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Serum MRGPRX2 and substance P levels are biomarkers of disease activity rather than an antihistamine response in chronic spontaneous urticaria

Nguyen Thi Kim Cuc[1,2]✉, Vu Nguyet Minh[2], Pham Thi Lan[2], Le Huyen My[1] & Le Huu Doanh[2]

In chronic spontaneous urticaria (CSU), the role of Mas-related G protein-coupled receptor X2 (MRGPRX2) and substance P (SP) as biomarkers of disease severity and the antihistamine response remains unclear. The study aims to examine the correlations between serum MRGPRX2 and SP levels, disease severity, and antihistamine response in patients with CSU. This study included 120 CSU patients and 30 healthy controls. Based on the Urticaria Activity Score over 7 days (UAS7), the patients with CSU were divided into two categories: severe (UAS7 ≥ 28) and non-severe (UAS7 < 28). Severe CSU patients received 20 mg of bilastine, titrated up to 80 mg based on Urticaria Control Test (UCT) results at days 15, 30, and 60. Serum MRGPRX2 and SP levels were measured at baseline for all participants and after two months in severe CSU patients. The Kruskal-Wallis test and Dunn's corrections were used to examine differences in multiple comparisons. Spearman's correlation assessed the relationships between MRGPRX2 and SP levels, age, and urticaria duration. Receiver Operating Characteristic (ROC) curves were created to identify the optimal serum levels of MRGPRX2 and SP for distinguishing severe CSU. Additionally, univariate and multivariate logistic regression analyses were conducted to identify risk factors associated with severe CSU. Serum levels of MRGPRX2 and SP were significantly higher in patients with severe CSU compared to non-severe patients ($P < 0.001$ and $P = 0.01$) but were comparable to controls ($P > 0.05$). These levels were positively correlated with the UAS7 ($P < 0.001$ and $P = 0.01$), with no correlation between MRGPRX2 and SP levels ($P = 0.28$). MRGPRX2 ≥ 11.67 ng/mL and SP ≥ 97.66 pg/mL were identified as independent risk factors for severe CSU (OR 48.21 95%CI 13.00–178.82; $P < 0.001$ and OR 3.19 95% CI 1.10–9.24, $P = 0.03$). Among the severe CSU patients, the baseline MRGPRX2 and SP levels did not significantly differ across the antihistamine response groups ($P > 0.05$); serum MRGRPX2 levels remained consistent over time after antihistamine treatment ($P = 0.41$), whereas serum SP concentrations significantly decreased ($P < 0.001$). Serum MRGPRX2 and SP levels are associated with disease severity in CSU patients but do not predict antihistamine response in severe cases.

Keywords Antihistamine response, Biomarkers, Chronic spontaneous urticaria, Disease severity, MRGPRX2, Substance P

Abbreviations

APAAACI	Asia Pacific Association of Allergy, Asthma, and Clinical Immunology
ASST	Autologous Serum Skin Test
CRP	C-reactive protein
CSU	Chronic spontaneous urticaria
EAACI	European Academy of Allergy and Clinical Immunology
ELISA	Enzyme-linked immunosorbent assay
Euroguiderm	European Dermatology Forum
GA2LEN	Global Allergy And Asthma European Network

[1]National Hospital of Dermatology and Venereology, Hanoi, Vietnam. [2]Ha Noi Medical University, Hanoi, Vietnam. ✉email: kimcuc190989m@gmail.com

MC	Mast cell
MRGPRX2	Mas-related G-protein-oupled receptor-X2
NC	Normal control
sgAH1	Second-generation H1 antihistamines
SP	Substance P
TPO	Thyroid Peroxidase
UAS7	Urticaria activity score over 7 days
UCT	Urticaria control test

Chronic spontaneous urticaria (CSU) is a disease driven by mast cells (MCs) and is clinically characterized by wheals, angioedema, or both. It occurs daily or almost daily and lasts for more than six weeks without an identifiable trigger. CSU can have an intermittent/recurrent course[1,2]. The disease is estimated to affect 0.5–1% of the general population and dramatically affects patient's quality of life[3]. Importantly, there is currently no cure for this disease. This problem arises from the complexity of the pathogenesis of CSU, which involves interactions between MCs and immune cells, the coagulation–complement system, and sensory nerves[2,4].

The Mas-related G-protein-coupled receptor X2 (MRGPRX2)-substance P (SP) axis is involved in MCs-sensory nerve interactions in the CSU[2,5]. MRGPRX2 is an essential receptor that regulates the IgE-independent MC degranulation pathway[6]. In particular, this receptor is expressed primarily on the membrane of human skin MCs[7]. Some studies have shown that MRGPRX2 expression is increased on skin MCs[5] and in the serum of CSU patients, directly correlated with disease severity[8,9]. SP is a neuropeptide released at the ends of specific sensory nerves in response to various stimulating factors, such as leukotrienes, prostaglandins, and histamine, which are products of MC degranulation[10]. The findings from studies on serum SP concentrations have been inconsistent. While some studies have indicated a significant difference in serum SP levels between patients with CSU and healthy controls[11,12], other studies have not reported any differences[13,14]. Additionally, some studies suggest a correlation between the SP concentrations and the severity of CSU[11,14]. High concentrations of SP induce rapid MC degranulation, whereas low concentrations promote the chemotaxis of immature MCs. SP-MRGPRX2 signaling determines the positioning and movement of MCs within tissues and enhances communication between MCs and nerve fibers[15]. The connection between serum MRGPRX2 levels and SPs and their relationship with treatment response, especially with second-generation H1 antihistamines (sgAH1) in CSU patients remains unexplored.

