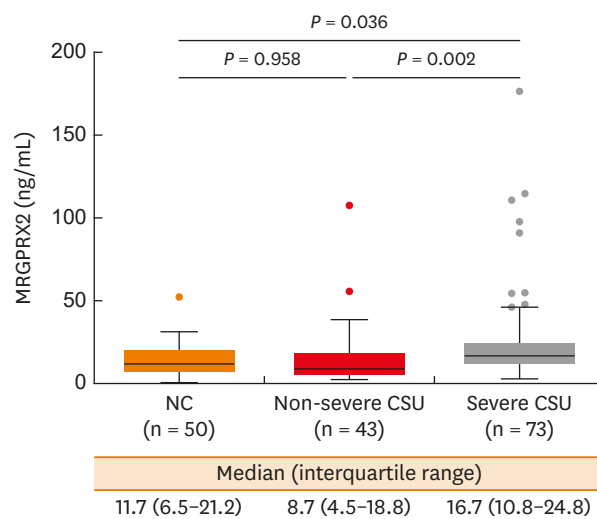


Fig. 1. Serum MRGPRX2 levels among the 3 study groups. *P* values were obtained by Kruskal-Wallis test with Dunn's multiple comparison test. MRGPRX2, mas-related G-protein coupled receptor X2; NC, normal healthy controls; CSU, chronic spontaneous urticaria.

Serum MRGPRX2 levels are higher in severe CSU patients and positively correlated with urticaria severity.

Serum MRGPRX2 levels were found to be significantly higher in the severe CSU group (median [interquartile range], 16.7 [10.8–24.8]) than in NCs (11.7 [8.3–21.2], $P = 0.036$) and non-severe CSU patients (8.7 [4.5–18.8], $P = 0.002$), whereas no difference was noted between NCs and the non-severe CSU group (**Fig. 1**).

As shown in **Fig. 2**, there were no significant differences in serum MRGPRX2 levels between the severe CSU and non-severe CSU groups according to the ASST positivity (15.5 [8.3–24.2] vs. 14.3 [7.9–21.5], $P = 0.854$), atopy (16.4 [8.5–28.1] vs. 12.1 [5.9–20.8], $P = 0.072$), combined angioedema (15.0 [9.1–24.1] vs. 13.8 [8.0–21.6], $P = 0.521$), and IgE sensitization to *D. farinae* (16.4 [9.9–29.4] vs. 12.3 [6.4–21.4], $P = 0.068$). Serum MRGPRX2 levels were positively correlated with UAS7 scores (Spearman' rho = 0.255, $P = 0.006$) and D2 levels (0.188, $P = 0.044$). No correlations were noted with total IgE levels, urticaria duration, or age (**Fig. 3**).

ROC curve analysis showed a serum MRGPRX2 value of 12 ng/mL as the optimal cutoff point for differentiating severe and active CSU among patients with CSU (AUC, 0.691; 95% CI, 0.586–0.797; $P = 0.001$). Using a serum MRGPRX2 level ≥ 12 ng/mL as the cutoff value, the sensitivity and specificity for detecting severe CSU were 67.1% and 60.5%, respectively. In logistic regression analysis, combined angioedema, serum total IgE level, peripheral basophil percentage, and a MRGPRX2 ≥ 12 ng/mL (odds ratio, 6.421; $P = 0.001$) were found to be significant determinants for severe CSU (**Table 2**).

