

Report

Thyrotropin receptor gene expression in the association between chronic spontaneous urticaria and Hashimoto's thyroiditis

Maria R. C. Silveiras¹, MD, PhD, Maria R. P. Fortes¹, PhD, Renata de A. M. L. Nascimento², PhD, Carlos R. Padovani³, PhD, Hélio A. Miot¹, MD, PhD, Célia R. Nogueira², MD, PhD, and Gláucia M. F. da S. Mazeto², MD, PhD

¹Department of Dermatology and Radiotherapy, Botucatu Medical School, Sao Paulo State University – Unesp, Botucatu, Sao Paulo, Brazil, ²Department of Internal Medicine, Botucatu Medical School, Sao Paulo State University – Unesp, Botucatu, Sao Paulo, Brazil, and ³Department of Biostatistics, Institute of Bioscience, Sao Paulo State University – Unesp, Botucatu, Sao Paulo, Brazil

Correspondence

Gláucia M. F. S. Mazeto, MD, PhD
Department of Internal Medicine
Botucatu Medical School, Unesp
18618-970 Botucatu
Sao Paulo
Brazil
E-mail: gmazeto@fmb.unesp.br

Conflicts of interest: The author(s) declare (s) that there is no conflict of interests regarding the publication of this paper.

doi: 10.1111/ijd.13487

[Correction made on 2/17/17, after first online publication: A new reference, Slominski A, Wortsman J, Kohn L, et al. Expression of hypothalamic-pituitary-thyroid axis related genes in the human skin. *J Invest Dermatol* 2002; 119: 1449–1455., was added as reference #18. All reference numbers were adjusted accordingly.]

Introduction

Chronic urticaria (CU), a severe and common condition, has a great impact on a patient's quality of life.^{1,2} In most cases, the cause is unknown, thus the condition is known as chronic spontaneous urticaria (CSU).^{3,4} Autoimmune phenomena are identified in approximately 25–45% of CSU cases characterized by the detection of IgG class histamine releasing antibodies directed against high affinity IgE receptors (antiFcεRI) or against IgE

Abstract

Background Chronic spontaneous urticaria (CSU) may be associated with autoimmune thyroid diseases, and the Autologous Serum Skin Test (ASST) is an autoreactivity marker. The thyrotropin (TSH) and TSH receptor (TSHR) could play a role in the pathogenesis of CSU. The aim of this study was to evaluate ASST positivity and TSHR gene expression in healthy skin and ASST wheals in euthyroid women with CSU, with (14 patients) and without (15 patients) Hashimoto's thyroiditis (HT).

Methods ASST was performed and TSHR gene expression studied in wheals induced by ASST and in healthy skin.

Results ASST presented greater positivity (86% × 40%) and larger diameter (10.8 × 9.6 mm) in the HT group ($P < 0.05$). TSHR gene expression was higher in the ASST area and healthy skin of HT group ($P < 0.01$). Positive correlation of antibodies levels with ASST wheal measurements and TSHR gene expression was seen.

Conclusions Women with CSU and HT presented greater positivity and larger measurements for ASST and higher TSHR expression in the skin, suggesting association between CSU, thyroid autoimmunity, and TSHR.

itself,^{3–5} and the Autologous Serum Skin Test (ASST) assesses such autoreactivity.^{1,6,7}

There are reports of association between CSU and autoimmune thyroid disease,^{8–10} specifically Graves disease (GD) and Hashimoto's thyroiditis (HT).^{10–12} HT is the most common autoimmune thyroid disease and may evolve to primary hypothyroidism. Autoantigens such as thyroperoxidase (TPO), thyroglobulin (TG), and thyrotropin (TSH) receptor have been proposed to be involved in its pathogenesis.^{13,14}

To strengthen the link between HT and CSU, it has been observed that this dermatological condition, when associated with the autoimmune thyroid disease, responds poorly to conventional treatment, and improvements with levothyroxine use were reported in some studies.^{11,15–17} These findings support the research of a new TSH role, and perhaps of its receptor, in immune response. Indeed, TSH receptor (TSHR) detection in dermis fibroblasts and melanocytes indicates that the skin is a target of thyroid axis.^{18–21} Thus, both thyroid autoimmunity and hormonal action on the TSHR could be involved in the pathogenesis of CSU. However, currently, the mechanisms involved in the relationship between CSU and thyroid disease, particularly HT, are unknown, which raises the question of whether both conditions have a cause–effect relationship or are two simultaneous autoimmune diseases.

