



Identification of antigenic epitopes of thyroperoxidase, thyroglobulin and interleukin-24. Exploration of cross-reactivity with environmental allergens and possible role in urticaria and hypothyroidism

Andrés Sánchez^{a,b}, Ricardo Cardona^b, Marlon Munera^a, Jorge Sánchez^{b,*}

^a Medical Research Group (GINUMED) University Corporation Rafael Nuñez, Immunology Department, Faculty of medicine, Cartagena, Colombia

^b Group of Clinical and Experimental Allergy (GACE), IPS Universitaria, University of Antioquia, Medellín, Colombia



ARTICLE INFO

Keywords:

Autoantibodies
Thyroperoxidase
Interleukin-24
Thyroglobulin
Urticaria
Hypothyroidism

ABSTRACT

Background: Human proteins such as interleukin-24 (IL24), thyroperoxidase (TPO) and thyroglobulin (Tg) are targets of IgE or IgG autoantibodies. Why these proteins are recognized by autoantibodies in some patients with chronic spontaneous urticaria (CSU) or hypothyroidism is unknown.

Objective: Through in silico analysis, identify antigen patches of TPO, Tg and IL24 and compare the sequences of these human proteins with some prevalent allergens.

Methods: The amino acids sequences of IL24, thyroperoxidase and thyroglobulin were compared between them and with 22 environmental allergens. Phylogenetic studies and multiple pairing were carried out to explore the degree of protein identity and cover. The proteins without 3D structure reported in the database, were modeled by homology with “Swiss Modeller” and compared through PYMOL. Residues conserved and accessible to the solvent (rASA > 0.25) were located in the 3D model to identify possible areas of cross-reactivity and antigen binding.

Results: We build a 3D model of the TPO and thyroglobulin protein base on proteins closely related. Five epitopes for TPO, six for IL24 and six for thyroglobulin were predicted. The amino acid sequences of allergens from different sources (*Dermatophagoides pteronyssinus*, *Blomia tropicalis*, *Betula verrucosa*, *Cynodon dactylon*, *Aspergillus fumigatus*, *Canis domesticus*, *Felis domesticus*) were compared with human TPO, Tg and IL24. The cover and alignments between allergens and human proteins were low.

Conclusion: We identify possible linear and conformational epitopes of TPO, Tg and IL24 that could be the target of IgE or IgG binding in patients with urticaria or hypothyroidism; These epitopes do not appear to be present among common environmental allergens, suggesting that autoreactivity to these human proteins are not by cross-reactivity.

1. Introduction

Chronic spontaneous urticaria (CSU) is a disease characterized by the presence of hives and angioedema that significantly affects the quality of life [1,2]. Patients with urticaria have a higher frequency of self-reactive autoantibodies IgE and IgG than healthy subjects [3–7]. Thyroperoxidase (TPO), interleukin 24 (IL24) and thyroglobulin (Tg) are some of the proteins that have been implicated in the pathogenesis of urticaria [8–10]. The frequency of autoantibodies against these proteins is low in healthy subjects [8,11] but Anti-Tg and anti-TPO antibodies are common in patients with thyroid carcinoma or Hashimoto's thyroiditis [12–14]. Why these human proteins are recognized by specific autoantibodies in patients with urticaria or

hypothyroidism is unknown.

Similar to what has been observed in other diseases [15–19], it is possible that in CSU and hypothyroidism the recognition of human proteins by IgG or IgE autoantibodies can be by cross-reactivity with environmental allergens.

This hypothesis is supported by some observations:

- 1) Patients with urticaria have a higher frequency of atopy and some allergic diseases like asthma and rhinitis than the population without CSU [20,21].
- 2) Patients with urticaria have a higher frequency of hypothyroidism than the population without urticaria [22–24].
- 3) A higher frequency of anti-TPO IgE is present in patients with CSU

* Corresponding author.

E-mail address: jorgem.sanchez@udea.edu.co (J. Sánchez).

<https://doi.org/10.1016/j.imlet.2020.02.003>

Received 2 January 2020; Received in revised form 30 January 2020; Accepted 2 February 2020

Available online 03 February 2020

0165-2478/ © 2020 European Federation of Immunological Societies. Published by Elsevier B.V. All rights reserved.

Table 1

Sequence of allergens used to compare with the sequences of TPO, IL-24 and Tg.

Source/Allergen	Entry Uniprot (3D Structure)	Codigo UNIPROT	GeneBank Nucleotide	GeneBank Protein	Aminoacids
<i>Dermatophagoides pteronyssinus</i>					
 Der p 1	P08176	PEPT1_DERPT	U11695	AAB60215.1	320
Der p 2	P49278	ALL2_DERPT	AF276239	AAF86462.1	146
Der p 5	P14004	ALL5_DERPT	S76337	AAB32841.1	132
Der p 10	O18416	TPM_DERPT	Y14906	CAA75141	284
<i>Blomia tropicalis</i>					
 Blo t 1	A1KX10	CYSP_BLOTA	AF277840	AAK58415	221
Blo t 2. 0101	Q1M2P1	Q1M2P1_BLOTA	AAQ73483	AY288141	144
Blo t 5	O96870	ALL5_BLOTA	U59102	AAD10850.1	134
Blo t 13	Q17284	FABP_BLOTA	AY283294.1	AAC80579	131
<i>Betula verrucosa</i>					
 Bet v 1	P15494	BEV1A_BETPN	Z80099	CAA33887	160
<i>Cynodon dactylon</i>					
 Cyn d 1	Q04701	MPAC1_CYNDA	S83343	AAB50734	246
<i>Aspergillus fumigatus</i>					
 Asp f 1	P67875	RNMG_ASPFU	M83781	AAB07779	176
Asp f 2	P79017	ALL2_ASPFU	U56938	AAC69357	310
Asp f 3	Q43099	PRX5_ASPFU	U58050	AAB95638	168
Asp f 5	P46075	ELM_ASPFU	Z30424	CAA83015	634
<i>Canis familiaris</i>					
 Can f 1	O18873	ALL1_CANLF	AF027177	AAC48794	174
Can f 2	O18874	ALL2_CANLF	AF027178	AAC48795	180
Can f 3	P49822	ALBU_CANLF	AB090854	BAC10663	608
Can f 4	D7PBH4	D7PBH4_CANLF	GU132996	ACY38525	174
Can f 5	P09582	ESTA_CANLF	Y00751	P09582	260
<i>Felis domesticus</i>					
 Fel d 1	P30438 (chain 1) P30440 (chain 2)	FEL1A_FELCA; FEL1B_FELCA	M74952 (chain 1) M77341 (chain 2)	AAC37318 (chain 1) AAC41616 (chain 2)	92; 109
Fel d 2	P49064	ALBU_FELCA	X84842	CAA59279	608
Fel d 3	Q8WNR9	CYTA_FELCA	AF238996	AAL49391	98

and hypothyroidism and the response against this human antigen can induce histamine release by basophils [10,22].

