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Original Article

Detection of circulating IgG autoantibody to FcεRIα in sera from chronic spontaneous urticaria patients

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Abstract *Backgrounds:* Chronic spontaneous urticaria (CSU) is a common skin disorder characterized by itchy wheals of at least 6 weeks in duration, wherein the autoimmune mechanism is involved to activate IgE receptors (FcεRIα) on mast cells. We aimed to assess levels of IgG autoantibody against FcεRIα in sera from CSU patients using dot-blot immunoassay.
Methods: We performed a hospital-based cross-sectional study of 125 CSU patients (64 ASST-positive, 61 ASST-negative) and 64 age-and sex-matched healthy controls. The cut-off value of IgG FcεRIα autoantibody was determined as the mean intensity plus two standard deviations of values in controls. Positivity for IgG autoantibody to FcεRIα was analyzed according to clinical parameters of disease duration, urticaria activity score (UAS), ASST, response to antihistamine treatment, complement levels, and the presence of other autoantibodies. Nonparametric tests were applied for statistical analyses.
Results: IgG positivity to FcεRIα was noted in 24.8% of CSU patients and was significantly more frequent in ASST-positive patients than in ASST-negative patients (32.8% vs 16.4%, $P = 0.040$). Only 3.1% of healthy controls had this autoantibody. Complement 3 levels were significantly lower in anti-FcεRIα antibody-positive patients than antibody-negative patients (109.8 ± 19.9 vs 123.1 ± 30.9, $P = 0.035$). No significant associations were found between IgG positivity to FcεRIα and UAS, serum total IgE levels, atopic status, clinical responses to antihistamines, or the presence of anti-thyroid and anti-nuclear antibodies.

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Conclusion: These findings suggest that circulating IgG autoantibody to FcεRIα in a subset of patients may be involved in the autoimmune mechanism of CSU. Further studies are needed to clarify its clinical significance.

Introduction

Chronic spontaneous urticaria (CSU) is defined as having wheals and/or angioedema for more than 6 weeks, with symptoms present almost daily, in individuals with no definite extrinsic causes.[1] Studies have indicated that CSU patients have specific factors in their blood that elicit urticarial wheals upon intradermal injections and the release of histamine from basophils *in vitro*.[2,3] Previous investigators have also reported that several autoimmune diseases, including thyroid disease, rheumatoid arthritis, type I diabetes mellitus, Sjörgen syndrome, Celiac disease, and systemic lupus erythematosus, are related to the prevalence of CSU.[4] An autoimmune background for CSU is supported by the observation of immediate wheals and flare responses after an autologous skin serum test (ASST) in CSU patients.[5] Since Hide et al. identified IgG against the α subunit of Fc receptor (FcεRIα) for the first time in the sera from CSU patients,[3] other studies have also confirmed that CSU patients have circulating FcεRIα or IgE antibody,[6–8] which can induce degranulation of basophils and mast cells.

Various methodologies are used to measure autoantibodies, including histamine release from donor basophils,[3,7–9] autologous skin serum test,[3,10] immunoblotting with purified IgG,[7,8,10] and ELISA.[11,12] Binding assays for FcεRIα have not proven useful as routine clinical assays, because of a low specificity and time-consuming nature. In a recent study,[13] however, a rapid dot-blot immunoassay was developed to detect autoantibody against FcεRIα with high specificity. Accordingly, we attempted to evaluate serum levels of IgG autoantibody against FcεRIα in CSU patients using dot-blot immunoassay and to investigate associations with disease severity and other clinical parameters, including ASST, urticaria activity score (UAS), responses to treatment, and the presence of other auto-antibodies.

Materials and methods

Clinical characteristics of the study subjects

We enrolled 64 healthy controls and 125 CSU patients who had urticaria symptoms for at least 6 weeks. CSU disease activity was assessed using UAS, which evaluates wheal characteristics, pruritus status, and symptom duration. Total scores ranged from 0 to 15, with higher scores indicating higher disease activity.[14] Refractoriness to antihistamines was defined as patients with persistent symptoms, even after increasing antihistamine doses up to 4-fold. Medication scores were calculated for systemic steroids and antihistamines for the first 3 months of treatment. Steroid and antihistamines uses were represented as equivalent doses of prednisolone (mg/week) and loratadine (mg/day), respectively.