This study investigated the relationship between serum MRGPRX2 and SP levels, disease severity, and response to sgAH1 treatment in patients with CSU.

Methods

Study design

A prospective observational study was conducted from March 2024 to September 2024 at the Chronic Urticaria and Urticaria Clinic within the Outpatient Department of the National Hospital of Dermatology and Venereology in Hanoi, Vietnam. The Hanoi Medical University Institutional Ethical Review Board (HMU IRB) approved the study protocol under approval number 1145/GCN-HMUIRB on December 28, 2023. We have previously reported data for some of the patients involved in this study[16]. All patients provided written informed consent for data and sample collection and their inclusion in the study, per the ethical standards outlined in the Declaration of Helsinki (2013) and its subsequent amendments.

Study population

Our study enrolled 120 CSU patients and 30 normal healthy controls (NCs). We recruited consecutive patients diagnosed with isolated CSU based on the EAACI/GA²LEN/EuroGuiDerm/APAAACI 2022 diagnostic criteria[1]. All participants were aged 16 years or older and had not used sgAH1 for at least 5 days; immunosuppressants (systemic corticosteroids, methotrexate, etc.) for at least 1 month, or NSAIDs and antibiotics for at least 1 week before storing the first serum sample.

Patients with the following conditions were excluded from the study: diseases that present with wheal and/or angioedema, such as urticarial vasculitis, urticaria pigmentosa, erythema multiforme, mastocytosis, hereditary angioedema, or drug-induced urticaria (a drug causes urticaria); other chronic itching skin disorders such as atopic dermatitis, bullous pemphigoid, dermatitis herpetiformis, senile pruritus, or psoriasis; and diseases that increase serum MRGPRX2 levels (asthma[17], allergic rhinitis[18]). Pregnant or nursing women were also excluded.

Thirty healthy volunteers, matched by age and sex, were enrolled as controls. These individuals had no history of inflammatory or allergic skin diseases, atopic conditions, infectious diseases, or other internal or surgical illnesses. Additionally, they had not used systemic antihistamines or immunosuppressive drugs for the same duration as the patients in the CSU group had before serum storage.

Study procedures

All serum samples from patients and controls were collected and stored at -80 °C for a maximum of two months to assess MRGPRX2 and SP levels via an ELISA kit (My BioSource, Inc., San Diego, CA, USA). The ELISA technique followed the manufacturer's ELISA procedure, performed at the Department of Hematology and Immunochemistry, National Hospital of Dermatology and Venereology, Hanoi, Vietnam.

Urticaria activity was determined by the Urticaria Activity Score over 7 days (UAS7). Patients were instructed to evaluate pruritus and wheal scores once daily, using a scale from 0 to 3 for each criterion. The UAS7 score was determined by summing the pruritus and wheal scores over 7 consecutive days. Patients with a UAS7 score of 28 or higher were classified as having severe CSU, whereas those with scores below 28 were classified as having non-severe CSU[19,20].

In the severe CSU group, patients were started on a standard dose of bilastine (Bilaxten 20 mg/day) according to the EAACI/GA²LEN/EuroGuiDerm/APAAACI guidelines[1]. Follow-up assessments were conducted on days 15, 30, and 60. Treatment responses and adjustments in the dose of sgAH1 were assessed using the Urticaria Control Test (UCT). CSU patients with a UCT score of 16 were considered completely controlled. Those with scores between 12 and 15 were classified as well-controlled. No changes to the bilastine dose were made in both groups. Patients with a UCT score of less than or equal to 11 were considered uncontrolled with bilastine and had their dosage increased to a maximum of four times, which is 80 mg per day[1,21]. The patient's serum sample was collected a second time after two months and stored at -80 °C for up to two months to assess MRGPRX2 and SP levels with an ELISA kit (My Biosource, Inc., San Diego, CA, USA). A flowchart depicting the study outline is presented in Fig. 1.

Statistical analysis

The data was entered into the REDCap platform (Vanderbilt University, Nashville, TN, USA)[22], and analyzed via Stata version 17.0 (StataCorp, College Station, TX, USA). The Kruskal-Wallis test and Dunn's corrections were used to examine differences in multiple comparisons. Spearman's correlation assessed the relationships