- 4) In some skin diseases like atopic dermatitis, IgE cross-reactivity have been detected between human antigens and proteins from pollen grains or fungus [25,26].
- 5) In some autoimmune diseases like thyroid disease, molecular mimicry has been detected between human antigens and some proteins from microorganisms [27–29]

The identification of the antigenic epitopes of IL24, TPO and Tg and the search of antigenic patches with cross-reactivity between these human proteins and common environmental allergens, could help to evaluate this hypothesis.

2. Methods

2.1. Selection of proteins and alignment

The amino acids sequences of TPO, IL24 and Tg were compared with prevalent allergens (Table 1) [30–33]. The sequences were obtained from the uniprot database (<https://www.uniprot.org/>). Sequences that were reported in the WHO/IUIS Allergen Nomenclature Sub-Committee (<http://www.allergen.org>) and presented a complete sequence were used. All the allergens that fulfilled these criteria were chosen, independent of the source (e.g pollen, animal) where it came from. Due to the variety of origin and length of amino acids between the molecules studied, cover and identity values were used to establish the degree of relationship between them. Identity and cover values between the molecule used in this study was determined by using BLAST from NCBI and praline web server (<http://www.ibi.vu.nl/>). Parameters to perform alignment were set up to use BLOSUM62 as exchange matrix and a conditional compositional score matrix adjustments. Not filter or Marking were used. Iterations used were 3 with an E-value of 0.01. Based in previous observations [34–36], we considered proper homology when both the identity and cover value were greater than 40

%.

2.2. Phylogenetic analysis

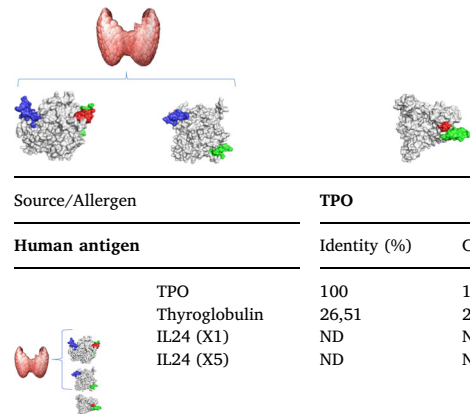
The program “Molecular Evolutionary Genetic Analysis” (MEGA) version X was used for the construction of the tree, using the method of reconstruction of Neighbor-Joining with support by Bootstrap with 1000 replications as a measure of reliability and robustness and a p – distance model, under the assumption of minimal or non-evolution in the topology [37,38]. This model uses a comparative matrix to find the similarity between the sequences. The matrix was constructed with all amino acid sequences of allergens chosen that were available in human proteins retrieved from Uniprot database and reported in WHO/IUIS Allergen Nomenclature Sub-Committee (<http://www.allergen.org>). Thus, the more positive identity values are found among the sequences, and they will be located in closer positions in the tree. All empty spaces were eliminated (full deletions). From the global comparison and the homologies, the sum of the length of branches (SBL) will be presented, which will determine the number of nodes and the position of the same, including the “clusters” of the evolutionarily closest sequences. The alignment for the phylogenetic analysis was carried out through the CLUSTAL W. program, which performs alignments.

2.3. Construction of 3D models and epitope prediction

The models of those proteins not reported in the protein data bank, were made by homology. The Swiss-model server (<https://swissmodel.expasy.org/>) was used [39]. The quality of the models was analyzed by ProSA-web. The models were refined in Deep-View (energy minimization and rotamer replacements). Its quality was evaluated by several tools, including the Ramachandran graphs, WHATIF, QMEAN4 index and energy values (GROMOS96 force field). The relative values of the area of accessible solvent (r-ASA) were determined by ASA-view. The models were used to identify residues exposed on the surface and conserved in the different groups. Those preserved and the residues

Table 2

Pairwise alignments results from TPO, IL-24, thyroglobulin and 22 allergens from different sources (Fel d 1 have two isoforms). The cover and identity values were taken to establish the degree of relationship between the proteins. The results are expressed as a percentage.



Source/Allergen	TPO		Thyroglobulin		IL24 (X1)		IL24 (X5)	
Human antigen	Identity (%)	Cover (%)	Identity (%)	Cover (%)	Identity (%)	Cover (%)	Identity (%)	Cover (%)
TPO	100	100	26,51	25	ND	ND	ND	ND
Thyroglobulin	26,51	25	100	100	20	17	ND	ND
IL24 (X1)	ND	ND	20	17	100	100	100	6
IL24 (X5)	ND	ND	ND	ND	100	6	100	100
Dermatophagoides pteronyssinus								
Der p 1	ND	ND	41,67	2	44	8	33,3	33
Der p 2	37,50	2	50	2	22	23	ND	ND
Der p 5	24,14	3	33,3	0	ND	ND	ND	ND
Der p 10	28,07	6	35,29	0	37	10	ND	ND
Blomia Tropicalis								
Blo t 1	26,10	11%	47,06	3	50	2	25	31
Blo t 2. 0101	ND	ND	50	2	29	17	28,57	33
Blo t 5	41,67	1%	32,14	0	ND	ND	ND	ND
Blo t 13	ND	ND	41,67	1	ND	ND	ND	ND
Betula verrucosa								
Bet v 1	ND	ND	42,11	2	ND	ND	40	7
Cynodon dactylon								
Cyn d 1	38,89	17	44	7	26,86	16	ND	ND
Aspergillus fumigatus								
Asp f 1	42,86	1	26,92	2	ND	ND	ND	ND
Asp f 2	36	4	ND	ND	ND	ND	ND	ND
Asp f 3	35,71	2	36,67	4	ND	ND	ND	ND
Asp f 5	ND	ND	42,11	2	23	31,25	ND	ND
Canis domesticus								
Can f 1	29,63	3	25,37	4	ND	ND	30,77	52
Can f 2	50	1	26,92	8	31	13	ND	ND
Can f 3	28,12	37	43,75	6	44	4	ND	ND
Can f 4	ND	ND	23,81	2	ND	ND	ND	ND
Can f 5	17,65	13	38,46	3	40	9	26,67	23
Felis domesticus								
Fel d 1	CHAIN 1: 50 CHAIN 2: ND	CHAIN 1: 0 CHAIN 2: ND	CHAIN 1: 32,4 CHAIN 2: 56,25	CHAIN 1: 4 CHAIN 2: 2	ND	ND	CHAIN 1: ND CHAIN 2: 50	CHAIN 1: ND CHAIN 2: 12
Fel d 2	28,12	24	20,29	4	ND	ND	ND	ND
Fel d 3	28,57	9	5	2	ND	ND	ND	ND