Autologous serum skin test and atopy

Antihistamines were withdrawn for at least 5 days before collecting autologous sera. ASST was performed according to European guidelines.[1] We defined a positive result as a wheal induced at 30 min after an intradermal injection of 50 μl of autologous serum that was 1.5 mm larger than a wheal induced by an injection of the same volume of saline. Atopy was defined as positivity to at least one or more allergens on a skin prick test with common inhalant allergens.

Rapid dot-blot immunoassay for detecting IgG to FcεRIα

To measure IgG to FcεRIα in sera from CSU patients and healthy controls, we performed immunoblot assay as described previously.[13] Extracellular fragments of FcεRIα protein (30 μg/ml in PBS) were dotted onto nitrocellulose membranes and air dried. The membranes were then blocked with 0.15% casein in PBS for 10 min and washed. After washing, diluted (1:4 casein) patient serum was added and incubated for 10 min. Bound IgG was detected using peroxidase conjugated goat anti-human IgG (Zymed, CA, USA). The reaction was developed using a chemiluminescent substrate solution (Applied Biosystems, Bedford, MA, USA), and signals were recorded by exposure to X-ray film. Quantitative analysis of the spots was conducted with a video densitometer using Kodak analyzer software. A cut-off value for IgG to FcεRIα positivity was determined as the mean value plus two standard deviations in healthy controls.

Measurement of other antibodies and complement

Serum total IgE levels were measured by the ImmunoCAP system (Thermo Fisher Scientific, Uppsala, Sweden) according to the manufacturer's instructions. Anti-thyroid autoantibodies, such as anti-thyroglobulin and thyroid microsomal antibodies, were detected by radioimmunoassay (BRAHMS Aktiengesellschaft, Hennigsdorf, Germany). Anti-nuclear antibody (ANA) was detected using HEp-2 cells and an indirect fluorescent antibody technique (Fluoro HEPANA test; Medical & Biological Laboratories, Nagoya, Japan). Complements 3 (90—180 mg/dl) and 4 (9—37 mg/dl) were measured quantitatively using an immunoturbidimetric assay (Roche Hitachi Cobas C system, Rotkreuz, Switzerland).

Statistical analysis

Mann–Whitney U-test was used for between-group comparisons of continuous variables. Categorical variables were compared using Fisher's exact test. Spearman's rho test was applied for correlation analysis. Statistical analyses were performed using IBM SPSS version 20 for Windows (SPSS Inc.,Chicago, IL, USA). *P*-values <0.05 were considered indicative of statistical significance.

Ethics statement

The study was approved by the Institutional Review Board of Ajou University Hospital (AJIRB-BMR-KSP-15-134).

Results

Clinical characteristic of the study subjects

There were no significant differences in mean age, sex, and atopy rate between CSU patients and controls (Table 1). To compare the prevalence of IgG to FcεRIα according to ASST results, we enrolled 64 ASST-positive and 61 ASST-negative patients in the present study. Of 125 CSU patients, 47 (37.6%) were identified as having refractory urticaria (Table 1). Median urticaria duration in CSU patients was 4.5 months, and 33.6% of CSU patients had an angioedema. The prevalences of ANA and anti-thyroid antibodies in CSU patients were 20.8% and 24.8%, respectively (Table 1).

Prevalence of IgG to FcεRIα in patients with CSU

To detect IgG to FcεRIα, a rapid immunodot assay was performed in sera from 125 patients with CSU and 64 healthy controls. We noted a significant correlation between human IgG concentration and intensity levels of standard spots (R^2 = 0.9542, *P* = 0.003). The mean intensity of dot signals in the sera from CSU patients was significantly greater than that in sera from NCs (167.9 ± 102.4 vs. 109.0 ± 45.5, *P* < 0.001, Fig. 1). The positive cutoff value for IgG to FcεRIα was determined as 200, which was equal to the mean plus two standard deviations of the intensity values of the serum samples from the 64 healthy subjects. The prevalence of IgG to FcεRIα was 24.8% in CSU patients, whereas just two (3.1%) of the 64 healthy controls had positive results on IgG to FcεRIα (*P* < 0.001, Fig. 2).The mean dot signal intensity was significantly higher in female patients with CSU than that in male patients (181.7 ± 111.7 vs. 129.5 ± 55.7, *P* = 0.006). However, no significant differences in levels of IgG to FcεRIα by sex were observed in NCs (111.6 ± 41.1 in 40 female controls vs. 106.0 ± 52.9 in 24 males, *P* = 0.441). Female sex (odds ratio 6.73, 95% confidence interval 1.43–31.67, *P* = 0.016) and a positive ASST response (2.53, 1.03–6.23, 0.043) were noted as key determinants of the presence of IgG to FcεRIα in CSU patients in logistic regression analysis (Table 3).

