

Substance P Is Upregulated in the Serum of Patients with Chronic Spontaneous Urticaria

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TO THE EDITOR

Chronic spontaneous urticaria (CSU) is characterized by short-lived itchy wheals, angioedema, or both for more than 6 weeks. Symptoms of CSU are mainly mediated by the release of histamine from mast cells, but as of yet it is not entirely understood how mast cells get activated in CSU. In most CSU patients, underlying causes such as intolerance to foodstuff, chronic infections, or autoreactivity (ie due to circulating IgG anti-FcεRI, IgG anti-IgE, or IgE antibodies to autoantigens) can be identified (Grattan *et al.*, 1986; Gruber *et al.*, 1988; Hide *et al.*, 1993; Wedi *et al.*, 1998; Magerl *et al.*, 2010; Altrichter *et al.*, 2011; Hatada *et al.*, 2013; Zuberbier *et al.*, 2014). Why some people in whom these potential causes are found develop urticaria and others don't is unclear, as is the question why CSU shows spontaneous remission at some point.

Disease activity of CSU is assessed using the UAS (urticaria activity score) and the AAS (angioedema activity score), and the impact of CSU on quality of life is assessed using the CU-QoL (chronic urticaria quality of life questionnaire) and the AE-QoL (angioedema quality of life questionnaire) (Zuberbier *et al.*, 2014). The recently introduced urticaria control test (UCT) enables a quick assessment of treatment efficacy and disease control (Weller *et al.*, 2014). All of these tools are, however, based on the subjective assessment of symptoms by the patient, and there is currently no objective measure to assess disease activity or severity. We and others have tried in

the past to identify biomarkers in CSU. Among these, D-dimer has been shown to correlate with disease severity and to be especially high in antihistamine-resistant urticaria (Asero *et al.*, 2008; Asero, 2013). However, plasma D-dimer concentrations are known to be rather nonspecific as they are known to be elevated in various inflammatory processes. A good biomarker in CSU should be elevated (only) in CSU patients and correlate with disease activity. As of yet, no good biomarker has been reported and confirmed by independent studies (Metz *et al.*, 2013).

We hypothesized that Substance P (SP), an 11-amino-acid peptide that acts primarily via the G-protein-coupled receptor neurokinin-1, could be both a biomarker and a factor involved in the pathogenesis of CSU since (1) at high concentrations, SP can induce the degranulation of mast cells *in vitro* (Kulka *et al.*, 2008); (2) SP induces a wheal and flare response *in vivo*, which is more pronounced in patients with CSU as compared to healthy controls (Borici-Mazi *et al.*, 1999); and (3) low concentrations of SP, ie, concentrations that are likely to occur in the skin of patients, can increase the responsiveness of mast cells to activating signals (Janiszewski *et al.*, 1994; Forsythe and Bienenstock, 2012).

To assess whether SP is a good biomarker in CSU, we first compared serum concentrations of SP in samples from 30 healthy subjects with 118 CSU patients from two independent urticaria clinics in Germany and observed a significant and more than fourfold

increase in SP levels in patients with CSU ($491 \pm 24 \text{ pg ml}^{-1}$) compared to healthy controls ($105 \pm 28 \text{ pg ml}^{-1}$, $P < 0.0001$, Figure 1a). Additionally, we have assessed SP levels in the serum of 20 patients with cold urticaria. Here, SP levels are also increased ($280.3 \pm 24 \text{ pg ml}^{-1}$), but significantly lower than in CSU patients (Figure 1a). All analyses have been carried out in adherence to the Helsinki Guidelines and after institutional approval with written and informed patient consent.

Next, using the UAS7 as activity marker, we found a significant correlation between SP and disease activity (Figure 1b). Furthermore, very high levels of SP ($> 800 \text{ pg ml}^{-1}$) were absent in healthy subjects and in mild CSU but found in every fourth patient with severe CSU (26%), while low levels of SP were predominant in healthy controls (77%) and rarely found in severe CSU patients (13%, Figure 1c). It has been shown that mast cells can be a source of SP (Katsuno *et al.*, 2003); hence it can be speculated that elevated SP is due to massive mast cell degranulation in patients with high UAS. To address this possibility, we have correlated SP level with urticaria activity at the day of blood withdrawal, which did not reveal significant differences (Figure 1d), indicating that elevated SP levels in CSU patients are not due to SP released from mast cells.