accessible to the solvent (rASA > 0.25) were located in the 3D model to identify pooled areas (> 4 residues) and possible cross-reactivity. Ellipro (<http://tools.iedb.org/ellipro/>) tools were used to predict lineal and discontinues epitopes on TPO, IL24 and thyroglobulin. Residues with larger scores are associated with greater solvent accessibility. Only residues with a score > 0,75 were selected.

3. Results

3.1. Sequences alignments

A total of 27 sequences including human TPO, Tg, two isoforms of IL24 (X1 and X5, due the different in length) and 22 prevalent allergens from seven different sources, were selected based on allergenic capacity reported (Table 1). The percentages of identity between the allergens Vs. TPO, IL24 and Tg are shown in Table 2. The cover between allergens and TPO was quite low, with a maximum cover and identity with Can f 3 (37 % and 28.1 % respectively). Most of the sequence's alignments had a coverage less than 5 %.

The IL24 isoform X5 and Can f 1 show the highest match among the

alignments (cover of 52 % and an identity of 30.77 %). For the case of the isoform X1, Asp f 5 showed the greatest identity among the allergens (cover 31.2 %, identity 23 %) followed by Blo t 2 (cover 17 %, Identity 29 %) (Table 2).

The thyroglobulin was the human antigen who show the lowest match with the allergens. Maximum cover of thyroglobulin was obtained with Can f 2 (8 %), but the identity was 26.9 %.

3.2. Phylogenetic analysis

The evolutionary history was inferred using the Neighbor-Joining method and involved a total of 27 amino acid sequences and is shown in the Fig. 1. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the representation of the sequence similarity degree between the proteins analyzed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test are shown next to the branches and all ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 2767 positions in the final dataset. The different molecules studied showed a very low relationship between them due to the protein

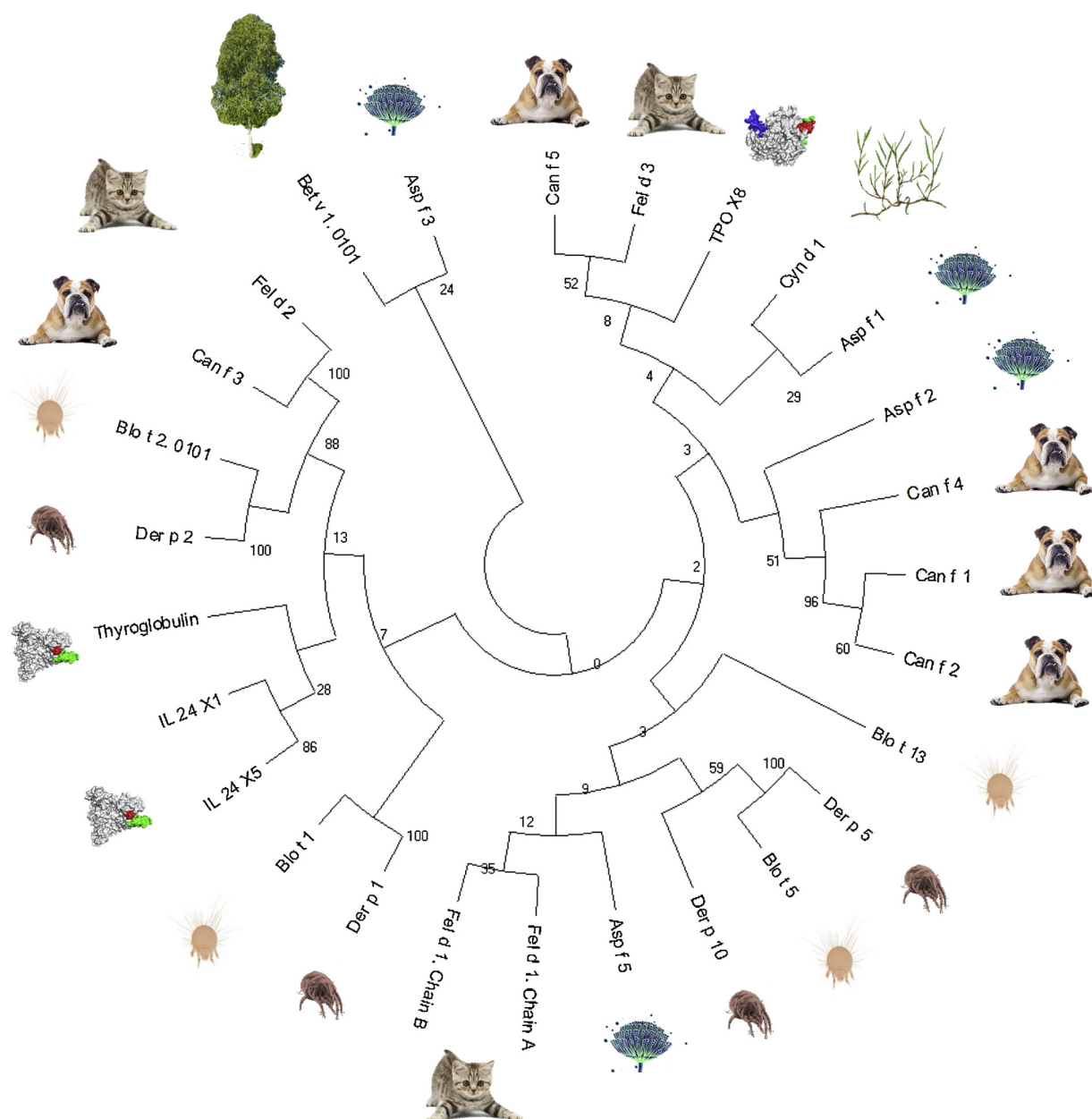


Fig. 1. In the analysis of the joining neighborhood methods, the branch represents the relationship among the proteins studied and the number beside represent the distant of the branch. The different molecules showed a very low relationship between them. Thyroglobulin and IL 24 isoforms were grouped in a single clade while the TPO showed a slight relationship between Fel d 3 and Can f 5 and in the next branch Cyn d 1 and Asp f 1.

diversity studied. In the analysis of the Neighbor-Joining method, the different molecules studied showed a very low relationship between them due to the protein diversity studied. Thyroglobulin and IL24 isoforms were grouped in a single clade while the TPO showed a slight relationship between Fel d 3 and Can f 5 and in the next branch Cyn d 1 and Asp f 1. Branches corresponding to partitions reproduced in less than 50 % bootstrap replicates are collapsed. The evolutionary distances were computed using the p-distance method and are in the units of the number of amino acid differences per site.