Table 1 Clinical characteristics of the study subjects.

	CSU n = 125	NC n = 64	*P* value
Age (years)[a]	39 (19–61)	38 (27–72)	0.089
Female	92 (73.6%)	40 (62.5%)	0.133
Atopy	68 (54.4%)	18 (41.9%)	0.163
Duration (months)[a]	4.5 (1.5–240)		
UAS[a]	10 (0–15)		
Angioedema	42 (33.6%)		
ANA	26 (20.8%)		
ATA	31 (24.8%)		
Total IgE (KU/L)[a]	117 (3–1188)		
Complement 3 (mg/dL)[a]	114 (77–229)		
Complement 4 (mg/dL)[a]	28 (14–62)		
Refractoriness to antihistamines	47 (37.6%)		

[a] Median (min-maximum).
ASST, autologous serum skin test; CSU, chronic spontaneous urticaria; NC, normal control; UAS, urticaria activity score (0–15); ANA, antinuclear antibody; ATA, anti-thyroid antibody.

Association of IgG to FcεRIα with other clinical parameters in patients with CSU

Positivity rates for IgG autoantibody to FcεRIα were significantly higher in ASST-positive CSU patients than in ASST-negative CSU patients (32.8% vs. 16.4%, *P* = 0.034, Fig. 2 and Table 2). Sex was found to be an important factor associated with positivity for IgG to FcεRIα, which was noted in 2 (6.1%) of 33 male versus 29 (31.5%) of 92 female patients with CSU (*P* = 0.004). Additionally, female preponderance was marked in patients with positive IgG to FcεRIα (93.5%), compared to IgG to FcεRIα-negative patients (67.0%, *P* = 0.004, Table 2). However, there were no

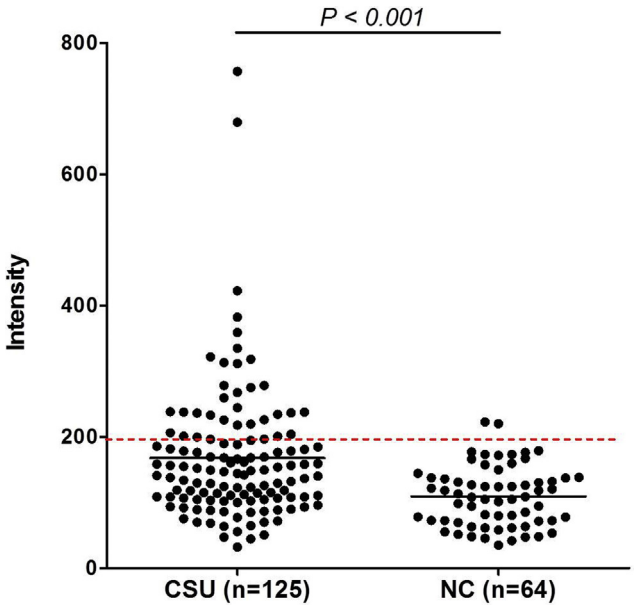

Figure 1. Dot-blot immunoassay of human IgG against recombinant FcεRIα in chronic spontaneous urticaria patients and healthy controls. FcεRIα, high-affinity IgE receptor; IgG, immunoglobulin G.