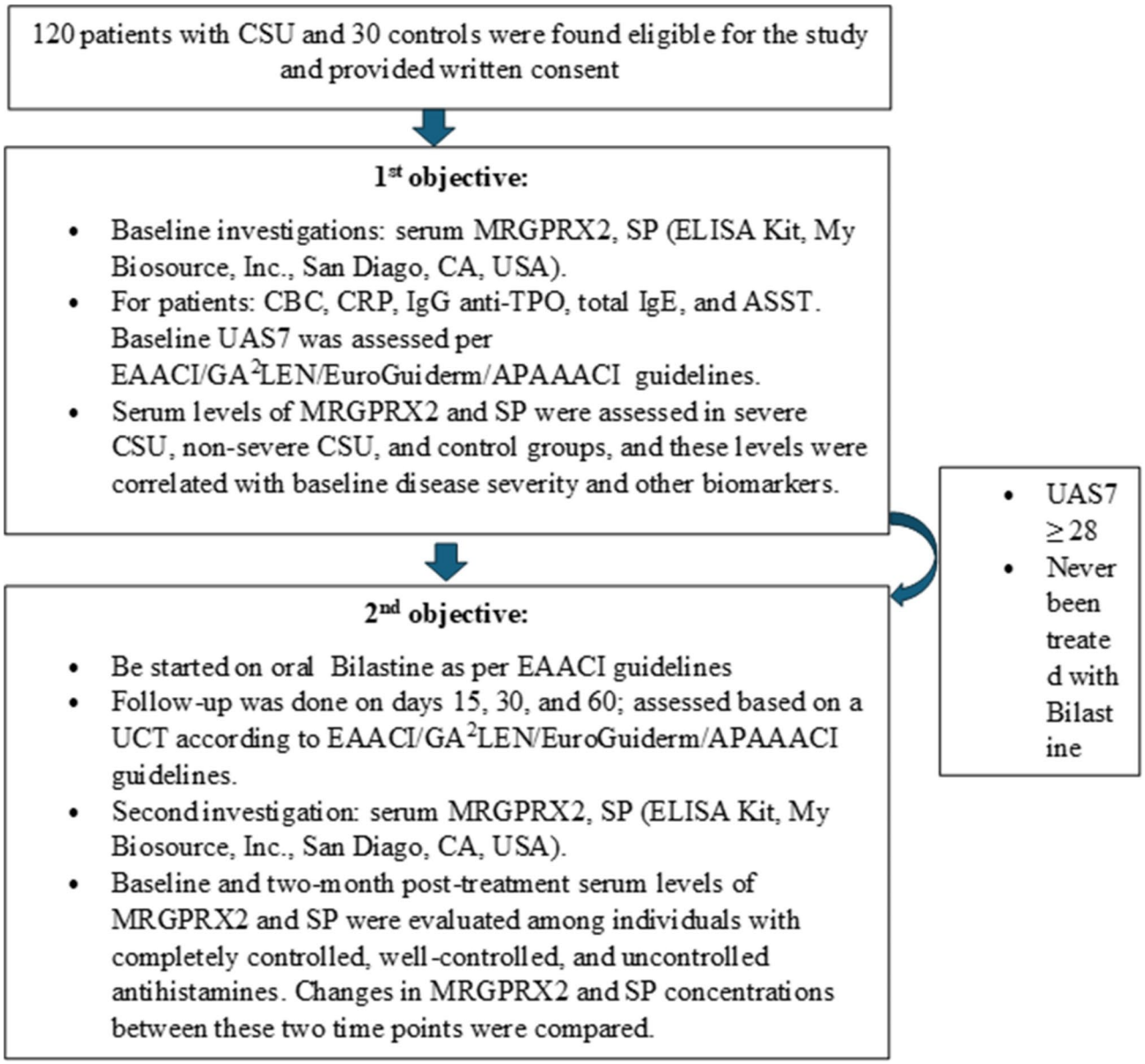

Fig. 1. Flowchart depicting the study outline. *ASST* autologous serum skin test, *CBC* complete blood count, *CRP* C-reactive protein, *CSU* chronic spontaneous urticaria, *EAACI* European Academy of Allergy and Clinical Immunology, *ELISA* Enzyme-linked immunosorbent assay, *IgE* immunoglobulin E, *IgG* immunoglobulin G, *MRGPRX2* Mas-related G-protein-coupled receptor-X2, *SP* Substance P, *TPO* Thyroid Peroxidase, *UAS7* urticaria activity score over 7 days, *UCT* Urticaria control test.

Variables	Severe CSU ($n=60$)	Non-severe CSU ($n=60$)	P value
Age (years)	40.1 ± 15.2	38.2 ± 15	0.49^{+}
Female	33 (55%)	40 (66.7%)	0.19**
UAS7 (0–42)	32.4 ± 5.3	20 (14–23.5)	0.000*
Urticaria duration (week)	20 (12.4–52)	19.5 (11.5–29)	0.31*
ASST (+)	34 (56.7%)	36 (61%)	0.63**
Angioedema	25 (41.7%)	19 (31.7%)	0.26**
Elevated CRP (> 5 mg/L)	8 (13.3%)	6 (10%)	0.57**
Elevated IgE (> 100 IU/mL)	49 (81.7%)	42 (70%)	0.33***
Elevated IgG anti-TPO (≥ 34kU/L)	3 (5%)	2 (3.3%)	1***
Eosinopenia ($< 0.05 \times 10^9$/L)	17 (28.3%)	13 (21.7%)	0.40**

Table 1. Characteristics of the study subjects. *ASST* autologous serum skin test, *CRP* C-reactive protein, *CSU* chronic spontaneous urticarial, *IgE* immunoglobulin E, *IgG* immunoglobulin G, *TPO* Thyroid Peroxidase, *UAS7* urticaria activity score over 7 days. *Mann–Whitney U test. **χ^2 test. ***Fisher Exact test. $^{+}$T-test.