Therefore, the objective of this study was to evaluate ASST positivity and TSHR gene expression in ASST wheals and healthy skin of euthyroid women with CSU, with and without HT.

Materials and methods

The current study was approved by the Botucatu Medical School Research Ethics Committee (protocol 341/2007), and all subjects gave their informed consent. All the procedures followed were in accordance with the Helsinki Declaration of 1975.

Patients

This was a transverse study with 29 female CSU patients seen at the Urticaria Outpatient Clinic, Botucatu Medical School (UNESP), Brazil, between July 2008 and May 2009. Of these, 14 had CSU and HT (HT group) and 15 had CSU without HT (non-HT group). The groups were compared for positivity and measurements of wheals caused by ASST and physiological solution as well as for TSHR gene expression in the wheal caused by the test and in healthy skin. Comparisons were also made for age and ethnicity, serum TSH, free thyroxine (FT₄), anti-TPO and anti-TG concentrations, and severity of CSU, through angioedema frequency evaluation.

The inclusion criteria were: female gender, over 18 years of age, with CSU diagnosis, euthyroid (normal serum TSH and FT), without the use of any medication for the thyroid, with or without HT diagnosis, no antihistamine use in the previous 5 days and no immunosuppression drug use in the previous 2 months.²² The following were excluded: those with acute urticaria, physical urticaria, urticaria vasculitis, those with CU of a known cause, or systemic diseases such as connectivopathies, other autoimmune or lymphoproliferative diseases, and those patients taking other medicines.

Chronic spontaneous urticaria diagnosis was defined by the presence of daily rashes, or one to three episodes *per* week, with and without angioedema, for a period of more than 6 weeks, without detectable cause.⁴ The investigation of the presence of HT was systematic, and no patient had symptoms

of thyroid disease. HT diagnosis was confirmed by at least three positive antithyroid antibody measurements (anti-TPO and/or anti-TG) at different occasions. All patients had normal serum TSH and FT₄, without the use of levothyroxine.

Hormone levels

Serum FT₄, TSH, anti-TPO, and anti-TG levels were obtained by the chemiluminescence method (reference values: 0.70–1.48 ng/dl; 0.35–4.94 μ lU/ml; <5.61 UI/ml; and <14.4 UI/ml, respectively) performed in the institution clinical laboratory.

Autologous serum skin test

ASST was performed after collecting 5 ml of peripheral blood and centrifuging it for 10 minutes. A quantity (0.05 ml) of the resulting serum was aspirated and intradermally injected into the arm contralateral to where the blood was collected. The injection was performed 5 cm from the cubital fossa with a BD Insulin Syringe and Ultra-Fine™ Needle 31Gx5/16" (0.3 mm caliber $\times$ 8 mm) (Becton Dickinson & Company, Franklin Lakes, NJ, USA). The size of the wheal caused by the autologous serum injection was measured 40 minutes after injection and compared to a wheal from an injection of 0.9% sodium chloride (physiological solution – PS) performed by the same technique 5 cm from the site of the serum injection. ASST was considered positive when the ASST wheal was 1.5 mm larger than the PS wheal.^{5,23–25}

All tests were performed by the main researcher (M.R.C.S.). Wheals were measured in millimeters with a pachymeter. Immediately after the test wheal measurements (area and diameter), a biopsy was performed on the wheal and on standard healthy skin 5 cm from the test. Biopsies were performed with a 4 mm diameter punch after asepsis, local anesthesia with 2% lidocaine, and administration of 1 : 1000 adrenalin. ASST wheal and skin fragments were placed in dry flasks and kept in liquid nitrogen for later study of tissue TSHR gene expression by real-time PCR (polymerase chain reaction).

TSHR mRNA expression

Total RNA was extracted from ASST and healthy skin using TRIzol (Invitrogen, São Paulo, Brazil) according to manufacturer's instructions. RNA concentration was determined by absorbance at 260 nm; all samples had an absorption ratio of approximately 2.0. One microgram of total RNA was used for the synthesis of 20 μ l of complementary DNA (cDNA) by Super Script II First-Strand Synthesis System for RT-PCR (Invitrogen). The mRNA levels of TSHR (HSO1053846 m1) were determined by real-time PCR. Quantitative measurements of cyclophilin (HS99999904 m1) were made with the commercial TaqMan PCR kit (Applied Biosystems, São Paulo, Brazil) in the detection system Applied Biosystems StepOne Plus (Applied Biosystems, Foster City, CA, USA). Cycling conditions were as follows: enzyme activation at 50 °C for 2 minutes, denaturation at 95 °C for 10 minutes, amplification of the cDNA in 40 cycles of