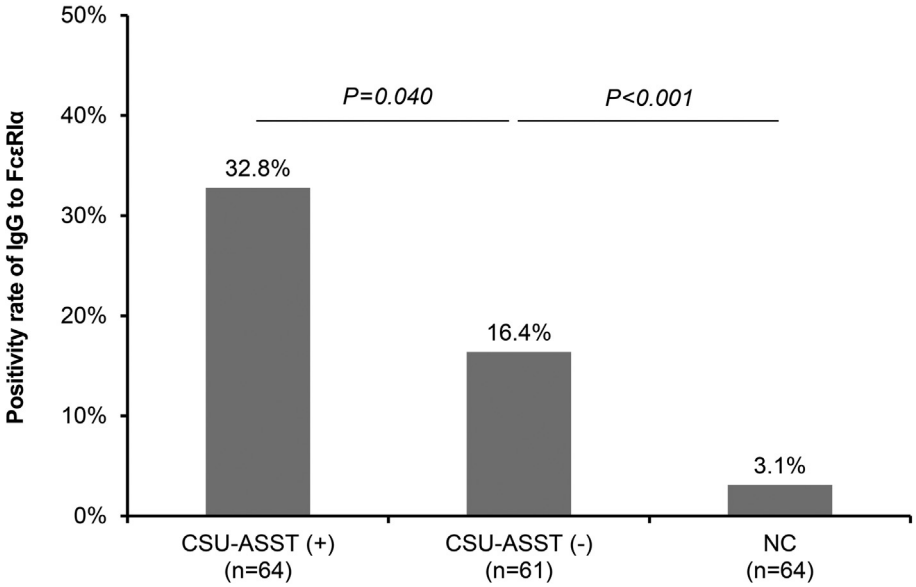

Figure 2. The prevalence of IgG autoantibody to FcεRIα according to ASST results in CSU patients. ASST, autologous serum skin test; CSU, chronic spontaneous urticaria; FcεRIα, high-affinity IgE receptor; IgG, immunoglobulin G; NC, healthy control.

Table 2 Comparison of clinical parameters according to the presence of IgG to FcεR1α in patients with chronic spontaneous urticaria.

	IgG to FcεR1α (+) n = 31	IgG to FcεR1α (−) n = 94	*P* value
Age (years)[a]	37 (19—61)	40 (20—60)	0.272
Female	29 (93.5%)	63 (67.0%)	0.004
Atopy	15 (48.4%)	53 (56.4%)	0.534
Duration (months)[a]	7.0 (1.5—240)	4.0 (1.5—240)	0.439
UAS (0—15)[a]	11 (5—14)	10 (1—15)	0.890
Angioedema presence	13 (41.9%)	29 (30.9%)	0.279
ASST positivity	21 (67.7%)	43 (45.7%)	0.040
ANA positivity	9 (29.0%)	17 (18.3%)	0.212
ATA positivity	11 (35.5%)	20 (21.3%)	0.149
Total IgE (KU/L)[a]	83 (9—1188)	127 (3—1003)	0.297
Complement 3 (mg/dL)[a]	108 (78—162)	116 (77—229)	0.035
Complement 4 (mg/dL)[a]	25 (14—59)	28 (14—62)	0.291
Refractoriness to antihistamines	13 (41.9%)	34 (36.2%)	0.670
Steroid (prednisolone mg/week)[b]	4.2 ± 10.3	3.8 ± 7.5	0.751
Antihistamines (loratadine mg/day)[b]	23.1 ± 11.1	25.4 ± 10.1	0.177

[a] Median (min-maximum).
[b] Mean ± standard deviation.
ANA, antinuclear antibody; ASST, autologous serum skin test; ATA, anti-thyroid antibody; FcεR1α; high-affinity IgE receptor; UAS, urticaria activity score.

significant differences in mean values of age, serum total IgE levels, urticaria duration, and UAS between IgG to FcεRIα-positive and FcεRIα—negative groups. The presence of angioedema (41.9% vs. 30.9%), ANA (29.0% vs. 18.3%), and anti-thyroid antibodies (35.5% vs. 21.3%) tended to be higher in patients with IgG autoantibody to FcεRIα, compared to those without IgG to FcεRIα, although statistical significance was lacking (Table 2). Meanwhile, however, complement 3 levels were significantly lower in CSU patients positive for anti-FcεRIα antibody than those with negative results on IgG to FcεRIα (109.8 ± 20.6 vs. 123.1 ± 30.9, $P = 0.035$, Table 2). Complement 3 levels were also significantly lower in ASST-positive patients than in ASST-negative patients (113.1 ± 25.7 vs. 126.5 ± 31.0, $P = 0.012$). The median UAS of patients having a positive result to IgG to FcεRIα was similar to that in patients with a negative result (11 vs. 10, $P = 0.890$, Table 2). The proportion of antihistamines-refractory patients was not different between IgG to FcεRIα positive and negative patients (41.9% vs. 36.2%, $P = 0.670$, Table 2). There were no significant differences in mean medication scores for steroid and antihistamines according to positivity of IgG to FcεRIα (4.2 ± 20.3 vs. 3.8 ± 7.5, $P = 0.751$ for steroid, 23.1 ± 11.0 vs. 25.4 ± 10.1, $P = 0.177$ for antihistamines,

