



Chemokines in hyperthyroidism

Silvia Martina Ferrari^a, Ilaria Ruffilli^a, Giusy Elia^a, Francesca Ragusa^a, Sabrina Rosaria Paparo^a, Armando Patrizio^a, Valeria Mazzi^a, Alessandro Antonelli^{a,*}, Poupak Fallahi^b

^a Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy

^b Department of Translational Research and of New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy

ARTICLE INFO

Keywords:

Chemokines
Cytokines
Hyperthyroidism
Graves' disease
CXCL10
CXCL9

ABSTRACT

The term “hyperthyroidism” indicates a condition due to an exaggerate production of thyroid hormone; the most frequent cause is Graves' disease (GD).

We review cytokines and chemokines in hyperthyroidism, with a special focus in GD.

In GD, recruited Th1 lymphocytes are responsible for enhanced IFN- γ and TNF- α production, which in turn stimulates Th1 chemokines release from thyrocytes, initiating and perpetuating the autoimmune process.

Circulating levels of these chemokines are associated with the active phase of GD.

Additional studies are necessary to investigate whether Th1 chemokines could be a novel therapeutic target in this disease.

Introduction

Hyperthyroidism

The terms “hyperthyroidism” and “thyrotoxicosis” are usually used in an interchangeable manner [1], but the first is a condition that occurs due to an exaggerate production of thyroid hormone by the thyroid [2], while the second is a condition occurring owing to excessive thyroid hormone by any cause, and for this reason it includes hyperthyroidism [2]. Different signs and symptoms exist: sleeping problems, irritability, heat intolerance, muscle weakness, hand tremor, a fast heartbeat, diarrhea, weight loss, and enlargement of the thyroid for Graves' disease (GD) and toxic multinodular goiter (TMN) (these signs and symptoms are lower in old people and in pregnancy) [3]. “Thyroid storm” is an unfrequent complication causing worsening symptoms, high temperature and confusion, and often leading to death [4].

In the United States, approximately 1.2% of the population is affected by hyperthyroidism [1]. The onset is frequently between 20 and 50 years of age, and more often in women, for GD [3,4], while in patients with TMN, or toxic adenoma, the disease is more frequent in people over the age of 50 years [3]. The diagnosis of hyperthyroidism is based on signs and symptoms and should be confirmed by blood tests [3], a low thyroid stimulating hormone (TSH), and raised triiodothyronine (T3) or thyroxine (T4) [3]. Moreover, to define the cause, radioactive iodine uptake by the thyroid and subsequent thyroid scan, ultrasonography, and thyroid-stimulating immunoglobulins (TSI) can

be of help [3].

In the United States, approximately 50–80% of the cases of hyperthyroidism is caused by GD [3,5]. Other causes are inflammation of the thyroid, multinodular goiter, toxic adenoma, an iodine excess, and the exaggerated administration of synthetic thyroid hormone [3,4]. Pituitary adenoma is a less frequent cause [3]. A complicated interaction of genetic, environmental and endogenous factors are at the basis of GD, that is a systemic autoimmune disorder, affecting thyroid and orbital connective tissue. The determinant role of the autoimmune response in the development of GD has been shown, as well as the pathogenetic role covered by thyroid stimulating autoantibodies [6,7].

Genetic factors are considered to be involved in the development of autoimmune thyroid diseases (AITDs) for approximately 70% [8]. The genes encoding for TSH receptor, thyroglobulin, CD40, cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), protein tyrosine phosphatase-22 (PTPN 22), CD25, and human leukocyte antigens (HLA) contribute to the pathogenesis of GD [9]. GD has been considered a HLA class II-associated disease. For example, the DR3 allele has been associated with GD and, slightly, Hashimoto thyroiditis (HT) [10]. In particular, the “DR3 haplotype” (i.e., DQA1*0501, DQB1*02, DRB1*03), is predisposing to GD, owing to the high degree of linkage disequilibrium among these DQA1, DQB1, and DRB1 loci, while the DR7 haplotype (i.e., DQA1*0201, DQB1*0302, DRB1*07 or DQA1*0201, DQB1*02, DRB1*07) is considered protective [11]. When DRB1*07 and DRB1*03 are present, the first seems to counteract the susceptibility to GD given by the second [12].

* Corresponding author at: Department of Clinical and Experimental Medicine, University of Pisa, School of Medicine, Via Savi, 10, I-56126 Pisa, Italy.
E-mail address: alessandro.antonelli@med.unipi.it (A. Antonelli).

Environmental factors contribute approximately for 30% to the development of the disease.

One environmental factor at the basis of GD is stress, that has long been considered a potential trigger for GD [11,13]. Infectious, physical stressful, and psychological events may induce the onset and, partly, the recurrences of hyperthyroidism in GD [14].