It has been hypothesized that SP is involved in the pathogenesis of angioedema in CSU (Akcali *et al.*, 2008). We, therefore, compared patients with ($n = 48$) or without ($n = 11$) angioedema and did not detect differences in SP levels between these groups (Figure 2a). Furthermore, comparison of SP levels among CSU patients based on the underlying etiology of CSU showed no differences between these groups (Figure 2b).

Abbreviations: AAS, angioedema activity score; AE-QoL, angioedema quality of life questionnaire; CSU, chronic spontaneous urticaria; CU-QoL, chronic urticaria quality of life questionnaire; SP, Substance P; UAS, urticaria activity score; UCT, urticaria control test

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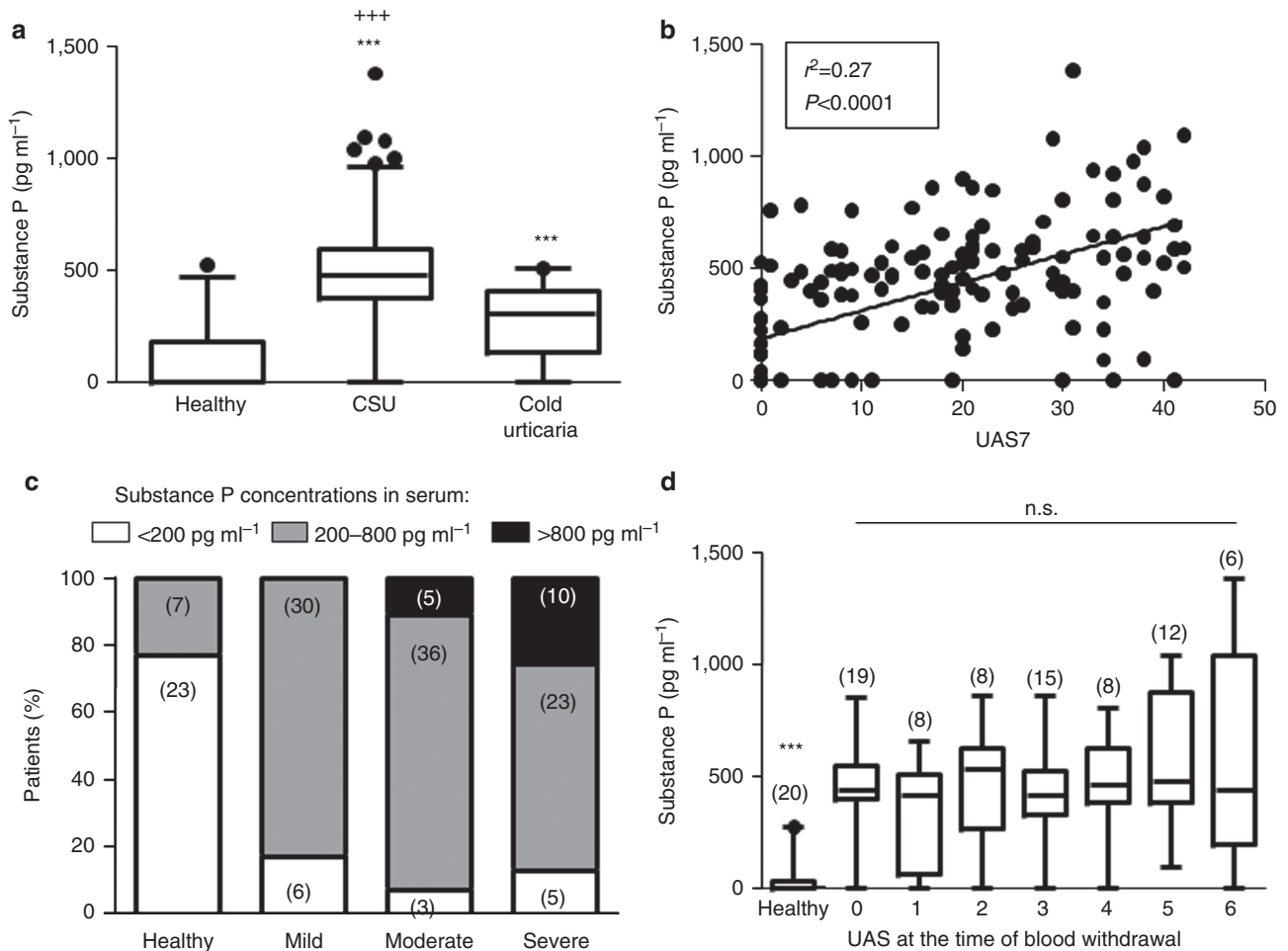