Table 3 Multivariate analysis for the presence of IgG to FcεR1α in patients with chronic spontaneous urticaria.

Parameter	OR	95% CI Lower, Upper	*P* value
Age	1.005	0.963, 1.048	0.827
Female	6.734	1.432, 31.669	0.016
UAS	0.956	0.836, 1.094	0.515
ASST	2.532	1.029, 6.232	0.043
ATA	0.652	0.254, 1.669	0.372

ASST, autologous serum skin test; ATA, anti-thyroid autoantibody; CI, confidence interval; FcεR1α; IgG; OR, odds ratio; UAS, urticaria activity score.

Table 2). In the present study, 11 (2 in the positive IgG to FceR1α group and 9 in the negative IgG to FceR1α group) patients were treated with anti-IgE treatment. Complete response to anti-IgE treatment was noted in 2 patients in each group. The remaining 7 in the negative IgG to FceR1α group were still unresponsive or partly responsive to anti-IgE treatment.

Discussion

Studies have indicated that 30–60% of patients with CSU has an autoimmune etiology on the basis of various laboratory and clinical evidence.[2–7,10,13,15–17] The prevalence of thyroid autoimmunity in patients with CU was previously reported as 12–30%.[16,17] In our study, 31 (24.8%) of 125 CSU patients exhibited a positive response to one or both of the anti-thyroglobulin and anti-microsomal antibodies. Similar to previous studies,[17] the frequency of anti-thyroid antibodies was found to be significantly higher in females (31.5%), compared to 6.1% in males. Positive associations between the presence of anti-thyroid antibodies and positivity to functional autoantibodies and ASST in CU patients has been reported.[15,18] However, no significant differences in the frequency of ATA according to ASST results and IgG to FcεRIα were observed in the present study. In agreement therewith, Mozena et al. demonstrated that an equivalent proportion of CU patients with and without thyroid autoimmunity had a positive ASST and anti-FcεRIα antibodies.[19] Moreover, they demonstrated that both the detection of anti-FcεRIα antibodies and mast cell activation were not affected by the addition of thyroid proteins.[19] Accordingly, we suggest that higher prevalences of anti-thyroid antibodies and IgG to FcεRIα in CSU patients indicate a common genetic predisposition concordant among different autoimmune diseases. Similarly, a strong association between HLA-DR4 and histamine-releasing functional antibodies has been reported not only in CU but also in other autoimmune diseases, including rheumatoid arthritis, vitiligo, and pernicious anemia.[20]

Anti-FcεRIα antibody was found in 24.8% of CSU patients in the present study, while it was detected in 3.1% of NCs. Furthermore, in patients with a positive ASST, the prevalence of IgG to FcεRIα was 32.8%, which was significantly higher than that in ASST-negative patients (16.4%). Previous studies reported various prevalences of anti-FcεRIα antibodies in CSU patients; in general, it was reported up to 50% in CSU patients. In a recent study that sought to compare the sensitivities and specificities of ELISA and immunodot assay for detecting anti-FcεRIα autoantibodies in 20 ASST-positive CSU patients, ELISA had 70% sensitivity and 82.5% specificity, while immunodot showed 55% sensitivity and 100% specificity.[13] We used the same immunodot method, though the positive rate of IgG to FcεRIα was significantly higher in patients with a positive ASST, although it was relatively lower than what has previously been reported (32.8% vs. 55%).[13] Notwithstanding, the larger number of study subjects in the present study, an ethnic difference, a variety of urticaria durations, and other clinical characteristics might be possible causes of this apparent discrepancy.