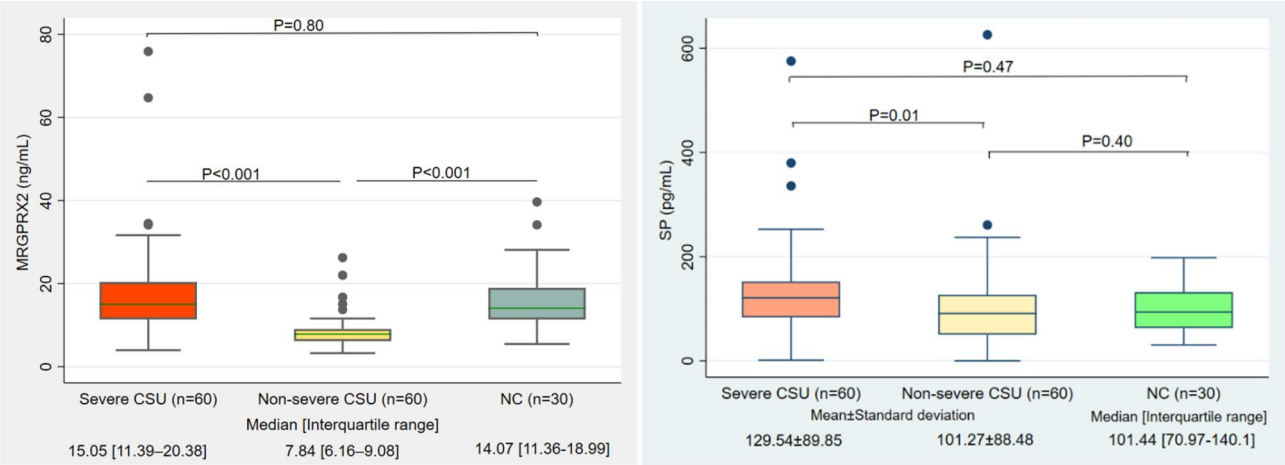

Fig. 2. Serum MRGPRX2 and SP concentrations between severe, non-severe CSU and controls (Kruskal-Wallis test with Dunn's multiple comparison test). *CSU* Chronic spontaneous urticaria, *MRGPRX2* Mas-related G-protein-coupled receptor-X2, *ng/mL* nanogram/milliliter, *NC* normal healthy controls, *pg/mL* picogram/milliliter, *SP* Substance P.

between MRGPRX2 and SP levels and various clinical and laboratory parameters. Univariate and multivariate logistic regression were applied to identify risk factors for severe CSU. ROC curves were drawn to determine the optimal serum MRGPRX2 and SP levels for distinguishing severe CSU. We evaluated the differences in the serum MRGPRX2 and SP concentrations before and after 2 months of treatment via the Wilcoxon signed-rank and sign tests. The significance level for all analyses was set at $P<0.05$.

Results

Characteristics of the study subjects

The study involved 120 patients with CSU and 30 healthy controls of similar age and sex. The NCs had an average age of 37.5 ± 12.7 years and a female proportion of 63.3%, which did not differ between severe CSU and non-severe CSU patients. There were no differences in mean urticaria duration, ASST positivity rates, combined angioedema, elevated CRP rates, elevated IgE rates, elevated IgG anti-TPO rates, or eosinopenia between the non-severe and severe CSU groups (Table 1).

Correlation of serum MRGPRX2 and SP levels with disease severity

Serum MRGPRX2 levels in patients with severe CSU (median [interquartile range]: 15.05 [11.39–20.38] ng/mL) were significantly higher than those in non-severe CSU patients (7.84 [6.16–9.08] ng/mL, $P<0.001$) but did not differ from those in NCs (14.07 [11.36–18.99] ng/mL, $P=0.80$). Similarly, the serum SP concentration in the severe CSU group (129.54 ± 89.85 pg/mL) was greater than that in the non-severe CSU group (101.27 ± 88.48 pg/mL, $P=0.01$), but there was no difference from that in the NCs (101.44 [70.97–140.1] pg/mL, $P=0.47$). (Fig. 2)

Serum MRGPRX2 levels were moderately correlated with UAS7 scores (Spearman's rho = 0.56, $P<0.001$). Moreover, serum SP concentrations were weakly associated with CSU disease activity (Spearman's rho = 0.22, $P=0.01$). An MRGPRX2 level of 11.67 ng/mL and an SP level of 97.66 pg/mL were the optimal cutoff points for

identifying severe and active CSU (AUC, 0.82; 95% CI, 0.74–0.91; $P<0.001$ with 75.0% sensitivity and 91.7% specificity for MRGPRX2 and AUC, 0.63; 95% CI, 0.53–0.73; $P=0.008$ with 66.67% sensitivity and 58.33% specificity for SP) (Fig. 3).

Compared with female patients, male patients had significantly greater serum concentrations of MRGRPX2 and SP, especially serum SP concentrations ($P=0.03$). There was no correlation between the serum concentrations of these two substances ($P=0.28$). No correlations between MRGPRX2 and SP concentration were found for age, duration of urticaria, combined angioedema, ASST positivity, eosinopenia, elevated CRP, high total IgE levels, or high IgG anti-TPO levels ($P>0.05$). (Table 2)

In logistic regression analyses, an MRGPRX2 level of ≥ 11.67 ng/mL and an SP level of ≥ 97.66 pg/mL were identified to predict severe CSU (OR 48.21 95% CI 13.00–178.82; $P<0.001$ for MRGPRX2 and OR 3.19 95% CI 1.10–9.24; $P=0.03$ for SP) (Table 3).

Correlation of serum MRGPRX2 and SP levels with antihistamines response

In the severe CSU group, after 2 months of treatment with bilastine, 21 (35%) patients did not respond despite receiving the highest dose (80 mg). The baseline MRGPRX2 and SP concentrations did not differ between the completely controlled, well-controlled, and uncontrolled groups ($P=0.72$ and $P=0.53$) (Fig. 4).

Only 33 serum samples were collected after 2 months of treatment. We observed no changes in the serum MRGPRX2 concentration before and after two months of treatment in severe CSU patients ($P=0.41$, Wilcoxon signed-rank test). In contrast, the serum SP concentration decreased after antihistamine treatment ($P=0.009$, sign test). (Fig. 5).