A study conducted serological HLA typing in 58 Caucasian patients with stress-related GD and in 130 matched healthy controls, and genomic HLA typing in 20/58 patients and in all controls [11]. Five HLA alleles and 3 loci were more common in patients compared to healthy controls: B8, Cw7, C*07, C*17, DR3, DR4, DRB1*04, and DQ2, while B14 was less frequent in patients than in controls. The Authors concluded that in GD patients with stress-triggered hyperthyroidism, HLA typing could be useful to predict the outcome of the disease [11].

Smoking is another well-established risk factor for GD. The odds ratio (OR) for Graves' hyperthyroidism is 3.30 (95% confidence interval (CI) = 2.09–5.22) in current smokers in comparison to never smokers [15,16].

The role of different viruses in the development of GD has been also investigated [17,18].

From an immune-pathogenetic point of view, GD is a predominantly Th1 cytokine disease, even if hyperthyroidism is due to the presence of thyroid-stimulating antibodies (TSAs). In fact, Th1 cytokines and chemokines are very important in its pathogenesis [19,20].

During the course of GD, T-helper cell 17 (Th17)/Treg cell infiltration, Th1/Th2 cytokine and chemokine production, and the presence of subtypes of immunoglobulins are some of the inflammatory events occurring.

Different treatment options are known, according to the cause and severity of the disease: medications, radioiodine therapy, and thyroid surgery [3]. Medications are beta blockers, that are able to control the symptoms, and anti-thyroid medications, such as methimazole (MMI) [3]. In case of opting for radioiodine therapy, the patient receives iodine-131 by mouth, that is absorbed into the gut, and then transported and concentrated into the thyroid, destroying it upon weeks to months, leading to hypothyroidism, that is then treated with synthetic thyroid hormone [3]. Surgery is used to remove the thyroid, in particular in presence of a large thyroid volume, or cancer [3].

Cytokines and chemokines

Cytokines are small proteins (~5–20 kDa), important in cell signaling. These peptides cannot cross the lipid bilayer and for this reason cannot simply enter the cytoplasm. Cytokines are involved in autocrine, paracrine and endocrine signaling as immunomodulating agents. Even if some overlap in the terminology exists, cytokines include interleukins (IL), chemokines, interferons (IFNs), tumor necrosis factors (TNFs), and lymphokines, but not growth factors or hormones. They are important in physiology as well as in certain diseases, in particular in host responses to infection, immune responses, inflammation, cancer, trauma, reproduction, and sepsis. Cytokines act through receptors, and modulate the balance between humoral and cell-based immune responses; they regulate the maturation, growth, and responsiveness of particular cell populations. Some cytokines enhance, or inhibit, the action of other cytokines. Cytokines are produced by various types of cells, as immune cells (macrophages, mast cells, B lymphocytes, and T lymphocytes), and endothelial cells, fibroblasts, and various stromal cells [21,22].

Chemokines (chemotactic cytokines) are a family of small cytokines, or signaling proteins secreted by cells, whose name derives from their ability to induce directed chemotaxis in nearby responsive cells. Chemokines are all about 8–10 kDa in mass and have 4 cysteine residues in conserved positions, that determine their 3-dimensional shape. Some chemokines are considered homeostatic and they control the migration of cells during normal processes of tissue maintenance, or development; while others are considered pro-inflammatory and can be induced during an immune response to recruit cells of the immune

system to a site of infection, or inflammation. Chemokines have been classified into 4 principal subfamilies: CXC, CC, CX3C and XC. All of them exert their biological effects by interacting with G protein-linked transmembrane receptors (called chemokine receptors), selectively present on the surfaces of their target cells [23]. The chemokine receptor (CXCR)3 belongs to the family of C-X-C chemokine receptors, with the 2 isoforms CXCR3-A and CXCR3-B, and binds the Th1 dependent chemokines, IFN- γ -inducible protein 10 (IP-10)/chemokine ligand 10 (C-X-C motif) (CXCL)10, monokine induced by IFN- γ (MIG)/CXCL9 and IFN-inducible T-cell α chemoattractant (I-TAC)/CXCL11 [24]. CXCR3 is expressed by different cells, as activated T lymphocytes, Natural Killer, some epithelial and endothelial cells, and it is highly expressed on Th1 cells, such as the chemokine receptor (C-C motif) (CCR)5. Th1 cells are attracted in the tissue by CXCL9, CXCL10 and CXCL11 chemokines that are released by the cells into the inflamed tissues. Hence, this mechanism underlined the central role played by both CXCR3 and its ligands in the recruitment of inflammatory cells [25,26].