Table 1 Clinical and laboratory data for patients with chronic spontaneous urticaria with or without HT

Clinical and laboratory data	Group		P-value
	HT (n = 14)	Non-HT (n = 15)	
Age (years) ^a	46.6 ± 14.0	42.8 ± 12.5	0.44
White ethnicity – N (%) ^b	13 (93)	14 (93)	0.99
Angioedema frequency – N (%) ^b	1 (7.1)	1 (6.7)	0.93
TSH (μUI/ml) ^c	1.1 (0.4–2.2)	1.4 (1.0–1.8)	0.30
FT ₄ (ng/dl) ^a	1.2 ± 0.2	1.1 ± 0.1	0.11
Antithyroperoxidase (UI/ml) ^c	414.5 (73.4–1000.0)	0.3 (0.3–0.8)	<0.01
Antithyroglobulin (UI/ml) ^c	15.4 (6.7–82.6)	1.5 (1.1–4.2)	<0.01

HT, Hashimoto's thyroiditis; TSH, thyrotropin; FT₄, free thyroxin.^aMean and standard deviation; Student *t* test.^bExact Fisher's test.^cMedian (1st and 3rd quartiles); Mann–Whitney test.

denaturation at 95 °C for 15 seconds, and annealing/extension at 60 °C for 1 minute. All samples were assayed in duplicate. Gene expression was quantified in relation to the values of the healthy skin group or in relation to the values of the non-HT group, after normalization by internal control (cyclophilin – HS99999904_m1; Applied Biosystems) using the $2^{-\Delta\Delta CT}$ method.²⁶

Statistical analysis

Sample size was calculated from the pre-test with 10 patients in each group, with an aim of estimating an alpha level of 0.05 and a power of 0.8 for each variable.

Comparison of clinical and laboratory data between groups was analyzed with Student's *t* test or the Mann–Whitney *U* test if normality was not identified by the Kolmogorov–Smirnov test (with Lilliefors' correction). Linear correlation between measurements of ASST, TSH, FT₄, anti-TPO, anti-TG, and

relative TSHR gene expression in ASST was estimated by the Spearman coefficient (ρ). Comparison of relative TSHR gene expression in the ASST wheal and the healthy skin of HT and without HT groups was performed using the paired Student's *t* test. Percentages relating to ethnicity and ASST positivity were compared by the chi squared, Fisher's exact, or Goodman test. Data were evaluated using SPSS 20.0 software (IBM Analytics, Armonk, NY, USA). Differences were considered statistically significant when $P < 0.05$.²⁷

Results

Clinical and laboratory data are shown in Table 1. Groups with and without HT did not differ for age, ethnicity, TSH, and FT₄ serum concentrations, or angioedema frequency. Anti-TPO and anti-TG antibody concentrations were significantly higher in the HT group.

The percentage of patients with positive ASST was higher and ASST wheal diameters were larger in the HT group than the non-HT group, with no difference between groups in the PS test (7.0 ± 1.6 vs. 7.9 ± 1.5 mm; $P = 0.15$; Table 2). Wheal areas were also larger in the HT group but without a difference between groups for PS test wheals (40.3 ± 16.6 vs. 49.9 ± 19.2 mm²; $P = 0.16$). TSHR gene expression was higher in the HT group than in the non-HT group, both in healthy skin and in ASST wheals (Table 2). TSHR relative gene expression was higher in ASST wheals than healthy skin in both the HT and non-HT groups (1.36 ± 0.36 vs. 1.00 ± 0.23 ; 1.46 ± 0.43 vs. 1.00 ± 0.27 , respectively; $P < 0.01$).

Positive correlation was seen between ASST measurements and antithyroid antibody levels (anti-TPO and anti-TG). TSHR gene expression correlated with anti-TG and anti-TPO levels. Gene expression in healthy skin correlated with ASST measurements and with anti-TPO serum concentrations (Table 3). Additionally, there was no correlation between TSHR gene expression in healthy skin and in ASST wheal, in both HT ($r = 0.03$; $P = 0.91$) and non-HT groups ($r = -0.04$; $P = 0.90$).