Fiebiger et al. demonstrated that 37% of CSU patients show IgG autoantibody against immobilized soluble FcεRIα in their sera using Western blot and that 60% of these sera are functionally active.[7] In another previous study that performed the same immunoblotting assay, 64% of 53 sera from CU patients showed a positive response to recombinant FcεRIα.[21] However, sera from some patients with negative results on immunoblotting were found to release histamine from basophils or cutaneous mast cells.[7,21] In addition, there was also a subset of patients with positive autoantibodies against IgE receptor or IgE itself who showed no response to basophil histamine releasing test.[21] Although we did not confirm the functional activity of IgG to FcεRIα, because no associations with the duration and severity of urticaria or with clinical responses to antihistamines were observed, we could not definitively confirm whether IgG to FcεRIα is genuinely pathogenic. Nonetheless, a recent study identified autoreactive CD4+ T-cells targeting FcεRIα in 27% of CSU patients, while autoantibodies to FcεRIα were detected in 47%.[22] However, the authors found no association between T-cell responses to FcεRIα and medication use.[22]

On the clinical implications of the presence of autoantibodies and ASST responses in CSU patients, the evidence is conflicting. Sabroe et al. reported that CU patients determined as having functional autoantibodies on the basis of basophil histamine releasibility show more and a wider distribution of wheals.[23] However, another study reported no significant difference in the use of prednisolone and cyclosporine in CU patients according to the presence of anti-thyroid antibodies and serum-evoked basophil activation.[17] With respect to ASST results, several studies have shown significant associations between ASST positivity and urticaria severity or duration,[24,25] whereas a prospective study revealed that ASST positivity was a significant predictor of CU control.[14] A recent systematic review also indicated no supportive evidence on an association between ASST positivity and urticaria duration.[26] Considering these heterogeneous results, we speculate that, in at least a third of patients with CSU, autoantibodies against FcεRIα can activate and degranulate mediators from basophils and mast cells; however, there may be subsets of CSU patients who have other circulating triggers to activate basophils and/or mast cells regardless of autoimmunity[14,27,28] or who have functionally inactive autoantibodies.[21]

In addition to a female predominance in the prevalence of IgG to FcεRIα, a significant decrease in complement 3

levels was also found in CSU patients with autoantibodies to FcεRIα, as commonly observed in other autoimmune diseases.[12,23,29] Complement 3 has been reported to have a synergic effect on IgG-mediated degranulation of mast cells.[30] While similar incidences of IgG to FcεRIα have been recorded for other autoimmune diseases, such as diabetes mellitus (36%), systemic lupus erythematosus (20%), and pemphigus vulgaris (39%), only sera from CSU patients with IgG to FcεRIα has been found to show histamine-releasing activity and complementation activation.[11]

The anti-IgE monoclonal antibody (omalizumab) treatment has been shown to be effective in antihistamine resistant chronic autoimmune urticaria.[31] Based on the mechanism of anti-IgE treatment is due to the reduction of free IgE levels and decreased FcεRIα expression on peripheral basophils and skin mast cells,[32,33] time to response to omalizumab treatment was evaluated in a previous study.[34] While the positivity in basophil histamine release assay and ASST were significantly associated with a slow response to omalizumab, however, the presence of IgG to FcεRIα was noted in 11.4% of all complete responders and it was not correlated with the time to urticaria remission with omalizumab.[34] Although small numbers of the study subjects had omalizumab treatment in the present study, there was no significant difference between the positivity of IgG to FcεRIα and the response rate or time to response to omalizumab. Therefore, further prospective studies are needed to evaluate whether IgG to FcεRIα can be a potential biomarker to predict therapeutic outcomes of anti-IgE treatment in CSU.

Taken together, we stress that further identification of autoimmune markers will help with understanding the pathogenic mechanisms of CSU. Furthermore, while the pathogenic relevance of circulating autoantibodies has been established, their clinical relevance against IgE receptors and IgE in CSU patients still remains to be elucidated.

In conclusion, we identified circulating IgG to FcεRIα in 24.8% of CSU patients, including 32.8% of ASST-positive patients. Our results reinforced findings from previous studies, particularly a female dominance and a higher prevalence in patients with positive ASST. Decreased C3 levels in patients with positive IgG to FcεRIα may be possibly associated with complement activation involved in autoimmunity in CSU. Further studies incorporating standardized measurements of autoantibodies and objective outcome measures on a larger study population could help with determining the exact clinical relevance of IgG to FcεRIα in patients with CSU.