3.3. Linear and discontinuous epitopes were predicted on TPO, IL24 and Tg

We build a 3D model of the TPO and Tg protein using proteins closely related; Three closet homologue protein of TPO were found: Lactoperoxidase (48 %), myeloperoxidase (47 %), and eosinophil peroxidase (47 %). Here, we used the myeloperoxidase as the structure

modelling due its dimers forms. By using Ellipro server, three lineal and two discontinuous epitopes on TPO were predicted (Table 3a). The first lineal epitope was located on a loops regions and the second and third lineal epitopes were located on α -helices (L1: 295–314; L2: 418–433; L3: 711–735), all with an area surface greater than 300 Å. None of these sequences were conserved in the allergens studied (Fig. 2a). Discontinuous epitopes 1 and 2 are conformed by 31 and 55 amino acid residues, respectively, located between two α -helices (Fig. 2b).

The IL 24 structure was previously described and analyzed by X-Ray methods and reported in Uniprot by the code 6DF3 [40], three lineal and three discontinuous epitopes were predicted and all the linear patches are found in β -sheets (LE1: 42–54; LE2: 218–229; LE3: 145–153) (Table 3b; Fig. 3a). The first discontinuous epitope is located in a loop, between two antiparallel β sheets, with a total of 6 residues. The other two patches are close located, DE2 is among to β -sheets and a loops with a total of 8 residues, meanwhile DE 3 cover more area and

Table 3
Lineal and discontinuous epitopes from TPO (3a), IL24 (3b), Tg (3c). A total of 5 antigenic patches was predicted for TPO (Table 3a), six for IL24 (Table 3b) and six for Tg (Table 3c). They contained more than three amino acids and a score > 0.75 excluding the second discontinuous patch from TPO and discontinuous patches from Tg but given the low number of epitopes it was included.

Table 3a						
TPO	Start	End	Peptide	Number of residues	Score	
	Lineal	295	314	ACGTGDQGFALFGNLSTANPR	20	0,838
		418	433	AAALKALNAHWSADAV	16	0,816
		711	735	VGKFPEDFESCDSITGMNLEAWRET	25	0,784
	Continues	338	735	A:R338, A:R341, A:N342, A:W343, A:T344, A:S345, A:A346, A:E347, A:G348, A:L349, A:L350, A:A418, A:A419, A:A420, A:L421, A:K422, A:A423, A:L424, A:N425, A:A426, A:H427, A:W428, A:S429, A:A430, A:D431, A:A432, A:V433, A:E436, A:R733, A:E734, A:T735	31	0,776
		137	732	A:M137, A:L138, A:P139, A:P140, A:K141, A:C142, A:P143, A:N144, A:T145, A:C146, A:L147, A:A148, A:N149, A:K150, A:Y151, A:A157, A:C158, A:N159, A:N160, A:R161, A:D162, A:H163, A:P164, A:W166, A:Y470, A:E471, A:G472, A:Y473, A:D474, A:S475, A:T476, A:A477, A:G667, A:W669, A:F670, A:W671, A:W672, A:E673, A:N674, A:S675, A:H676, A:V677, A:F678, A:T679, A:D680, A:A681, A:Q682, A:R683, A:R684, A:E685, A:N728, A:L729, A:E730, A:A731, A:W732	55	0,714
Table 3b						
IL24	Start	End	Peptide	Number of residues	Score	
	Lineal	42	54	DSTPDTVYS	9	0,844
		218	229	RVKTLPRDRTWTG	12	0,841
	Continues	147	153	RAGDGHHR	7	0,816
		147	152	L:R147, L:A148, L:G149, L:D150, L:G151, L:H152	6	0,865
		170	178	L:Q170, L:V171, L:N172, L:R173, L:T174, L:Y175, L:Q176, L:H178	8	0,785
	129	229	L:I129, L:S130, L:K131, L:V132, L:R133, L:S134, L:F189, L:G190, L:L191, L:T192, L:P193, L:D194, L:T195, L:E196, L:F197, L:R218, L:V219, L:K220, L:T221, L:L222, L:P223, L:D224, L:R225, L:T226, L:W227, L:T228, L:G229	27	0,759	
Table 3c						
Tg	Start	End	Peptide	Number of residues	Score	
	Lineal	2719	2731	SKYISLKTSADG	13	0,803
		2522	2545	AVKQFEESRGRTSSKTAIFYQALQN	24	0,792
		2197	2228	SVPSVPISTHGRLLGRSQAIVGTSWKQVDQF	32	0,781
	Continues	2547	2579	LGGEDSDARVEAAATWYYSLHSTDDYASFRA	33	0,769
		2674	2676	A:F2674, A:S2675, A:R2676	3	0,979
2528		2530	A:E2528, A:S2529, A:R2530	3	0.883	