Table 2 ASST measurements and TSHR relative gene expression^a in patients with chronic spontaneous urticaria with or without Hashimoto's thyroiditis (HT)

ASST measurements and TSHR gene expression data	Groups		P-value
	HT (n = 14)	Non-HT (n = 15)	
Positive ASST n (%) ^b	12 (85.7)	6 (40.0)	0.02
ASST wheal diameter (mm) ^c	10.8 ± 1.4	9.6 ± 1.4	0.02
ASST wheal areas (mm ²) ^c	92.8 ± 21.9	73.0 ± 20.7	0.02
TSHR gene expression in healthy skin ^c	1.58 ± 0.36	1.00 ± 0.27	<0.01
TSHR gene expression in ASST wheal ^c	1.47 ± 0.38	1.00 ± 0.29	<0.01

ASST, Autologous Serum Skin Test; TSHR, thyrotropin receptor; HT, Hashimoto's thyroiditis.

^aTSHR gene expression was quantified in relation to the values of the non-HT group.^bGoodman test.^cMean ± standard deviation; Student *t* test.

Table 3 Coefficients of linear correlation (Spearman) between ASST measurements, TSH, free-T₄, antithyroperoxidase, antithyroglobulin, and TSH receptor gene expression in ASST wheal

	TSH	FT ₄	Anti-TG	Anti-TPO	TSHRw	TSHRh
ASST wheal diameter	0.00	0.02	0.41*	0.53*	0.30	0.43*
ASST wheal area	0.00	0.02	0.39*	0.54*	0.28	0.47*
TSH	–	–0.56*	–0.08	0.07	–0.12	–0.26
FT ₄	–	–	0.12	0.23	0.21	0.20
Anti-TG	–	–	–	0.61*	0.49*	0.24
Anti-TPO	–	–	–	–	0.37*	0.52*
TSHRw	–	–	–	–	–	0.22

ASST, autologous serum skin test; TSH, thyrotropin; FT₄, free thyroxine; anti-TPO, antithyroperoxidase antibody; anti-TG, antithyroglobulin antibody; TSHRw, TSH receptor in ASST wheal; TSHRh, TSH receptor in adjacent healthy skin.

* $P < 0.05$.

Discussion

Concomitance of CSU and HT implies a more severe picture of urticaria, in which the disease is less responsive to conventional treatment, has a varied response to levothyroxine, and has a higher positivity to both ASST and antiFcεRI and/or against IgE autoantibodies.^{15,17,24,28–30} Moreover, the frequency of thyroid autoantibodies is significantly higher in patients with CU than in healthy controls, and the urticaria duration is associated with the positivity of these antibodies.^{31,32} On the other hand, a recent study comparing patients with CSU and autoimmune hypothyroidism treated with levothyroxine, and euthyroid subjects with CSU, did not observe differences between the groups regarding the Urticaria Activity Score after 3 and 6 months of the hormone treatment.³³

The truth is that the mechanisms which connect CSU and HT have not yet been explained. In addition, the TSHR represents one of the HT autoantigens,¹³ and it is present in skin cells, suggesting that the epidermis is a TSH signaling target.^{18–21} Indeed, thyroid hormones alter skin architecture and homeostasis.^{9,21,34} Therefore, the purpose of this study was to evaluate ASST positivity and TSHR gene expression in ASST wheals and healthy skin of women with CSU, with and without HT. Patients were found to be euthyroid, with differences only in the presence of specific thyroid antibodies, thus minimizing the possibility of hormone impact in the results.

ASST measurements were larger in the HT group than in the non-HT group. In the literature, there is a lack of ASST measurement standardization. Most studies have evaluated test positivity or negativity based on means of measurements.^{3,23,30} In this study, we observed that ASST measurements correlated with antithyroid antibodies and with TSHR gene expression.

Grattan *et al.* (1991) considered ASST area as the measure of test positivity but did not investigate its relationship with thyroid autoimmunity.³⁵ Similarly, Sabroe *et al.* (1999) observed larger wheal diameter, volume, and erythema in addition to reflex erythema in CSU patients compared to healthy individuals.³⁰ Only Saab *et al.* (2006) investigated the correlation of ASST measurements with positivity for anti-TPO and anti-TG in CSU and in healthy controls; they observed larger antithyroid antibody concentrations and wheal diameters in CU compared to controls, suggesting a relationship between thyroid autoimmunity and ASST diameter.³⁶ A recent study has shown an indirect correlation between urticaria and anti-TG.³⁷ However, the exact role of antithyroid antibodies in CSU remains unclear.