Conflicts of interest

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

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References

1. Zuberbier T, Aberer W, Asero R, Bindslev-Jensen C, Brzoza Z, Canonica GW, et al. The EAACI/GA(2) LEN/EDF/WAO Guideline for the definition, classification, diagnosis, and management of urticaria: the 2013 revision and update. *Allergy* 2014;**69**:868—87.
2. Grattan CE, Francis DM, Hide M, Greaves MW. Detection of circulating histamine releasing autoantibodies with functional properties of anti-IgE in chronic urticaria. *Clin Exp Allergy* 1991;**21**:695—704.
3. Hide M, Francis DM, Grattan CE, Hakimi J, Kochan JP, Greaves MW. Autoantibodies against the high-affinity IgE receptor as a cause of histamine release in chronic urticaria. *N Engl J Med* 1993;**328**:1599—604.
4. Confino-Cohen R, Chodick G, Shalev V, Leshno M, Kimhi O, Goldberg A. Chronic urticaria and autoimmunity: associations found in a large population study. *J Allergy Clin Immunol* 2012;**129**:1307—13.
5. Sabroe RA, Grattan CE, Francis DM, Barr RM, Kobza Black A, Greaves MW. The autologous serum skin test: a screening test for autoantibodies in chronic idiopathic urticaria. *Br J Dermatol* 1999;**140**:446—52.
6. Tong LJ, Balakrishnan G, Kochan JP, Kinet JP, Kaplan AP. Assessment of autoimmunity in patients with chronic urticaria. *J Allergy Clin Immunol* 1997;**99**:461—5.
7. Fiebiger E, Maurer D, Holub H, Reininger B, Hartmann G, Woisetschlager M, et al. Serum IgG autoantibodies directed against the alpha chain of Fc epsilon RI: a selective marker and pathogenetic factor for a distinct subset of chronic urticaria patients? *J Clin Invest* 1995;**96**:2606—12.
8. Kikuchi Y, Kaplan AP. Mechanisms of autoimmune activation of basophils in chronic urticaria. *J Allergy Clin Immunol* 2001;**107**:1056—62.
9. Niimi N, Francis DM, Kermani F, O'Donnell BF, Hide M, Kobza-Black A, et al. Dermal mast cell activation by autoantibodies against the high affinity IgE receptor in chronic urticaria. *J Invest Dermatol* 1996;**106**:1001—6.
10. Sabroe RA, Fiebiger E, Francis DM, Maurer D, Seed PT, Grattan CE, et al. Classification of anti-FcepsilonRI and anti-IgE autoantibodies in chronic idiopathic urticaria and correlation with disease severity. *J Allergy Clin Immunol* 2002;**110**:492—9.
11. Fiebiger E, Hammerschmid F, Stingl G, Maurer D. Anti-FcepsilonRIalpha autoantibodies in autoimmune-mediated disorders. Identification of a structure-function relationship. *J Clin Invest* 1998;**101**:243—51.
12. Zuberbier T, Henz BM, Fiebiger E, Maurer D, Stingl G. Anti-FcepsilonRIalpha serum autoantibodies in different subtypes of urticaria. *Allergy* 2000;**55**:951—4.
13. Lee MF, Lin TM, Liu SW, Chen YH. A rapid method of detecting autoantibody against FcepsilonRIalpha for chronic spontaneous urticaria. *PLoS One* 2014;**9**, e109565.
14. Ye YM, Park JW, Kim SH, Ban GY, Kim JH, Shin YS, et al. Prognostic factors for chronic spontaneous urticaria: a 6-month