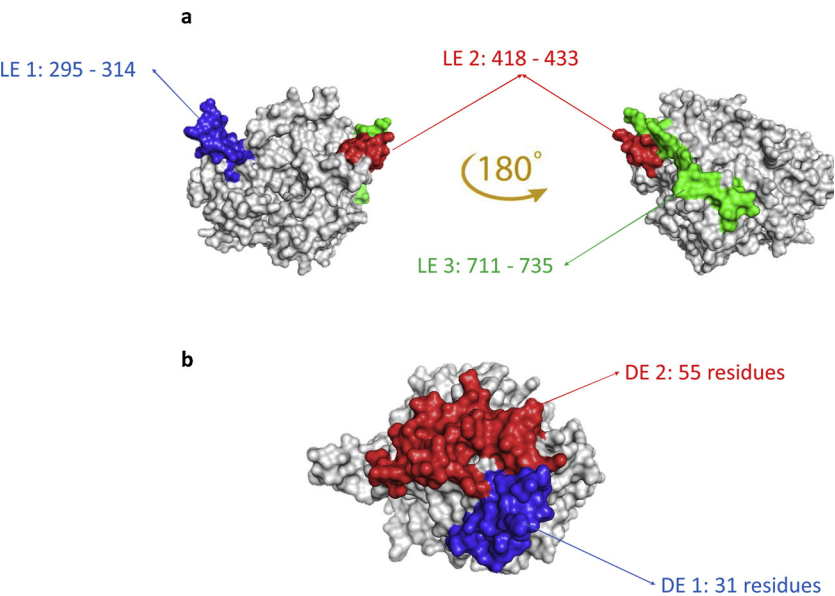


Fig. 2. Lineal (Fig. 2a) and discontinuous (Fig. 2b) epitopes of the TPO. The patches present a score > 0.75, showing a high probability of antibody accessibility. The color blue, red and green represent the first, second and third patch, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

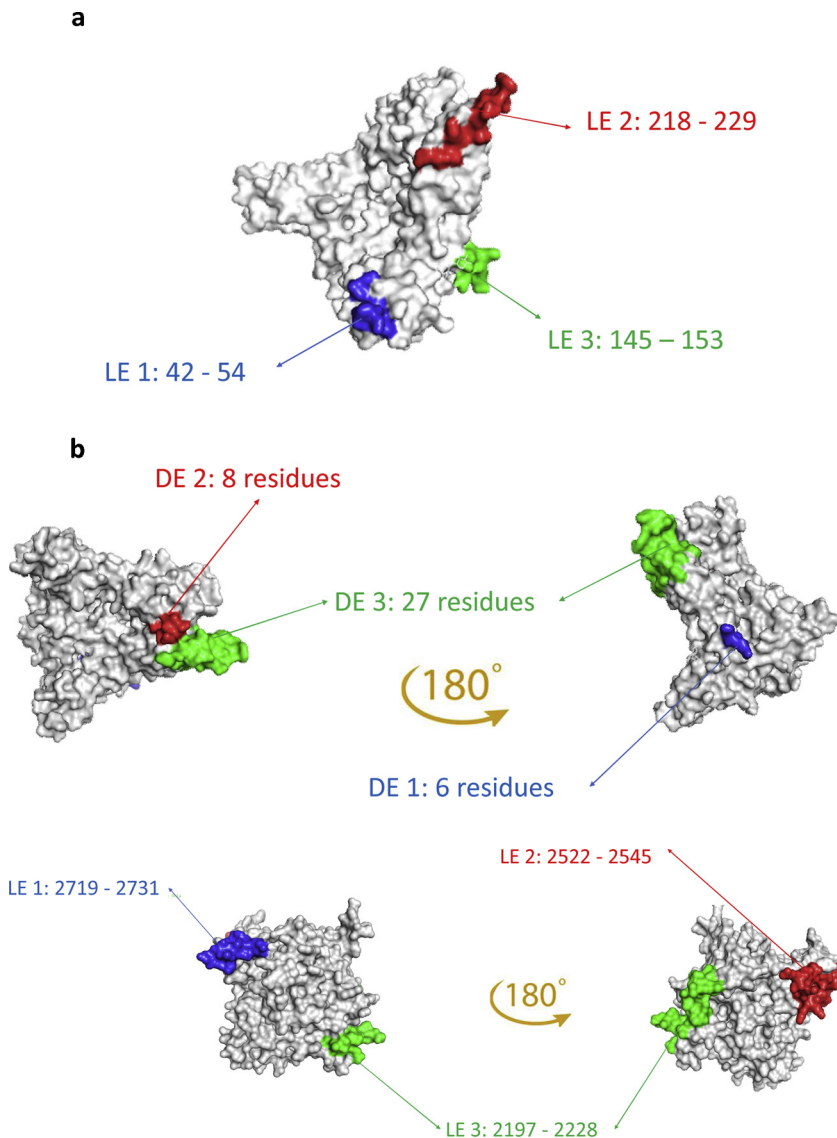


Fig. 3. Linear (Fig. 3a) and discontinuous (Fig. 3b) epitopes of the IL24. The patches present a score > 0.75, showing a high probability of antibody accessibility. The color blue, red and green represent the first, second and third patch, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Fig. 4. Linear epitopes of the thyroglobulin. The patches present a score > 0.75, showing a high probability of antibody accessibility. The color blue, red and green represent the first, second and third patch, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

has part of 3 antiparallel β -sheets with a total of 27 residues (Fig. 3b).

The model construction of the monomer Tg used the acetylcholinesterase as the template due it presented the greatest identity with a 36 %. Four linear epitopes and two discontinuous epitopes were found in the thyroglobulin (Table 3c). Three of the linear epitopes are found in different α helices while only one is part of β -sheets structure (LE1: 2719–2731; LE2: 2522–2545; LE3: 2197–2228; LE4: 2547–2579) (Fig. 4). The first two discontinuous epitopes were excluded since they did not meet the conditions because one of them had only three residues and the other present a score < 0.75.

4. Discussion

CSU is a disease with multiple triggers and mechanisms [3,4,41,42]. Different autoimmune mechanisms have been identified, being the IgG and IgE autoantibodies the most studied mechanisms [5,43–46].