Discussion

The MRGPRX2-SP axis is key to the bidirectional interaction between MCs and sensory nerves in the CSU[23,24]. Our study revealed elevated MRGPRX2 and SP levels in the serum of patients with severe CSU, which correlated

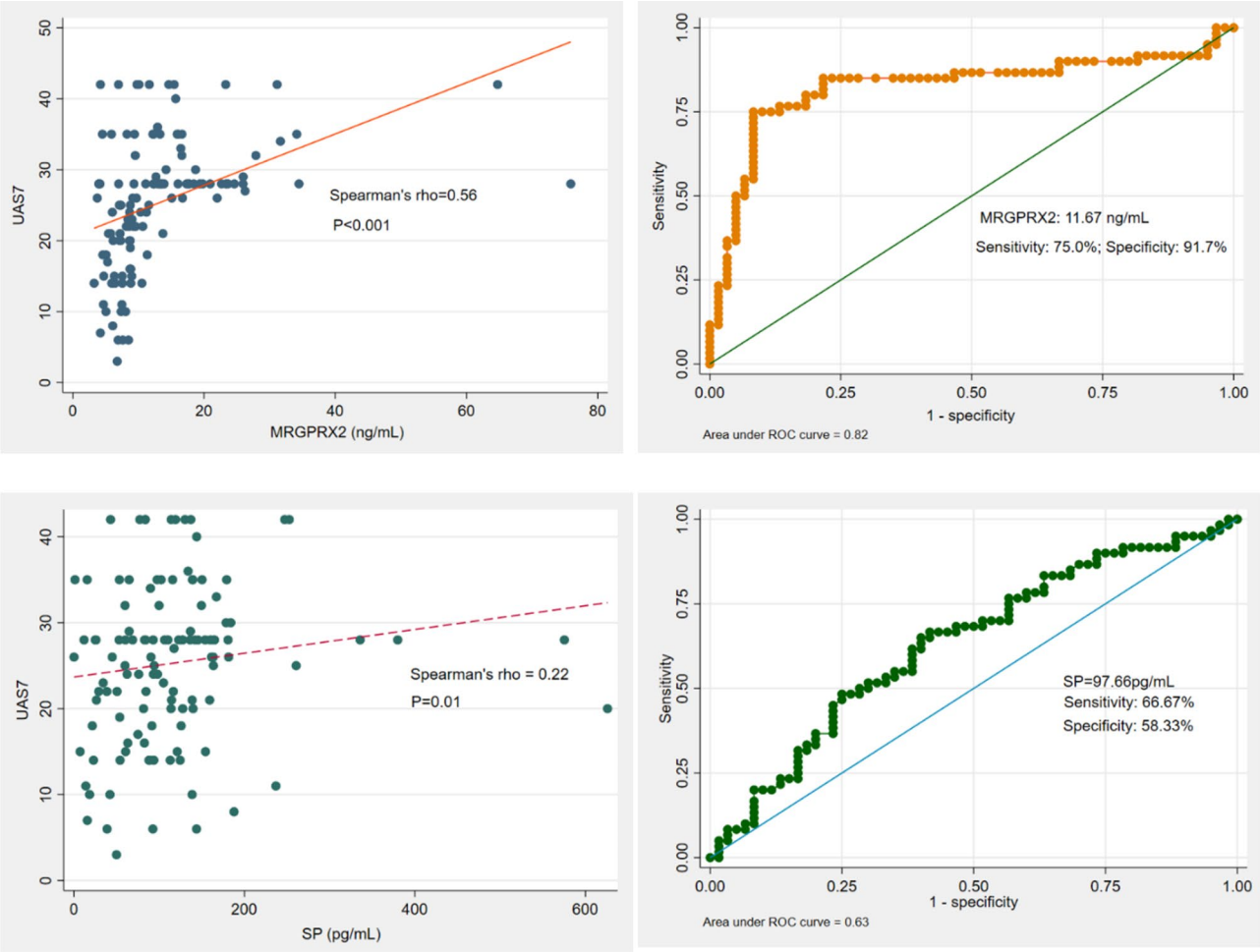

Fig. 3. Correlation between serum MRGPRX2 and SP concentrations and CSU disease activity by Spearman correlation and ROC curve analysis. *CSU* Chronic spontaneous urticaria, *MRGPRX2* Mas-related G-protein-coupled receptor-X2, *ng/mL* nanogram/milliliter, *pg/mL* picogram/milliliter, *SP* Substance P, *UAS7* Urticaria activity score over 7 days.

Variables	Spearman's rho		*P* value	
	MRGPRX2	SP	MRGPRX2	SP
Age (years)	0.04	0.10	0.68	0.26
Urticaria duration (weeks)	− 0.11	0.43	0.24	0.07
SP (pg/mL)	0.10	1	0.28	< 0.001