DISCUSSION

In this study, we found that MRGPRX2 levels were higher in the sera from severe CSU patients and were significantly correlated with UAS7, compared to NCs. This result is consistent with that of a previous report on increased expression of MRGPRX2 on the skin biopsy specimens from severe CSU patients.⁷

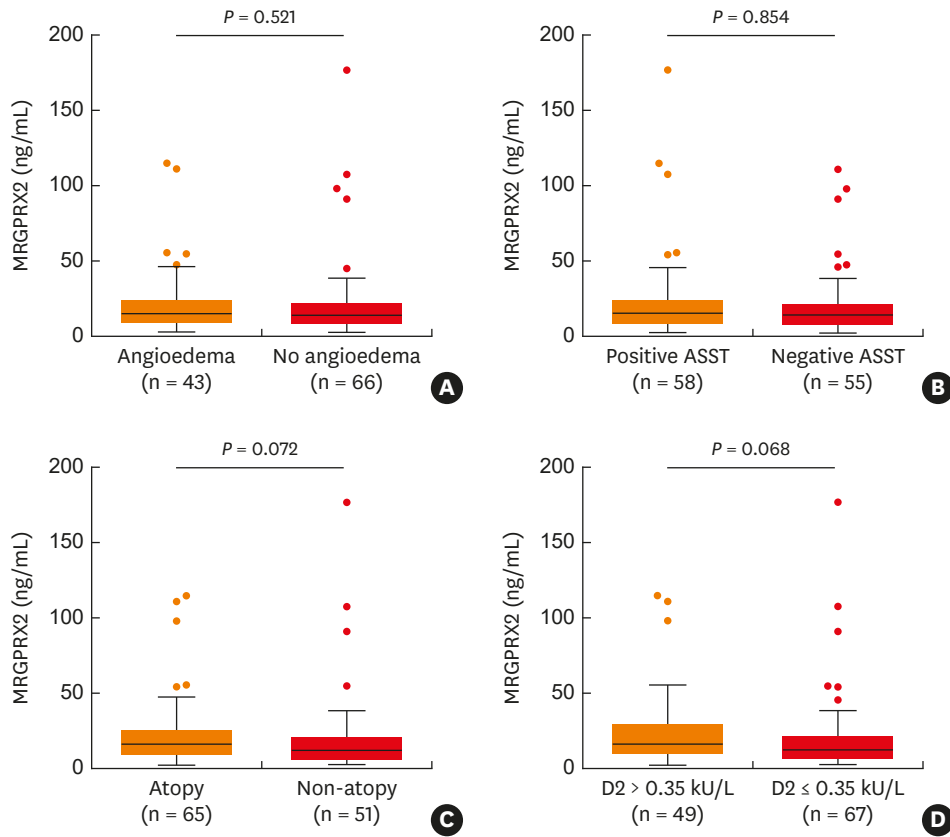


Fig. 2. Comparison of serum MRGPRX2 levels according to the presence of angioedema (A), ASST positivity (B), atopy (C), and sensitization to *D. farinae* (D). P values were evaluated by Mann-Whitney test. MRGPRX2, mas-related G-protein coupled receptor X2; ASST, autologous serum skin test; D2, specific IgE to *Dermatophagoides farinae*.