The Th1 lymphocytes recruited in the inflamed tissue increase the IFN- γ and TNF- α production, that leads to a Th1 chemokines secretion by several cells, creating an amplification feedback loop [27,28]. The high level of Th1 chemokines in peripheral fluids can be considered as a marker for the host immune response [27,28]. High serum and tissue levels of Th1 chemokines have been reported in specific autoimmune diseases, as: autoimmune thyroiditis (AT) [20,29–36], GD [37], Graves' ophthalmopathy [38,39], type 1 diabetes [40], or systemic rheumatological disorders, like SLE [41], rheumatoid arthritis [42], systemic sclerosis [43], sarcoidosis [44], psoriasis or psoriatic arthritis [45,46], HCV-related cryoglobulinemia [47–49], other HCV-immune-mediated disorders [31,50,51], other disorders, and also in cancers [16,52].

Here, we review cytokines and chemokines in hyperthyroidism, with a special focus in GD.

Hyperthyroidism and cytokines

Different circulating cytokines are increased in autoimmune (as IL-6 and IL-18) [53–55] as well as non-autoimmune hyperthyroidism (as IL-6, TNF- α , and IL-8) [56–58], indicating that this could result from the chronic effects of thyroid hormone excess, rather than from the accompanying autoimmune, inflammatory condition present in GD. The matter is complicated, and conclusions should be drawn carefully, as several demographic, clinical and therapeutical variables, which have not always been cautiously investigated, could lead to biased results. For instance, very few studies establishing the importance of serum cytokines in hyperthyroidism have taken into account the role of age, that seems to be a determinant variable, significantly and directly correlated with several cytokines, and particularly CXCL10, as earlier reported [59]. Hence, it should be considered that toxic nodular goiter (TNG) patients are usually older than GD patients. Furthermore, serum thyroid hormones, whose levels are generally higher in GD, and the use of corticosteroid therapy (used in GD patients with ophthalmopathy), should be considered when comparing serum cytokines in GD and TNG patients.

Th1 chemokines in GD (Table 1)

In GD, unlike CXCL10 which was observed either in infiltrating inflammatory and endothelial cells and thyrocytes, CXCR3 receptor turned out to be more expressed only in the first two types of cells [60]. Another study showed that in GD, the recruitment of inflammatory cells and the following amplification of inflammation are dependent on CXCR3-binding chemokine CXCL10, and suggested that at the beginning of GD the recruitment of CXCR3-expressing Th1 cells can be caused by the production of this chemokine by resident follicular epithelial cells [61].

Higher circulating CXCL10 levels have been observed in GD patients

Table 1
Role of cytokines/chemokines in Graves' disease (GD) pathogenesis: results of *in vitro* and *in vivo* studies.

Cytokines	<i>In vivo</i> studies	<i>In vitro</i> studies
CXCL10	<ul style="list-style-type: none"> - Important role in the recruitment of inflammatory cells and in the amplification of inflammation in AITDs [61]. - High serum levels in relapsing hyperthyroid GD patients, and in the newly diagnosed ones [37]. - High circulating serum levels in GD patients [62]. - Different expression patterns of chemokines in the various thyroid diseases [63]. 	<ul style="list-style-type: none"> - Thyroid follicular cells represent an important source of CXCL10, which can mediate the recruitment of activated CXCR3+ lymphocytes to the thyroid in AITDs [60]. - Under the Th1 cytokine IFN-γ stimulation, thyrocytes participate to the induction and perpetuation of inflammation by releasing CXCL10; PPAR-γ inhibited this effect [64]. - PPAR-α activation had an inhibitory role on CXCL10 secretion by thyroid cells [38,66].
CXCL9	<ul style="list-style-type: none"> - Important role in the recruitment of inflammatory cells and in the amplification of inflammation in AITDs [61]. - Different expression patterns of chemokines in the various thyroid diseases [63]. - Association of the serum chemokines levels with the active phase of GD [67]. - Association between the intractability of GD and the MIG rs2276886 AG genotype [68]. 	<ul style="list-style-type: none"> - Thyroid follicular cells represent an important source of CXCL9, which can mediate the recruitment of activated CXCR3+ lymphocytes to the thyroid in AITDs [60]. - Under the Th1 cytokine IFN-γ stimulation, thyrocytes participate to the induction and perpetuation of inflammation by releasing CXCL9; PPAR-γ inhibited this effect [64]. - PPAR-α activation had an inhibitory role on CXCL9 secretion by thyroid cells [38,66].
CXCL11	<ul style="list-style-type: none"> - Association of the serum chemokines levels with the active phase of GD [67]. 	<ul style="list-style-type: none"> - Under the Th1 cytokine IFN-γ stimulation, thyrocytes participate to the induction and perpetuation of inflammation by releasing CXCL11; PPAR-γ inhibited this effect [64]. - PPAR-α activation had an inhibitory role on CXCL11 secretion by thyroid cells [38,66].
CCL2	Different expression patterns of chemokines in the various thyroid diseases [63].	
CCL5	Different expression patterns of chemokines in the various thyroid diseases [63].	
IL-37	IL-37 mRNA levels were increased in GD patients with respect to patients with inactive disease, or healthy controls [69].	In PBMCs of GD, IL-37 inhibited the production of IL-6, IL-17 and TNF- α [69].
IL-21	An increased serum concentration of IL-21 in untreated GD and HT patients was present [70].	IL-21 could take part in the pathogenesis of the disease, as the expression of IL-21 and IL-21R was up-regulated in PBMCs from GD patients [70].
CCL21	Clinical correlation between GD and plasma CCL21 [71].	
IL-23	IL-23A gene could be a genetic risk marker in Han Chinese population [72].	
IL-6	A significant association between the IL6 -174 G/C polymorphism and the risk for GD [73].	
TNF- α	The promoter SNP rs1800629 within the TNF- α gene is associated with increased risk for developing GD [74].	