It has been possible to detect autoantibodies against multiple human antigens in CSU [8–10,15,47], the most studied is the thyroperoxidase. In one recent study we demonstrated that thyroperoxidase could induce hives in patients with urticaria and anti-TPO IgE [10]; It has also been observed that the suppression of anti-TPO IgE avoid hives [10,48]. The demonstration of this causal relationship, opened the possibility that other autoantibodies can do the same in this disease. Nevertheless, it is

not known why this auto-reactive response occurs. Some hypotheses could explain this self-response;

4.1. Patients with CSU have an increased risk of developing autoimmune diseases possibly due to genes isoforms influencing the immune response, which would facilitate the recognition of autoantigens by clones of B lymphocytes with IgE or IgG receptors

This hypothesis is supported by epidemiological investigations that show a higher frequency of hypothyroidism in patients with CSU (15–30%) compared to subjects without CSU (5–7%). The high frequency of hypothyroidism is present in patients with CSU and anti-TPO IgE or anti-Fc ϵ R1a IgG. Mozena, et al., [49] investigated whether the high incidence of Hashimoto's thyroiditis (HT), observed in patients with CSU could be explained by epitope cross-reactivity of anti-Fc ϵ R1a antibodies with thyroid proteins (TPO and Tg); they did not find that thyroid proteins could generate an inhibition of the response observed with anti-Fc ϵ R1a IgG, so they concluded that cross-reactivity does not explain the increased prevalence of HT found in CSU patients with anti-Fc ϵ R1a IgG. Nevertheless, they didn't compare the IgE response or the identity of the epitopes among thyroid proteins. The results of Mozena, et al., also indicate that IgG against TPO and Tg had no capacity to induce mast cell activation, however, other studies have shown that

recognition of these antigens by IgE can lead to cell activation [9,10]. All these results suggest that the autoimmune response IgE and IgG in urticaria may have some points in common, but also differences in the recognized antigens. If there is a common epitope for the IgG response in HT and for the IgE response in CSU is unknown but, we identify some antigenic epitopes that could be recognized by both antibodies (IgG and IgE), and we believe that during an inflammatory process secondary to the recognition of self-proteins as occurs in these diseases, the formation of multiple B lymphocytes cell clones IgG and IgE is enhanced and this could explain the epidemiological relationship that has been found in both diseases.

We identify some possible antigenic epitopes for both TPO and Tg; These epitopes did not share a common sequence, so they are unlikely to have cross-reactivity and possibly its formation is by independent mechanisms. As presented in Table 2, the identity and cover of these proteins and the IL24 was also low. No epitopes shared between IL24 and thyroid antigens were found, so cross reactivity is unlikely. There is still little information about the role of IL24 in hives; In one study, IgE-anti-IL24 was found in a large number of patients with CSU (90 %), but the possible association with other autoimmune diseases has not been evaluated.

4.2. Human thyroperoxidase could share antigenic epitopes with prevalent allergens (e.g., house dust mites, pets, fungal spores or pollen)

Epidemiological studies that show a higher prevalence of atopy in patients with CSU versus subjects without urticaria support this hypothesis [50–52]. It is also supported in some studies with IgE skin diseases; In atopic dermatitis, human auto-antigens that have cross-reactivity with similar environmental allergens have been detected in patients with severe clinical conditions [25,26]. According with this hypothesis, first, the body would mount an IgE response to common allergenic sources such as mite proteins, dog epithelia, grains of pollen etc., subsequently, after a chronic inflammatory process these antibodies would recognize similar epitopes in humans previously unexposed and, through cross-reactivity, they would mount an auto-reactive response.

To explore this hypothesis, we compared the sequence of TPO, Tg and IL24 with multiple allergenic proteins. Proteins with a close phylogenetic relationship are more likely to share common segments in their sequence, however the phylogenetic relationship between the proteins studied was low. Through *in-silico* prediction analysis, we describe several epitopes in TPO, Tg and IL24 with antigenic potential that could be the targets for antibodies, however none of these epitopes seems to be present in the primary or tertiary structure of the allergens studied. Therefore, according to these results, cross-reactivity between exogenous allergens and endogenous antigens seems unlikely. The high prevalence of atopy in patients with CSU could then be due to other causes; for example, the polyclonal response that can be generated after a chronic inflammatory response may boosting IgE response to different unrelated antigens. However, we cannot rule out that other allergenic proteins not included in this study could share cross-reactivity with TPO, Tg, IL24; for example, *Helicobacter pylori* or *Toxocara canis* have proteins capable of binding to IgE and IgG autoantibodies, and have been associated as possible triggers of chronic urticaria [53,54]. However, we were unable to explore this possibility since their proteins have not yet been reported in NCBI or Uniprot. In addition, we chose multiple frequent allergens but it is possible that other less prevalent allergens from mites, pets or fungi could have a greater identity with TPO, Tg or IL24.

It is also necessary to note that our study has some weaknesses that could explain the lack of cross-reactivity between the auto-antigens and the allergens evaluated; In the case of TPO, we model its tertiary structure based on other homologous proteins since its tertiary structure is unknown, however *in-silico* constructions are not exact and it is possible that its natural form is different from what we propose. In the

same direction, the antigen patches require further confirmation through studies in biological models. However, bioinformatic analyzes have many advantages to prioritize the resources of an investigation and serve in the initial evaluation of a hypothesis and as support in defining whether or not it is justified to perform *in-vitro* or *ex-vivo* analysis.

In conclusion, through *in-silico* modeling we identify possible linear and conformational epitopes of TPO, Tg and IL24 that could be the target of IgE or IgG binding in patients with urticaria or hypothyroidism; These epitopes do not appear to be present among the major allergens from mites, common grass, dog, cat and *Aspergillus fumigatus*, suggesting that they are by a specific reaction and not by cross-reactivity.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We appreciate the funding of the University of Antioquia and the “IPS Universitaria” foundation; This research was carried out as part of the 2017-19 inter-institutional grant (2017-16193 / ES84170191).