Figure 1. Substance P is markedly increased in chronic spontaneous urticaria (CSU) and correlates with disease activity. Substance P was measured by ELISA (R&D Systems, Minneapolis, MN) in the serum of CSU patients ($n=118$), age- and sex-matched healthy controls ($n=30$), and patients with cold urticaria ($n=20$). (a) Whiskers show 5–95th percentiles, with the dots representing outliers; *** $P<0.001$ vs. ‘Healthy’ and +++ $P<0.001$ vs. cold urticaria. (b) The urticaria activity score (UAS7) was calculated as a weekly score (minimum 0, maximum 42) and a linear regression was used to correlate Substance P levels with UAS. (c) Patients were arbitrarily categorized into mild, moderate, and severe CSU based on their UAS (mild 1–15, moderate 16–29, severe 30–42), and the percentage of patients showing low, medium, or high levels of substance P is shown. Total numbers are given in brackets. (d) The urticaria activity score (UAS) at the time of blood withdrawal was calculated from the score at the day of blood withdrawal (minimum 0, maximum 6). The number of patients is shown in parentheses; n.s. = no significance between any of the UAS groups, *** $P<0.001$ vs. each of the UAS groups.

CSU patients have a high prevalence of psychosomatic comorbidity and often report exacerbation of disease under stress (Staubach *et al.*, 2006). As SP has been implicated not only in inflammatory diseases but also in the pathophysiology of depression and anxiety (Rosenkranz, 2007); we next examined SP in patients with or without increased levels of anxiety, depressive mood, or history of exacerbation of disease in stress. In the investigated population, we did not observe differences in SP between either group (Figure 2c and d). To rule out other potential bias, we also correlated SP levels with duration of disease

and with the age and sex of the CSU patients. As expected, no correlation was observed for any of these groups (data not shown).

Here, we show that SP is increased in CSU patients and that only disease activity but not underlying cause of disease, occurrence of angioedema, or psychosomatic comorbidities are correlated with serum concentrations of SP. Whether SP also contributes to the pathophysiology of CSU is as of yet unclear and cannot be answered by our findings. However, hints from the literature allow us to hypothesize about a potential role of SP: Unlike human skin mast cells, mucosal mast cells in

the intestine do not constitutively express neurokinin-1, the main receptor for SP (Bischoff *et al.*, 2004). Urticaria symptoms are mainly restricted to the skin, and despite the abundance of mast cells in the intestine urticaria does usually not affect the gastrointestinal tract. A role for SP in urticaria could explain this phenomenon. Furthermore, SP has been shown to act as a sensitizer for mast cells, i.e., increased SP levels may reduce the activation threshold of mast cells (Janiszewski *et al.*, 1994; Zuberbier *et al.*, 2002; Forsythe and Bienenstock, 2012). Here, we have identified elevated SP regardless of the underlying cause of urticaria. We could