The percentage of patients with positive ASST was higher in the HT group, which is in line with the findings of other authors.^{1,36} Gaig *et al.* (2000) also observed 55.6% ASST positivity in a series of cases.³⁸ Fusari *et al.* (2005) observed positive ASST in 86.7 and 8% of patients with and without HT, respectively.²³ However, Mozena *et al.* (2010) reported no difference in test positivity between patients with and without HT.³⁹

Higher positivity and measurements for ASST found in HT cases and higher anti-TPO concentrations in CSU patients support the idea of a relationship between thyroid autoimmunity and ASST.^{28,40,41} There are no studies linking ASST area with antithyroid antibodies, and this relationship therefore requires further investigation. In 2006, Saab *et al.* evaluated positivity but not ASST dimensions in 68 patients,³⁶ while O'Donnell *et al.* (2005) observed that the main influencing factor in anti-TPO positivity was ASST positivity.¹ Recently, Turkoglu *et al.* (2012), when evaluating individuals without CU, observed a greater ASST positivity in HT cases than in healthy volunteers. They concluded that the patients with HT have more skin mast cell autoreactivity, regardless of CU presentation.⁴²

Thyrotropin concentrations were not different between the two groups, and this hormone was not correlated with the ASST. Thus, it does not seem that the TSH has been responsible for the observed differences in test responses. On the other hand, the correlation observed between TSHR gene expression and antithyroid antibodies with ASST response suggests that TSHR may play a role in CSU pathogenesis, particularly when there is an association with thyroid autoimmunity. Therefore, the mechanisms involved in this relationship, whether by antibodies directed against TSHR or by the TSH stimulation route, or both, need further clarification. Bodó *et al.* (2010) reported immunoreactivity for TSH in the epidermis of normal individuals, which is upregulated by hypothalamic TRH and downregulated by thyroid hormones, and for TSHR in the dermis adjacent to the epidermis.³⁴ Csaba and Pallinger (2009) observed that stimulus with TSH provoked increased intracellular T₃ concentrations in lymphocytes, suggesting that the pituitary hormone can regulate thyroid hormone concentration in immune cells and influence the course and development of inflammatory

dermatitis.⁴³ However, Bianco *et al.* (1983) observed that mast cells presented a regulatory role in the secretion of TSH by the pituitary gland.⁴⁴ Therefore, although histopathological evaluation was not performed in this study, based on previously quoted literature, the authors suggest that the expression of the TSHR was present in the fibroblasts and other cells of the healthy skin and also in the inflammatory cells from the wheal caused by ASST. This would indicate a relationship between TSH, fibroblasts and, particularly, the inflammatory response, as suggested by the higher expression of TSHR in the ASST wheal compared to adjacent skin. Thus, considering that ASST also indicates mast cell autoreactivity,⁴² the authors hypothesize that these cells may represent skin targets for both TSH and autoantibody action, influencing the clinical picture of CSU associated with HT. In this context, the greater TSHR expression also observed in healthy skin of the HT group could indicate a greater influx of mast cells and other inflammatory cells to the nearby regions of ASST wheal.

Finally, the authors propose that the ASST wheal can represent a model to study the involvement of thyroid autoimmunity in CSU pathogenesis, based on the histological similarity of inflammatory infiltrate,^{40,45} and on the observation that TSH induces histamine liberation from intrathyroid mast cells,^{41,42} as well as on TSH's capacity to bind to polymorphonuclear and mononuclear cells and lymphocytes from peripheral blood.^{40,41,43,45}

The small sample size, the exclusive use of adult women, and the lack of a control group of HT patients without CSU are limitations in this study. However, the consistent results, correlation between variables, and the reproduction of observations from other publications add strength to the findings.

In conclusion, women with CSU and HT presented higher ASST positivity and wheal dimensions with higher TSHR expression in the skin. Positive correlations between antithyroid antibodies and both ASST measurements and TSHR gene expression in ASST strengthens the evidence for an association between these two conditions. Moreover, these findings could suggest a pathogenetic link between thyroid autoimmunity (particularly, the presence of the anti-TPO), TSHR, and CSU, which highlights the need for further investigations.

Acknowledgments

The authors are grateful to the Research Support Foundation of the State of São Paulo (Fundação de Amparo à Pesquisa do Estado de São Paulo, Fapesp; grant no. 08/50303) for the financial support.