	MRGPRX2 (ng/mL)	SP (pg/mL)		
	Median (interquartile range) or mean ± standard deviation		MRGPRX2	SP
Sex				
Male (*n* = 47)	12.68 (7.1–18.71)	133.90 (75.17–153.99)	0.07*	0.03*
Female (*n* = 73)	8.85 (7.21–13.58)	93.30 (53.77–127.61		
Angioedema				
Positive (*n* = 44)	9.47 (7.26–14.95)	102.45 (48.00–140.39)	0.81*	0.51*
Negative (*n* = 76)	9.74 (7.15–16.35)	111.40 (64.03–144.69)		
ASST				
Positive (*n* = 70)	9.43 (7.34–13.93)	101.11 (53.36–139.81)	0.47*	0.55*
Negative (*n* = 50)	11.175 (6.43–19.35)	106.79 (64.85–145.54)		
Eosinophils				
Eosinopenia (*n* = 30)	8.96 (5.91–16.63)	113.07 ± 52.53	0.42*	0.55*
Non-Eosinopenia (*n* = 90)	9.74 (7.56–15.91)	103.71 (53.77–143.83)		
CRP				
Elevated (*n* = 14)	13.29 (9.3–15.69)	90.41 (64.85-139.42)	0.16*	0.58*
Normal (*n* = 106)	9.37 (7–16.46)	106.79 (60.67–143.83)		
Total IgE				
Low (*n* = 5) (< 40 IU/mL)	6.97 (6.34–8.71)	154.52 ± 67.58	0.47++	0.22++
Normal (*n* = 24)	9.33 (8.5–16.33)	95.67 (78.94–126.44)		
Elevated (*n* = 91)	10.1 (7.16–15.91)	117.37 ± 99.71		
IgG anti-TPO				
Normal (*n* = 115)	9.71 (7.29–16.04)	105.05 (60.67–142.13)	0.41*	0.47*
Elevated (*n* = 5)	6.91 (6.43–12.93)	133.90 (83.74–143.83)		

Table 2. Serum MRGPRX2 and SP concentrations in CSU according to some clinical/paraclinical characteristics. *ASST* autologous serum skin test, *CRP* C-reactive protein, *CSU* chronic spontaneous urticarial, *IgE* immunoglobulin E, *IgG* immunoglobulin G, *MRGPRX2* Mas-related G-protein-coupled receptor-X2, *SP* Substance P, *TPO* Thyroid Peroxidase. *Mann–Whitney U test. ++Kruskal–Wallis test.

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI	*P* value	OR	95% CI	*P* value
Age	1.00	0.98–1.03	0.49	1.01	0.98–1.05	0.53
Female (sex)	0.61	0.29–1.28	0.19	1.71	0.53–5.57	0.37
Combined angioedema	1.54	0.73–3.26	0.26	1.91	0.65–5.59	0.24
Eosinopenia ($< 0.05 \times 10^9$/L)	1.43	0.62–3.29	0.40	1.26	0.39–4.07	0.70
Elevated total IgE (> 100 IU/mL)	1.91	0.81–4.49	0.14	2.19	0.61–7.85	0.23
Elevated CRP (> 5 mg/L)	1.38	0.45–4.26	0.58	0.41	0.07–2.43	0.33
Elevated anti-TPO (≥ 34kU/L)	1.53	0.26–9.48	0.65	1.61	0.14–18.07	0.70
MRGPRX2 ≥ 11.67 ng/mL	30.25	10.28–89.04	**< 0.001**	48.21	13.00–178.82	**< 0.001**
SP ≥ 97.66 pg/mL	2.60	1.24–5.44	**0.01**	3.19	1.10–9.24	**0.03**

Table 3. Logistic regression analysis of risk factors for severe chronic spontaneous urticaria. *CRP* C-reactive protein, *IgE* immunoglobulin E, *IgG* immunoglobulin G, *MRGPRX2* Mas-related G-protein-coupled receptor-X2, *OR* Odds ratio, *SP* Substance P, *TPO* Thyroid Peroxidase.

with the UAS7 score compared with patients with non-severe CSU. This finding is consistent with the findings of studies by Cao et al.[8], Lao et al.[9]. , Metz et al.[11], and Nishimori et al.[14]. However, these studies only evaluated each substance in the CSU separately, and no study evaluated both substances simultaneously similar to our study. Cao et al. reported a weak correlation between the serum MRGPRX2 concentration and the UAS7 score (Spearman's rh0 = 0.255; $P < 0.001$).[8] An association between the serum SP concentration and the UAS7 score

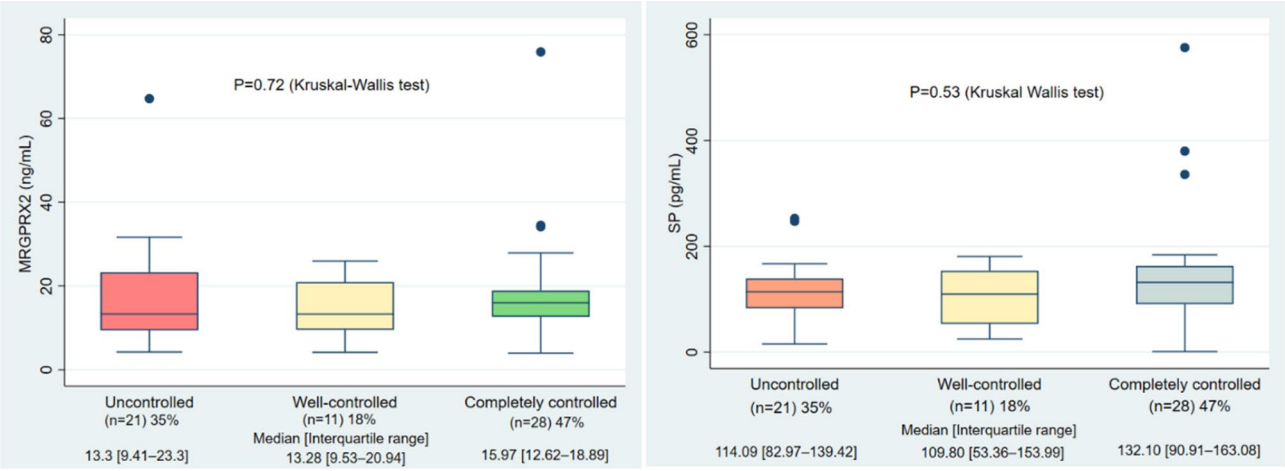

Fig. 4. Baseline serum MRGPRX2 and SP concentrations among treatment response groups to antihistamine in the severe CSU (Kruskal–Wallis test). *CSU* Chronic spontaneous urticarial, *MRGPRX2* Mas-related G-protein-coupled receptor-X2, *ng/mL* nanogram/milliliter, *pg/mL* picogram/milliliter, *SP* Substance P.