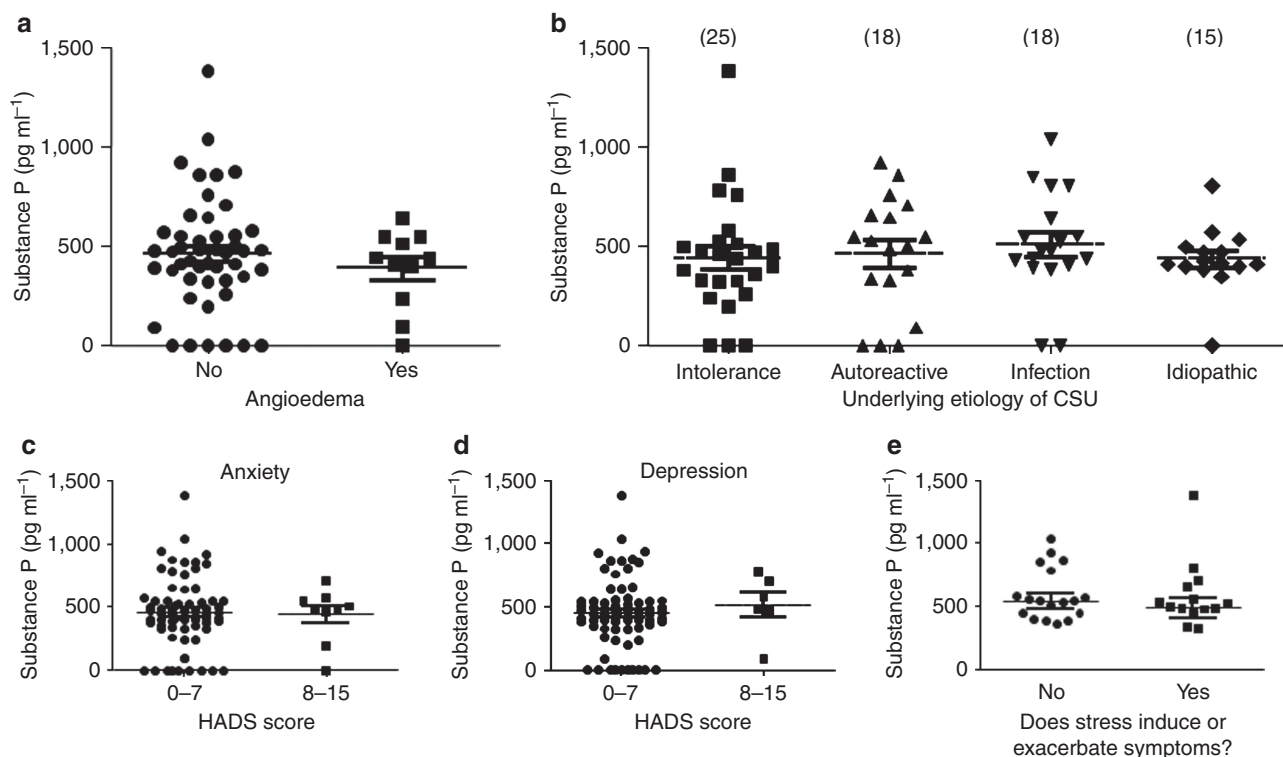


Figure 2. Substance P is elevated independently of co-factors present in chronic spontaneous urticaria (CSU) patients. Some patients underwent extensive diagnostic work-up, including pseudoallergen- and histamine-low diet, search for infections (i.e., ear, nose and throat, teeth, gastrointestinal), and autologous serum skin test, to identify the underlying etiology of CSU, and questionnaires such as the Hospital Anxiety and Depression Scale (HADS) to assess potential psychosomatic comorbidities. Overall, no differences in substance P expression were observed (a) depending on whether patients developed wheals with ($n = 11$) or without ($n = 48$) angioedema; (b) depending on the underlying cause of CSU (n given in parentheses); (c, d) depending on whether patients showed low (0–7) or high (8–15) values on the anxiety ($n = 69$ and 9 , respectively) (c) or the depression scale ($n = 72$ and 6 , respectively) (d) of the Hospital Anxiety and Depression Scale (HADS); and (e) independent of whether patients answered ‘yes’ ($n = 20$) or ‘no’ ($n = 17$) to the question if stress induces or exacerbates their symptoms.

thus hypothesize that elevated SP may be a unifying underlying sensitizer enabling other factors (e.g., complement, pseudoallergens, autoreactive substances) to reach threshold levels for activating mast cells.