References

- O'Donnell BF, Francis DM, Swana GT, *et al.* Thyroid autoimmunity in chronic urticaria. *Br J Dermatol* 2005; **153**: 331–335.
- Silvares MR, Fortes MR, Miot HA. Quality of life in chronic urticaria: a survey at a public university outpatient clinic, Botucatu (Brazil). *Rev Assoc Med Bras* 2011; **57**: 577–582.
- Zuberbier T, Bindsley-Jensen C, Canonica W, *et al.* EAACI/GA2LEN/EDF guideline: definition, classification and diagnosis of urticaria. *Allergy* 2006; **61**: 316–320.
- Zuberbier T, Aberer W, Asero R, *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**: 868–887.
- Kaplan AP, Greaves M. Pathogenesis of chronic urticaria. *Clin Exp Allergy* 2009; **39**: 777–787.
- Konstantinou GN, Asero R, Maurer M, *et al.* EAACI/GA(2) LEN task force consensus report: the autologous serum skin test in urticaria. *Allergy* 2009; **64**: 1256–1268.
- 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.
- Confino-Cohen R, Chodick G, Shalev V, *et al.* Chronic urticaria and autoimmunity: associations found in a large population study. *J Allergy Clin Immunol* 2012; **129**: 1307–1313.
- Dreskin SC, Andrews KY. The thyroid and urticaria. *Curr Opin Allergy Clin Immunol* 2005; **5**: 408–412.
- Missaka RFBG, Penalti HC, Silvares MR, *et al.* Autoimmune thyroid disease as a risk factor for angioedema in patients with chronic idiopathic urticaria: a case-control study. *Sao Paulo Med J* 2012; **130**: 294–298.
- Bagnasco M, Minciullo PL, Saraceno GS, *et al.* Urticaria and thyroid autoimmunity. *Thyroid* 2011; **21**: 401–410.
- Ruggeri RM, Imbesi S, Saitta S, *et al.* Chronic idiopathic urticaria and Graves' disease. *J Endocrinol Invest* 2013; **36**: 531–536.
- Davies TF, Yin X, Latif R. The genetics of the thyroid stimulating hormone receptor: history and relevance. *Thyroid* 2010; **20**: 727–736.
- Tunbridge WM, Vanderpump MP. Population screening for autoimmune thyroid disease. *Endocrinol Metab Clin North Am* 2000; **29**: 239–253.
- Aversano M, Caiazzo P, Iorio G, *et al.* Improvement of chronic idiopathic urticaria with L-thyroxine: a new TSH role in immune response? *Allergy* 2005; **60**: 489–493.
- Bangash SA, Bahna SL. Resolution of chronic urticaria and angioedema with thyroxine. *Allergy Asthma Proc* 2005; **26**: 415–417.
- Rumbyrt JS, Katz JL, Schocket AL. Resolution of chronic urticaria in patients with thyroid autoimmunity. *J Allergy Clin Immunol* 1995; **96**: 901–905.
- Slominski A, Wortsman J, Kohn L, *et al.* Expression of hypothalamic-pituitary-thyroid axis related genes in the human skin. *J Invest Dermatol* 2002; **119**: 1449–1455.
- Cianfarani F, Baldini E, Cavalli A, *et al.* TSH receptor and thyroid-specific gene expression in human skin. *J Invest Dermatol* 2010; **130**: 93–101.
- Daumerie C, Ludgate M, Costagliola S, *et al.* Evidence for thyrotropin receptor immunoreactivity in pretibial connective tissue from patients with thyroid-associated dermopathy. *Eur J Endocrinol* 2002; **146**: 35–38.
- Ellerhorst JA, Sendi-Naderi A, Johnson MK, *et al.* Human melanoma cells express functional receptors for thyroid-stimulating hormone. *Endocr Relat Cancer* 2006; **13**: 1269–1277.
- Grattan CE, Sabroe RA, Greaves MW. Chronic urticaria. *J Am Acad Dermatol* 2002; **46**: 645–657.