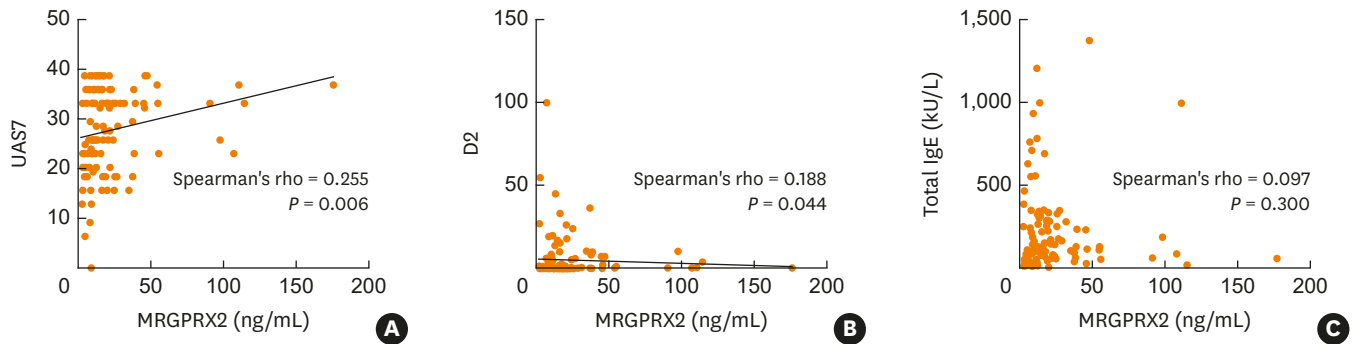


Fig. 3. Correlations for MRGPRX2 with UAS7, D2 and total IgE levels in chronic spontaneous urticaria patients. UAS7, urticaria activity score over 7 days; MRGPRX2, mas-related G-protein coupled receptor X2; D2, specific IgE level to *Dermatophagoides farinae*; IgE, immunoglobulin E.

Table 2. Logistic regression analysis of risk factors for severe chronic spontaneous urticaria

Variables	Odds ratio	95% CI	P value
Age	0.988	0.934–1.046	0.687
Female (sex)	0.372	0.118–1.175	0.092
Combined angioedema	4.317	1.477–12.620	0.008
Serum total IgE level	1.005	1.001–1.008	0.012
D2 > 0.35 kU/L	1.995	0.634–6.279	0.238
Peripheral basophil (%)	0.014	0.001–0.158	0.001
MRGPRX2 > 12.0 ng/mL	6.421	2.149–19.181	0.001

CI, confidence interval; IgE, immunoglobulin E; D2, specific IgE level to *Dermatophagoides farinae*; MRGPRX2, mas-related G-protein coupled receptor X2.

Some mast cell secretagogues, such as complement, neuropeptides, host defense peptides, or drugs, bind to G-protein coupled receptors to induce mast cell exocytosis and the release of mediators.¹⁰ MRGPRX2 as a G-protein coupled receptor is selectively and highly expressed in human skin mast cells, and research has shown MRGPRX2 to be a crucial receptor for pseudo-allergic and non-IgE-mediated drug reactions^{6,11} as well as neurogenic inflammation, pain, and itch.¹² SP, an agonist of MRGPRX2, which is released from sensory nerve endings and activated mast cells, can provide a positive feedback mechanism for further mast cell activation and its production.⁵ Interestingly, a significant elevation in serum SP levels in severe CSU patients has been reported.¹³ Moreover, SP and interleukin (IL)-33 have been shown to synergistically potentiate the production of cytokines including tumor necrosis factor- α , vascular endothelial growth factor, and IL-1 β by human mast cell lines.¹⁴ Consequently, increased MRGPRX2 expression in skin mast cells and the sera from severe CSU patients may indicate that MRGPRX2-mediated, non-IgE dependent mechanisms are involved in the pathogenesis of CSU. We found that 56% of CSU patients had atopy, which did not differ from those of prior investigations reporting positivity in skin prick test to inhalant to be from 27.4% to 64% in CU patients.¹⁵

Peripheral basopenia and the presence of angioedema have been reported to be linked to urticaria severity, longer urticaria duration, and type IIb autoimmunity.^{16,17} Similarly, fewer basophils and a higher prevalence of angioedema were found in the severe CSU group than in the non-severe CSU group in the present study. Moreover, we found that a serum MRGPRX2 level of > 12 ng/mL was a significant determinant of severe CSU, independent of other well-known clinical parameters such as increased total IgE, decreased basophils, and concomitant angioedema. However, no significant correlations between MRGPRX2 levels and peripheral basophil percentages, the presence of angioedema were observed, which suggests that elevated MRGPRX2 levels can be indicative of high disease activity associated with greater mast cell activation.