In a previous report, SP has not been found to be elevated in CSU (Tedeschi *et al.*, 2005). In our hands there was a very robust and reproducible upregulation of SP in CSU patients from two independent centres as compared to healthy controls; we can therefore not explain this discrepancy. However, it has been shown before that bovine SP is markedly reduced in blood samples processed within 1 hour because of rapid degradation by enzymes (Mosher *et al.*, 2014). To avoid degradation of SP, we have processed blood within 30 minutes and stored serum at -80°C . Possibly, differences in the blood processing have resulted in the observed discrepancies. Furthermore,

we compared SP levels in serum with or without the addition of the enzyme inhibitor aprotinin and EDTA and did not observe differences in SP concentrations (data not shown).

Taken together, our results indicate that SP has the potential to be used as a biomarker in CSU and that it could be a therapeutic target in CSU treatment.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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REFERENCES

- Akcali C, Ozkur M, Erbagci Z *et al.* (2008) Association of insertion/deletion polymorphism of the angiotensin-converting enzyme gene with angio-oedema accompanying chronic urticaria but not chronic urticaria without angio-oedema or the autologous serum skin test response. *J Eur Acad Dermatol Venereol* 22:83–6
- Altrichter S, Peter HJ, Pisarevskaja D *et al.* (2011) IgE mediated autoallergy against thyroid peroxidase—a novel pathomechanism of chronic spontaneous urticaria? *PloS One* 6: e14794
- Asero R (2013) D-dimer: a biomarker for antihistamine-resistant chronic urticaria. *J Allergy Clin Immunol* 132:983–6
- Asero R, Tedeschi A, Riboldi P *et al.* (2008) Severe chronic urticaria is associated with elevated plasma levels of D-dimer. *Allergy* 63:176–80
- Bischoff SC, Schwengberg S, Lorentz A *et al.* (2004) Substance P and other neuropeptides do not induce mediator release in isolated human intestinal mast cells. *Neurogastroenterol Motil* 16:185–93

- Borici-Mazi R, Kouridakis S, Kontou-Fili K (1999) Cutaneous responses to substance P and calcitonin gene-related peptide in chronic urticaria: the effect of cetirizine and dimethindene. *Allergy* 54:46–56
- Forsythe P, Bienenstock J (2012) The mast cell-nerve functional unit: a key component of physiologic and pathophysiologic responses. *Chem Immunol Allergy* 98:196–221
- Grattan CE, Wallington TB, Warin RP et al. (1986) A serological mediator in chronic idiopathic urticaria—a clinical, immunological and histological evaluation. *Br J Dermatol* 114: 583–90
- Gruber BL, Baeza ML, Marchese MJ et al. (1988) Prevalence and functional role of anti-IgE autoantibodies in urticarial syndromes. *J Invest Dermatol* 90:213–7
- Hatada Y, Kashiwakura J, Hayama K et al. (2013) Significantly high levels of anti-dsDNA immunoglobulin E in sera and the ability of dsDNA to induce the degranulation of basophils from chronic urticaria patients. *Int Arch Allergy Immunol* 161(Suppl 2):154–8
- Hide M, Francis DM, Grattan CE et al. (1993) Autoantibodies against the high-affinity IgE receptor as a cause of histamine release in chronic urticaria. *N Engl J Med* 328: 1599–604
- Janiszewski J, Bienenstock J, Blennerhassett MG (1994) Picomolar doses of substance P trigger electrical responses in mast cells without degranulation. *Am J Physiol* 267:C138–45
- Katsuno M, Aihara M, Kojima M et al. (2003) Neuropeptides concentrations in the skin of a murine (NC/Nga mice) model of atopic dermatitis. *J Dermatol Sci* 33:55–65
- Kulka M, Sheen CH, Tancowny BP et al. (2008) Neuropeptides activate human mast cell degranulation and chemokine production. *Immunology* 123:398–410
- Magerl M, Pisarevskaja D, Scheufele R et al. (2010) Effects of a pseudoallergen-free diet on chronic spontaneous urticaria: a prospective trial. *Allergy* 65:78–83
- Metz M, Krull C, Maurer M (2013) Histamine, TNF, C5a, IL-6, -9, -18, -31, -33, TSLP, neopterin, and VEGF are not elevated in chronic spontaneous urticaria. *J Dermatol Sci* 70:222–5
- Mosher RA, Coetzee JF, Allen PS et al. (2014) Effects of sample handling methods on substance P concentrations and immunoreactivity in bovine blood samples. *Am J Vet Res* 75: 109–16
- Rosenkranz MA (2007) Substance P at the nexus of mind and body in chronic inflammation and affective disorders. *Psychol Bull* 133: 1007–37
- Staubach P, Eckhardt-Henn A, Dechene M et al. (2006) Quality of life in patients with chronic urticaria is differentially impaired and determined by psychiatric comorbidity. *Br J Dermatol* 154:294–8
- Tedeschi A, Lorini M, Asero R (2005) No evidence of increased serum substance P levels in chronic urticaria patients with and without demonstrable circulating vasoactive factors. *Clin Exp Dermatol* 30:171–5
- Wedi B, Wagner S, Werfel T et al. (1998) Prevalence of Helicobacter pylori-associated gastritis in chronic urticaria. *Int Arch Allergy Immunol* 116:288–94
- Weller K, Groffik A, Church MK et al. (2014) Development and validation of the urticaria control test: a patient-reported outcome instrument for assessing urticaria control. *J Allergy Clin Immunol* 133:1365–72
- Zuberbier T, Aberer W, Asero R et al. (2014) The EAACI/GA²LEN/EDF/AAAAI/WAO Guideline for the definition, classification, diagnosis and management of Urticaria The 2013 revision and update. *Allergy* 69:868–87
- Zuberbier T, Pfrommer C, Specht K et al. (2002) Aromatic components of food as novel eliciting factors of pseudoallergic reactions in chronic urticaria. *J Allergy Clin Immunol* 109:343–8