References

- [1] A. Arias-Cruz, S.N. González-Díaz, A. Macías-Weinmann, J.A. Ibarra-Chávez, D. Sánchez-Guerra, L. Leal-Villarreal, et al., Quality of life in chronic urticaria and its relationship with economic impact and disease control in patients attended to at the University Hospital of Monterrey, Mexico, *Rev. Alerg. Mex.* 65 (3) (2018) 170–178.
- [2] M. Maurer, K. Houghton, C. Costa, F. Dabov, L.F. Ensina, A. Giménez-Arnau, et al., Differences in chronic spontaneous urticaria between Europe and Central/South America: results of the multi-center real world AWARE study, *World Allergy Organ. J.* 11 (1) (2018) 32.
- [3] J. Sánchez Jorge, A. Sánchez, R. Cardona, Prevalence of drugs as triggers of exacerbations in chronic urticaria, *J. Investig. Allergol. Clin. Immunol.* (2018) 0.
- [4] J. Sánchez, A. Sánchez, R. Cardona, Dietary habits in patients with chronic spontaneous urticaria: evaluation of food as trigger of symptoms exacerbation, *Dermatol. Res. Pract.* 2018 (2018) 6703052.
- [5] M. Hide, D.M. Francis, C.E. Grattan, J. Hakimi, J.P. Kochan, M.W. Greaves, Autoantibodies against the high-affinity IgE receptor as a cause of histamine release in chronic urticaria, *N. Engl. J. Med.* 328 (22) (1993) 1599–1604.
- [6] M.F. Lee, T.M. Lin, S.W. Liu, Y.H. Chen, A rapid method of detecting autoantibody against FcεR1α for chronic spontaneous urticaria, *PLoS One* 9 (10) (2014) e109565.
- [7] B. Ulambayar, Y.H. Chen, G.Y. Ban, J.H. Lee, C.G. Jung, E.M. Yang, et al., Detection of circulating IgG autoantibody to FcεR1α in sera from chronic spontaneous urticaria patients, *J. Microbiol. Immunol. Infect.* (2017).
- [8] O. Schmetzer, E. Lakin, F.A. Topal, P. Preusse, D. Freier, M.K. Church, et al., IL-24 is a common and specific autoantigen of IgE in patients with chronic spontaneous urticaria, *J. Allergy Clin. Immunol.* (2017).
- [9] S. Altrichter, H.J. Peter, D. Pisarevskaja, M. Metz, P. Martus, M. Maurer, IgE mediated autoallergy against thyroid peroxidase—a novel pathomechanism of chronic spontaneous urticaria? *PLoS One* 6 (4) (2011) e14794.
- [10] J. Sánchez, A. Sánchez, R. Cardona, Causal relationship between Anti-TPO IgE and chronic urticaria by, *Allergy Asthma Immunol. Res.* 11 (1) (2019) 29–42.
- [11] N.J. Poindexter, E.T. Walch, S. Chada, E.A. Grimm, Cytokine induction of interleukin-24 in human peripheral blood mononuclear cells, *J. Leukoc. Biol.* 78 (3) (2005) 745–752.
- [12] R.T. Posselt, V.N. Coelho, T.L. Skare, Hashimoto thyroiditis, anti-thyroid antibodies and systemic lupus erythematosus, *Int. J. Rheum. Dis.* 21 (1) (2018) 186–193.
- [13] W.L.C.J. Pereira, E.M.V. Reiche, A.P. Kallaur, S.R. Oliveira, A.N.C. Simão, M.A.B. Lozovoy, et al., Frequency of autoimmune disorders and autoantibodies in patients with neuromyelitis optica, *Acta Neuropsychiatr.* 29 (3) (2017) 170–178.
- [14] H. Nakamura, T. Usa, M. Motomura, T. Ichikawa, K. Nakao, E. Kawasaki, et al., Prevalence of interrelated autoantibodies in thyroid diseases and autoimmune disorders, *J. Endocrinol. Invest.* 31 (10) (2008) 861–865.
- [15] M. Maurer, S. Altrichter, O. Schmetzer, J. Scheffel, M.K. Church, Metz M. Immunoglobulin E-Mediated autoimmunity, *Front. Immunol.* 9 (2018) 689.
- [16] B. Dema, C. Pellefigues, S. Hasni, N. Gault, C. Jiang, T.K. Ricks, et al., Autoreactive IgE is prevalent in systemic lupus erythematosus and is associated with increased disease activity and nephritis, *PLoS One* 9 (2) (2014) e90424.
- [17] R. Valenta, S. Natter, S. Seiberler, S. Wichlas, D. Maurer, M. Hess, et al., Molecular characterization of an autoallergen, Hom s 1, identified by serum IgE from atopic dermatitis patients, *J. Invest. Dermatol.* 111 (6) (1998) 1178–1183.