The differentiation, phenotypes, and function of mast cells in tissues are greatly determined by the tissue microenvironment, comprising cytokines, chemokines, and regulatory stimuli.¹⁸ For other allergic diseases, An *et al.*⁴ reported that allergic asthmatic patients had higher levels of serum MRGPRX2 than non-allergic asthmatics. They used the same commercial ELISA kit that we used in the present study, and serum MRGPRX2 levels in their control group were comparable with those in our NCs, although they were higher in the sera from the asthmatics in their study than in ours. Therefore, circulating MRGPRX2 levels might depend not only on the disease activity but also on the disease entity and inflamed tissues. Furthermore, as MRGPRX2 is associated with basophil and eosinophil activation, an inflammatory milieu may affect the expression of MRGPRX2 as well as the extent of mast cell degranulation, in various diseases. Meanwhile, although Fujisawa *et al.*⁷ described larger numbers and higher percentages of MRGPRX2-expressing mast cells in skin specimens from CU patients than from NCs, and the mean percentage of MRGPRX2-positive skin mast cells from severe CU patients was only 47.0%, compared to 21.6% in NC skin samples, indicating that not all skin mast cells express MRGPRX2 even in severe CSU patients. How MRGPRX2 expression is regulated in mast cells, basophils, and eosinophils remains unknown, and whether elevated total IgE levels in CSU patients are generally observed or play any physiologic roles in CSU has not been established. Notwithstanding the fact, previous studies suggested the role of both IgE-mediated autoimmunity (anti-TPO^{19,20} and anti-IL-24²¹) and specific IgE to candida²² and staphylococcal enterotoxins^{23,24} in the pathogenesis of CSU, and also reported increased total IgE in CSU compared to controls. Particularly, Altrichter

*et al.*²⁴ found that IgE-anti-SEB was linked to total IgE and disease activity in CSU. In our study, we noted significant but weak correlations between specific IgE to *D. farinae* and serum MRGPRX2/UAS7, while total IgE was not correlated with MRGPRX2. A prior report measuring serum MRGPRX2 in asthmatic patients showed that allergic patients had higher levels of MRGPRX2, although no correlation was noted between total IgE and MRGPRX2 levels.⁴ Research has demonstrated that *D. farinae* directly induces skin allergic inflammation *in vivo* by stimulating the release of SP from nociceptors and signaling through MRGPRB2 (a murine ortholog of MRGPRX2).²⁵ Song *et al.*²⁶ reported significantly higher urticaria activity scores in house dust mite (HDM)-sensitive CU patients than in those with negative skin tests to HDM. Furthermore, although in anecdotal experience and case reports, a clinical benefit from mite immunotherapy in CU patients with mite hypersensitivity has been suggested.^{27,28} However, when we compared serum MRGPRX2 levels between CSU patients with or without *D. farinae* sensitivity at a cutoff point of 0.35 kU/L, no significant difference was found. In addition, D2 positivity was not an independent factor for distinguishing severe CSU in a multivariate regression analysis. Consequently, we suspect that the presence of HDM-specific IgE in CSU patients may simply reflect the epiphenomenon of exposure to HDM, but not a cause of urticaria. Therefore, the clinical relevance of *D. farinae* sensitivity to the development and progression of CSU needs to be confirmed.

Omalizumab, a recombinant and humanized anti-IgE antibody, is an effective treatment option for antihistamine-refractory severe CSU. However, in around 30% of CSU patients, inadequate control of itchy wheals and/or angioedema has been reported even with 150 and 300 mg of omalizumab over 6 months.²⁹ Considering that MRGPRX2 is remarkably expressed in skin lesions and peripheral blood of severe CSU patients and is specifically involved in IgE-independent activation of mast cells, modulating MRGPRX2-related signals can be an alternative therapeutic option for severe CSU patients who remain symptomatic after anti-IgE treatment.

Taken together, this study showed that elevated serum MRGPRX2 levels in CSU patients were correlated with urticaria severity. A MRGPRX2 level of > 12 ng/mL could potentially be a novel marker for identifying severe CSU, along with total IgE, basopenia, and angioedema. Further studies are needed to investigate how MRGPRX2 expression is increased in severe CSU patients.

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