prospective observational study. *Allergy Asthma Immunol Res* 2016;**8**:115—23.
15. Kikuchi Y, Fann T, Kaplan AP. Antithyroid antibodies in chronic urticaria and angioedema. *J Allergy Clin Immunol* 2003;**112**:218.
16. Leznoff A, Sussman GL. Syndrome of idiopathic chronic urticaria and angioedema with thyroid autoimmunity: a study of 90 patients. *J Allergy Clin Immunol* 1989;**84**:66—71.
17. Najib U, Bajwa ZH, Ostro MG, Sheikh J. A retrospective review of clinical presentation, thyroid autoimmunity, laboratory characteristics, and therapies used in patients with chronic idiopathic urticaria. *Ann Allergy Asthma Immunol* 2009;**103**:496—501.
18. O'Donnell BF, Francis DM, Swana GT, Seed PT, Kobza Black A, Greaves MW. Thyroid autoimmunity in chronic urticaria. *Br J Dermatol* 2005;**153**:331—5.
19. Mozena JD, Tinana A, Negri J, Steinke JW, Borish L. Lack of a role for cross-reacting anti-thyroid antibodies in chronic idiopathic urticaria. *J Invest Dermatol* 2010;**130**:1860—5.
20. Di Lorenzo G, Leto-Barone MS, La Piana S, Seidita A, Rini GB. Chronic spontaneous urticaria: an autoimmune disease? A revision of the literature. *Clin Exp Med* 2013;**13**:159—64.
21. Ferrer M, Kinet JP, Kaplan AP. Comparative studies of functional and binding assays for IgG anti-Fc(epsilon)RIalpha (alpha-subunit) in chronic urticaria. *J Allergy Clin Immunol* 1998;**101**:672—6.
22. Auyeung P, Mittag D, Hodgkin PD, Harrison LC. Autoreactive T cells in chronic spontaneous urticaria target the IgE Fc receptor Iα subunit. *J Allergy Clin Immunol* 2016;**138**:761—8.
23. Sabroe RA, Seed PT, Francis DM, Barr RM, Black AK, Greaves MW. Chronic idiopathic urticaria: comparison of the clinical features of patients with and without anti-FcepsilonRI or anti-IgE autoantibodies. *J Am Acad Dermatol* 1999;**40**:443—50.
24. Toubi E, Kessel A, Avshovich N, Bamberger E, Sabo E, Nusem D, et al. Clinical and laboratory parameters in predicting chronic urticaria duration: a prospective study of 139 patients. *Allergy* 2004;**59**:869—73.
25. Alyasin S, Hamidi M, Karimi AA, Amiri A, Ghaffarpasand F, Ehsaei MJ. Correlation between clinical findings and results of autologous serum skin test in patients with chronic idiopathic urticaria. *South Med J* 2011;**104**:111—5.
26. Rabelo-Filardi R, Daltro-Oliveira R, Campos RA. Parameters associated with chronic spontaneous urticaria duration and severity: a systematic review. *Int Arch Allergy Immunol* 2013;**161**:197—204.
27. Kim JH, Lee HY, Ban GY, Shin YS, Park HS, Ye YM. Serum clusterin as a prognostic marker of chronic spontaneous urticaria. *Med Baltim* 2016;**95**:e3688.
28. Trinh HK, Pham DL, Ban GY, Lee HY, Park HS, Ye YM. Altered systemic adipokines in patients with chronic urticaria. *Int Arch Allergy Immunol* 2016;**171**:102—10.
29. Fine LM, Bernstein JA. Guideline of chronic urticaria beyond. *Allergy Asthma Immunol Res* 2016;**8**:396—403.
30. Yu Y, Blokhuis BR, Garssen J, Redegeld FA. Non-IgE mediated mast cell activation. *Eur J Pharmacol* 2016;**778**:33—43.
31. Kaplan AP, Joseph K, Maykut RJ, Geba GP, Zeldin RK. Treatment of chronic autoimmune urticaria with omalizumab. *J Allergy Clin Immunol* 2008;**122**:569—73.
32. Saavedra MC, Sur S. Down regulation of the high-affinity IgE receptor associated with successful treatment of chronic idiopathic urticaria with omalizumab. *Clin Mol Allergy CMA* 2011;**9**:2.
33. Metz M, Staubach P, Bauer A, Brehler R, Gericke J, Kangas M, et al. Clinical efficacy of omalizumab in chronic spontaneous urticaria is associated with a reduction of FcεRI-positive cells in the skin. *Theranostics* 2017;**7**:1266—76.
34. Gericke J, Metz M, Ohanyan T, Weller K, Altrichter S, Skov PS, et al. Serum autoreactivity predicts time to response to omalizumab therapy in chronic spontaneous urticaria. *J Allergy Clin Immunol* 2017;**139**:1059—61. e1.