Biomechanical Properties of the Skin in Cutis Laxa

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TO THE EDITOR

Cutis laxa (CL) is a heterogeneous group of disorders characterized by loose, redundant, inelastic, or prematurely wrinkled skin (Berk et al., 2012; Uitto et al., 2013). Several inherited forms of CL have been identified (Urban and Davis, 2014), with nine causative genes known to date (*ALDH18A1*, *ATP6V0A2*, *ATP7A*, *EFEMP2/FBLN4*, *ELN*, *FBLN5*, *LTBP4*, *PYCR1*, *RIN2*). A shared feature of all types of inherited CL is a reduced or abnormal deposition of elastic fibers in the skin and other tissues (Berk et al., 2012). The consequences of this disorder for the biomechanics of the skin has received little attention (Grahame and Beighton, 1971). Such studies are essential for a fundamental understanding of the contribution of

elastic fibers to the mechanical properties of the skin, and for an improved, objective diagnosis of CL.

In the present study, 118 participating controls and 17 CL patients were inclu-

ded with informed, written consent. The same control cohort was used in a concurrent study on the mechanical properties of the skin in Williams–Beuren syndrome (Kozel et al., 2014).

Table 1. Demography and elasticity parameters in the participants

	Controls (n = 118)	Patients (n = 17)	P-value
Age	33.22 ± 1.58	29.21 ± 5.47	0.490 ^a
Gender (male %)	37.3%	35.3%	0.874 ^b
E (MPa)	11.61 ± 0.15	7.85 ± 0.60	<0.0001 ^a
Retraction time (ms)	622.82 ± 21.20	1,152.82 ± 211.21	0.024 ^a
VE (MPa)	5.35 ± 0.14	2.51 ± 0.32	<0.0001 ^a

Abbreviations: E, elastic modulus; VE, viscoelastic modulus.

Continuous variables: mean ± standard error of mean.

^aIndependent t-test.

^bChi-square test.

Abbreviations: ADCL, autosomal dominant cutis laxa; CL, cutis laxa; E, elastic modulus; ROC, receiver operating characteristic; RT, retraction time; VE, viscoelastic modulus

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