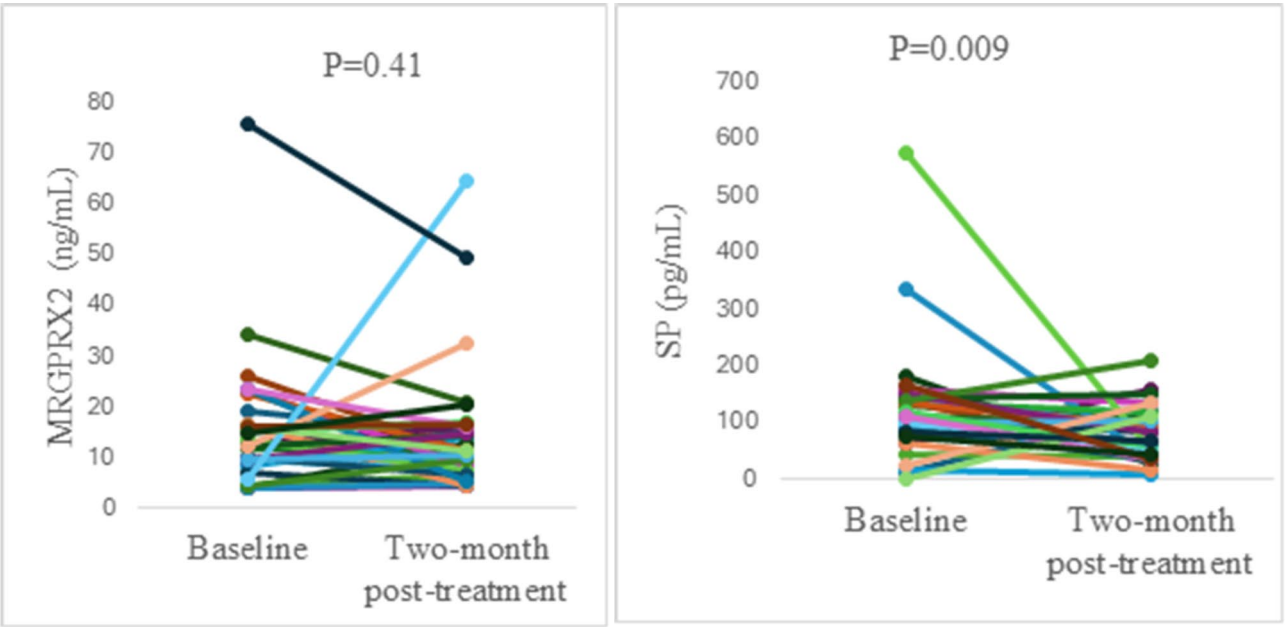

Fig. 5. Changes in serum MRGPRX2 and SP levels before and after treatment with antihistamine in the severe CSU. *CSU* Chronic spontaneous urticaria, *MRGPRX2* Mas-related G-protein-coupled receptor-X2, *ng/mL* nanogram/milliliter, *SP* Substance P.

was noted in the studies of Metz et al. (Spearman's rho = 0.52, $P < 0.001$)[11] and Nishimori et al. (Spearman's rho = 0.18, $P = 0.043$).[14] In another study, Lao et al. reported that the concentration of serum MRGPRX2 in the severe CSU group was greater than that in the non-severe group, but this difference was detected only in the treatment-naive group (not taking any regular CSU medications for at least 1 month) ($P = 0.03$) and not in the treated group ($P = 0.09$). The authors suggested that the drugs used to treat CSU may affect the concentration of serum MRGPRX2, but there was no comparison of MRGPRX2 concentrations before and after treatment[9]. A comparison of the serum MRGPRX2 and SP concentrations before and after 2 months of therapy with those of sgAH1 in 33 patients with severe CSU revealed that sgAH1 had no significant effect on the serum concentration of this receptor ($P = 0.41$), but it did lead to a significant decrease in the serum SP level ($P = 0.009$). Therefore, we suggest that sgAH1 affects only the UAS7 score and serum SP levels but not the serum MRGRPX2. Histamine is the primary mediator released from cutaneous MCs during degranulation, occurring through IgE-dependent and IgE-independent pathways[4]. sgAH1 functions as an inverse agonist of the H1 receptor. This means that they are not structurally related to histamine and do not block histamine from binding to the H1 receptor. Instead, sgAH1 binds to different sites on the H1 receptor to maintain its inactive state[25]. H1 receptor is expressed mainly

in nerves, endothelial cells, respiratory epithelium, and some immune cells such as T lymphocytes, eosinophils, and basophils but not human skin MCs[26]. As a result, sgAH1 decreases the stimulation of sensory nerves on H1 receptors, reducing the secretion of SP from these nerve fibers. However, they do not directly affect the MC degranulation pathway, unlike MRGPRX2. Our study also showed that even levels of SP (MRGPRX2 agonist) had no association with MRGPRX2 concentration ($P=0.28$). In another study, West et al. demonstrated that low levels of SP play a role in immature MC chemotaxis and increase the number of MRGPRX2-expressing MCs in inflammatory skin diseases[15]. Further studies are needed to determine the factors influencing the serum MRGPRX2 concentration in CSU.