- [18] A.A. Fedorov, T. Ball, N.M. Mahoney, R. Valenta, S.C. Almo, The molecular basis for allergen cross-reactivity: crystal structure and IgE-epitope mapping of birch pollen profilin, *Structure* 5 (1) (1997) 33–45.
- [19] R. Valenta, S. Natter, S. Seiberler, M. Grote, Isolation of cDNAs coding for IgE autoantigens: a link between atopy and autoimmunity, *Int. Arch. Allergy Immunol.* 113 (1–3) (1997) 209–212.
- [20] K.L. Chang, Y.H. Yang, L.J.H. Yu HH, L.C. Wang, B.L. Chiang, Analysis of serum total IgE, specific IgE and eosinophils in children with acute and chronic urticaria, *J. Microbiol. Immunol. Infect.* 46 (1) (2013) 53–58.
- [21] K. Kulthanan, S. Jiamton, N.O. Rutnin, M. Insawang, S. Pinkaew, Prevalence and relevance of the positivity of skin prick testing in patients with chronic urticaria, *J. Dermatol.* 35 (6) (2008) 330–335.
- [22] P. Kolkhir, M. Metz, S. Altrichter, M. Maurer, Comorbidity of chronic spontaneous urticaria and autoimmune thyroid diseases: a systematic review, *Allergy* 72 (10) (2017) 1440–1460.
- [23] R. Confino-Cohen, G. Chodick, V. Shalev, M. Leshno, O. Kimhi, A. Goldberg, Chronic urticaria and autoimmunity: associations found in a large population study, *J. Allergy Clin. Immunol.* 129 (5) (2012) 1307–1313.
- [24] E. Magen, J. Mishal, The effect of L-thyroxine treatment on chronic idiopathic urticaria and autoimmune thyroiditis, *Int. J. Dermatol.* 51 (1) (2012) 94–97.
- [25] J. Sánchez, A. Sánchez, R. Cardona, Particular characteristics of atopic eczema in tropical environments. The Tropical Environment Control for Chronic Eczema and Molecular Assessment (TECCEMA) cohort study, *An. Bras. Dermatol.* 92 (2) (2017) 177–183.
- [26] S. Altrichter, E. Kriehuber, J. Moser, R. Valenta, T. Kopp, G. Stingl, Serum IgE autoantibodies target keratinocytes in patients with atopic dermatitis, *J. Invest. Dermatol.* 128 (9) (2008) 2232–2239.
- [27] M.A. Sousa, R. Paraná, L.J. Andrade, Sequence similarity between thyroid self-protein and hepatitis C virus polyprotein: possible triggering mechanism of autoimmune thyroiditis, *Arq. Gastroenterol.* 53 (3) (2016) 185–191.
- [28] S. Benvenha, F. Guarneri, Molecular mimicry and autoimmune thyroid disease, *Rev. Endocr. Metab. Disord.* 17 (4) (2016) 485–498.
- [29] S. Benvenha, L. Santarpia, F. Trimarchi, F. Guarneri, Human thyroid autoantigens and proteins of *Yersinia* and *Borrelia* share amino acid sequence homology that includes binding motifs to HLA-DR molecules and T-cell receptor, *Thyroid* 16 (3) (2006) 225–236.
- [30] L. Caraballo, J. Zakzuk, B.W. Lee, N. Acevedo, J.Y. Soh, M. Sánchez-Borges, et al., Particularities of allergy in the tropics, *World Allergy Organ. J.* 9 (2016) 20.
- [31] B.I. Nwaru, S. Suzuki, L. Ekerljung, S. Sjölander, R. Mincheva, E.P. Rönmark, et al., Furry animal allergen component sensitization and clinical outcomes in adult asthma and rhinitis, *J. Allergy Clin. Immunol. Pract.* (2018).
- [32] J. Sánchez, S. Diez, R. Cardona, [Frequency of sensitization to animals in a tropical area], *Rev. Alerg. Mex.* 61 (2) (2014) 81–89.
- [33] J. Sanchez, S. Diez, R. Cardona, Sensibilización a aeroalergenos en pacientes alérgicos de Medellín, Colombia *Revista Alergia México* 59 (3) (2012) 139–147.
- [34] B. Rost, Twilight zone of protein sequence alignments, *Protein Eng.* 12 (2) (1999) 85–94.
- [35] G. Armano, L. Milanese, A. Orro, Multiple alignment through protein secondary-structure information, *IEEE Trans. Nanobiosci.* 4 (3) (2005) 207–211.
- [36] Y. Hong, S.V. Chintapalli, K.D. Ko, G. Bhardwaj, Z. Zhang, D. van Rossum, et al., Predicting protein folds with fold-specific PSSM libraries, *PLoS One* 6 (6) (2011) e20557.
- [37] O. Gascuel, M. Steel, Neighbor-joining revealed, *Mol. Biol. Evol.* 23 (11) (2006) 1997–2000.
- [38] J.A. Studier, K.J. Keppler, A note on the neighbor-joining algorithm of Saitou and Nei, *Mol. Biol. Evol.* 5 (6) (1988) 729–731.
- [39] A. Waterhouse, M. Bertoni, S. Bienert, G. Studer, G. Tauriello, R. Gumienny, et al., SWISS-MODEL: homology modelling of protein structures and complexes, *Nucleic Acids Res.* 46 (W1) (2018) W296–W303.
- [40] J. Lubkowski, C. Sonmez, S.V. Smirnov, A. Anishkin, S.V. Kotenko, A. Wlodawer, Crystal structure of the labile complex of IL-24 with the extracellular domains of IL-22R1 and IL-20R2, *J. Immunol.* 201 (7) (2018) 2082–2093.
- [41] J. Sánchez, E. Amaya, A. Acevedo, A. Celis, D. Caraballo, R. Cardona, Prevalence of Inducible Urticaria in patients with chronic spontaneous urticaria: associated risk factors, *J. Allergy Clin. Immunol. Pract.* 5 (2) (2017) 464–470.
- [42] C. Grattan, Autoimmune chronic spontaneous urticaria, *J. Allergy Clin. Immunol.* 141 (3) (2018) 1165–1166.
- [43] C.E. Grattan, D.M. Francis, M. Hide, M.W. Greaves, Detection of circulating histamine releasing autoantibodies with functional properties of anti-IgE in chronic urticaria, *Clin. Exp. Allergy* 21 (6) (1991) 695–704.
- [44] C.E. Grattan, Histamine-releasing autoantibodies in chronic urticaria, *Skin Pharmacol.* 4 (Suppl 1) (1991) 64–70.
- [45] R. Asero, A.V. Marzano, S. Ferrucci, M. Cugno, D-dimer plasma levels parallel the clinical response to Omalizumab in patients with severe chronic spontaneous urticaria, *Int. Arch. Allergy Immunol.* 172 (1) (2017) 40–44.
- [46] P. Auyeung, D. Mittag, P.D. Hodgkin, L.C. Harrison, Autoreactive T cells in chronic spontaneous urticaria target the IgE Fc receptor α subunit, *J. Allergy Clin. Immunol.* 138 (3) (2016) 761–768 e4.
- [47] Y.S. Shin, D.H. Suh, E.M. Yang, Y.M. Ye, H.S. Park, Serum specific IgE to thyroid peroxidase activates basophils in aspirin intolerant urticaria, *J. Korean Med. Sci.* 30 (6) (2015) 705–709.
- [48] M. Maurer, S. Altrichter, T. Bieber, T. Biedermann, M. Bräutigam, S. Seyfried, et al., Efficacy and safety of omalizumab in patients with chronic urticaria who exhibit IgE against thyroperoxidase, *J. Allergy Clin. Immunol.* 128 (1) (2011) 202–209 e5.
- [49] J.D. Mozena, A. Tiñana, J. Negri, J.W. Steinke, L. Borish, Lack of a role for cross-reacting anti-thyroid antibodies in chronic idiopathic urticaria, *J. Invest. Dermatol.* 130 (7) (2010) 1860–1865.
- [50] M. Maurer, M. Metz, R. Brehler, U. Hillen, T. Jakob, V. Mahler, et al., Omalizumab treatment in patients with chronic inducible urticaria: a systematic review of published evidence, *J. Allergy Clin. Immunol.* 141 (2) (2018) 638–649.
- [51] K. Weller, T. Ohanyan, T. Hawro, A. Ellrich, G. Sussman, J. Koplowitz, et al., Total IgE levels are linked to the response of chronic spontaneous urticaria patients to omalizumab, *Allergy* (2018).
- [52] M. Sánchez-Borges, A. Capriles-Hulett, F. Caballero-Fonseca, L. González-Aveledo, Biomarkers of treatment efficacy in patients with chronic spontaneous urticaria, *Eur. Ann. Allergy Clin. Immunol.* 50 (1) (2018) 5–9.
- [53] M. Burak Selek, O. Baylan, A. Kutlu, M. Özyurt, Toxocara Canis igg seropositivity in patients with chronic urticaria, *Iran. J. Allergy Asthma Immunol.* 14 (4) (2015) 450–456.
- [54] T. Yoshimasu, F. Furukawa, Eradication therapy for urticaria with high titers of anti H. pylori IgG antibody, *Allergol. Int.* 63 (1) (2014) 37–40.