- 23 Fusari A, Colangelo C, Bonifazi F, *et al.* The autologous serum skin test in the follow-up of patients with chronic urticaria. *Allergy* 2005; **60**: 256–258.
- 24 Kandeel AA, Zeid M, Helm T, *et al.* Evaluation of chronic urticaria in patients with Hashimoto thyroiditis. *J Clin Immunol* 2001; **21**: 335–347.
- 25 Sabroe RA, Greaves MW. Chronic idiopathic urticaria with functional autoantibodies: 12 years on. *Br J Dermatol* 2006; **154**: 813–819.
- 26 Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta C(T)) method. *Methods* 2001; **25**: 402–408.
- 27 Zar JH. *Biostatistical Analysis*. Englewood Cliff, NJ: Prentice-Hall, 2009.
- 28 Kozel MM, Sabroe RA. Chronic urticaria: aetiology, management and current and future treatment options. *Drugs* 2004; **64**: 2515–2536.
- 29 Paus R. Exploring the “thyroid-skin connection”: concepts, questions, and clinical relevance. *J Invest Dermatol* 2010; **130**: 7–10.
- 30 Sabroe RA, Seed PT, Francis DM, *et al.* Chronic idiopathic urticaria: comparison of the clinical features of patients with and without anti-FcεRI or anti-IgE autoantibodies. *J Am Acad Dermatol* 1999; **40**: 443–450.
- 31 Verneuil L, Leconte C, Ballet JJ, *et al.* Association between chronic urticaria and thyroid autoimmunity: a prospective study involving 99 patients. *Dermatology* 2004; **208**: 98–103.
- 32 Toubi E, Kessel A, Avshovich N, *et al.* Clinical and laboratory parameters in predicting chronic urticaria duration: a prospective study of 139 patients. *Allergy* 2004; **59**: 869–873.
- 33 Magen E, Mishal J. The effect of L-thyroxine treatment on chronic idiopathic urticaria and autoimmune thyroiditis. *Int J Dermatol* 2012; **51**: 94–97.
- 34 Bodó E, Kany B, Gaspar E, *et al.* Thyroid-stimulating hormone, a novel, locally produced modulator of human epidermal functions, is regulated by thyrotropin-releasing hormone and thyroid hormones. *Endocrinology* 2010; **151**: 1633–1642.
- 35 Grattan CE, Francis DM, Hide M, *et al.* Detection of circulating histamine releasing autoantibodies with functional properties of anti-IgE in chronic urticaria. *Clin Exp Allergy* 1991; **21**: 695–704.
- 36 Saab NGJ, Vera JG, Tiro JJJ, *et al.* Prevalencia de urticaria crónica de origen autoinmunitario en el Hospital Regional Adolfo López Mateos. *Rev Alerg Mex* 2006; **53**: 58–63.
- 37 Sun L, Erxun K, Li J, *et al.* Correlations between anti-mast cell autoantibodies and chronic idiopathic urticaria. *Ann Dermatol* 2014; **26**: 145–149.
- 38 Gaig P, Garcia-Ortega P, Enrique E, *et al.* Successful treatment of chronic idiopathic urticaria associated with thyroid autoimmunity. *J Invest Allergol Clin Immunol* 2000; **10**: 342–345.
- 39 Mozena JD, Tinana A, Negri J, *et al.* Lack of a role for cross-reacting anti-thyroid antibodies in chronic idiopathic urticaria. *J Invest Dermatol* 2010; **130**: 1860–1865.
- 40 Coutelier JP, Kehrl JH, Bellur SS, *et al.* Binding and functional effects of thyroid stimulating hormone on human immune cells. *J Clin Immunol* 1990; **10**: 204–210.
- 41 Ericson LE, Hakanson R, Melander A, *et al.* TSH-induced release of 5-hydroxytryptamine and histamine rat thyroid mast cells. *Endocrinology* 1972; **90**: 795–801.
- 42 Turkoglu Z, Zindanci I, Turkoglu O, *et al.* Skin autoreactivity in Hashimoto’s thyroiditis patients without urticaria: autologous serum skin test positivity correlation with thyroid antibodies, sonographical volume and grading. *Eur J Dermatol* 2012; **22**: 345–350.
- 43 Csaba G, Pallinger E. Thyrotropic hormone (TSH) regulation of triiodothyronine (T(3)) concentration in immune cells. *Inflamm Res* 2009; **58**: 151–154.
- 44 Bianco AC, Nunes MT, Douglas CR, *et al.* Influence of mast cells on thyroid function. *Endocrinol Exp* 1983; **17**: 99–106.
- 45 Kiss E, Balazs C, Bene L, *et al.* Effect of TSH and anti-TSH receptor antibodies on the plasma membrane potential of polymorphonuclear granulocytes. *Immunol Lett* 1997; **55**: 173–177.