Interestingly, we did not observe any difference in MRGPRX2 or SP concentrations between severe CSU patients and healthy controls. The lower serum MRGPRX2 and SP levels in Vietnamese individuals may explain the lack of this significant difference. This effect on the serum SP concentration has also been noted by Tedeschi et al.[13] and Nishimori et al.[14] This observation seems to challenge the essential role of SP as a histamine-releasing factor in the CSU, but it does not eliminate the possibility of its involvement. Compared with the controls, the absence of increased serum SP levels in the severe CSU group may be attributed to the release of SP, which primarily occurs at the skin level, its rapid inactivation, or its quick binding to the MRGPRX2 on MCs. Interestingly, Tedeschi's study reported 3 CSU patients with very high serum levels of SP (ranging from 910 to 1100 pg/mL).[13] In contrast, Nishimori's study identified two patients with serum SP levels exceeding 600 pg/mL.[14] These findings suggest that SP may play a role in the pathogenesis of CSU in specific individuals. Our study also revealed this phenomenon, with 5 CSU patients showing high serum SP concentrations greater than 300 pg/mL, whereas in the healthy controls, this threshold value was less than 200 pg/mL. This result for serum MRGPRX2 levels contrasts with the findings of Fujisawa et al. (2014)[5] and Cao et al. (2021)[8]. Fujisawa et al. demonstrated that the number of skin MCs expressing the marker MRGPRX2 was more significant in patients with severe CSU (UAS > 30) than in healthy individuals. Specifically, they reported an average of 116 ± 19 cells/mm² in severe CSU patients versus 68.5 ± 51.1 cells/mm² in the healthy control group ($P<0.001$). Additionally, the percentage of MCs expressing MRGPRX2 was significantly more significant in the severe CSU group (47.0% ± 6.9%) than in the control group (21.6% ± 7.8%)[5]. In a separate study, Cao et al. reported that the concentration of MRGPRX2 was statistically more significant than that in the healthy controls ($P=0.04$)[8]. We suggest that variations in MRGPRX2 expression may not fully account for the mechanisms underlying CSU and that MRGPRX2 polymorphisms could play a role. Shtessel et al. demonstrated that mild CSU patients presented stronger wheal responses to MRGPRX2 drug ligands than healthy controls[27]. In the context of pseudo-allergic drug reactions (non-IgE-mediated hypersensitivity reactions), more than 30 naturally occurring protein-coding variants of MRGPRX2 have been identified[28]. These variations may influence how MRGPRX2 responds to its agonists[29]. Further research is needed to clarify the influence of MRGPRX2 polymorphisms on CSU.

Our study revealed a fascinating finding: serum MRGPRX2 and SP concentrations were higher in males than in females ($P=0.07$ for MRGPRX2 and $P=0.03$ for SP). This observation may help explain why the proportion of males in the severe CSU group (45%) was greater than that in the non-severe CSU group (33.3%).

Our study is the first to investigate the relationship between baseline serum MRGPRX2 and SP levels and the response to sgAH1 therapy in severe CSU patients. The results indicate that the levels of MRGPRX2 and SP are not reliable predictors of response to sgAH1 in patients with severe CSU. Therefore, sgAH1 remains the first-line treatment for CSU, including severe CSU[1]. Other treatments should be combined when patients are poorly controlled with sgAH1. MRGPRX2 antagonists, such as EP262 (phase 2, NCT06077773) and EVO756 (phase 1), are used in these patients[30,31].

Our study had several limitations. First, we did not compare the serum concentration of MRGPRX2 and its expression in skin MCs between patients with CSU and healthy subjects, nor did we directly compare the two groups. Additionally, we did not analyze the amino acid sequence of MRGPRX2. There is currently no research worldwide on MRGPRX2 mutations in CSU. Finally, we did not compare the serum SP concentration with the SP levels in the skin lesions of patients with CSU.

Conclusion

In conclusion, serum concentrations of MRGPRX2 and SP correlate with the severity of urticaria in Vietnamese patients with chronic spontaneous urticaria. A serum MRGPRX2 level greater than 11.67 ng/mL and an SP level greater than 97.66 pg/mL can help identify cases of severe chronic spontaneous urticaria; however, these levels do not reliably predict the response to antihistamines. While MRGPRX2 levels remain unchanged, SP levels significantly decrease in severe cases when patients are treated with antihistamines. Further research is needed to clarify the role of MRGPRX2 polymorphisms and determine the factors influencing serum MRGPRX2 concentrations in chronic spontaneous urticaria patients.

Data availability

The corresponding author will provide the raw data to any qualified researcher upon request.

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Author contributions

N.T.K.C., V.N.M., and P.T.L.: conceptualization; N.T.K.C., V.N.M., L.H.M., and P.T.L.: investigation and methodology; N.T.K.C., V.N.M., L.H.D., and P.T.L.: formal analysis; N.T.K.C., V.N.M., and P.T.L.: writing – original draft, and all authors: writing – review & editing. All the authors have read and agreed to the published version of the manuscript.

Declarations

Ethics approval and consent to participate

The Hanoi Medical University Institutional Ethical Review Board (HMU IRB) No. 1145/GCN-HMUIRB approved the study protocol on December 28, 2023. Written informed consent was obtained from all study participants before data and sample collection. All participants had the right to withdraw from the study at any time.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to N.T.K.C.

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