in comparison to the controls (matched by age and sex) [62]. Differently from GD patients with untreated hyperthyroidism, subjects with euthyroid or hyperthyroid GD under MMI treatment had lower serum CXCL10 levels; meanwhile these levels were higher in hyperthyroid GD patients compared to hypothyroid or euthyroid GD ones [37]. Serum CXCL10 levels turned out to be similar both in untreated patients with relapsed hyperthyroidism who previously took MMI, and in recently diagnosed untreated hyperthyroid GD subjects. These results showed that either in relapsing hyperthyroid patients, or in the newly diagnosed ones, the active phase of GD is associated with elevated serum CXCL10 levels [37].

Serum levels of CCL2, CCL5, CXCL9 and CXCL10 in patients with GD, HT and nontoxic nodular thyroid disease (NNT) have been assessed in a further study [63]. Despite CCL2 and CXCL9, whose concentrations were similar in patients with AITD and NNT, CCL5 was substantially higher in GD patients than in the HT or NNT ones. Conversely, CXCL10 levels were lower in patients with GD, with a difference that reach the significance only when compared to HT subjects. Of note, different statistically significant levels of CXCL9 were observed in GD patients who relapsed or went into remission. These findings suggested that the distinct immune responses in GD and HT can be related to the different expression patterns of chemokines [63].

Other studies assessed the action of peroxisome proliferator-activated receptor (PPAR)- γ and of the IFN- γ and TNF- α stimulation on the CXCL10, CXCL9, CXCL11 release, in primary cells of GD thyrocytes [62,64,65]. Basally, the secretion of these chemokines was absent, and they were dose-dependently released treating cells with IFN- γ , while TNF- α alone had no effect. The treatment with TNF- α + IFN- γ had a synergistic effect on the CXCL10, CXCL9 and CXCL11 secretion. The co-treatment with the PPAR- γ agonist, rosiglitazone, or pioglitazone suppressed this effect in a dose-dependent manner. These results demonstrated that thyrocytes from patients with GD under the cytokines stimulation participate to the self-protraction of inflammation by

releasing CXCL9, CXCL10, and CXCL11, and PPAR- γ inhibited this effect. Among the CXC chemokines, the CXCL9 leading role was suggested by its high response to the IFN- γ + TNF- α -stimulation [64].

The effect of PPAR- α activation on CXCL10, CXCL9 and CXCL11 chemokines in general, or on the secretion of these chemokines in thyrocytes has been evaluated by other studies [38,66]. The presence of PPAR- α and PPAR- γ in GD and control cells in primary culture has been shown by real-time reverse transcription-polymerase chain reaction (RT-PCR). The role of PPAR- α and PPAR- γ activation on chemokines secretion has been also evaluated after the treatment of GD and control cells with IFN- γ and TNF- α . A strong dose-dependent inhibition on the cytokines-stimulated secretion of CXCL11, CXCL10, and CXCL9 by PPAR- α -agonists has been observed in both primary culture cells. The effect of PPAR- α -agonists was higher on the release of CXCL9 (85% of inhibition with ciprofibrate, 90% with fenofibrate). The compounds had a different potency for each chemokine; for instance, the inhibition exerted by gemfibrozil on CXCL11 was of 55%, while it exerted a lower inhibition on CXCL9 secretion, of 40%. In thyrocytes, the inhibition of CXCL10, CXCL9 and CXCL11 secretion was greater with PPAR- α agonists than with PPAR- γ agonists. These findings suggested that the immune response in thyroid may be modulated by PPAR- α [38,66].

In another study [67], CXCL9 and CXCL11 levels were measured in the sera of 91 GD patients, 91 AT, 34 nontoxic multinodular goiters (MNGs), 31 TNGs, and 91 healthy subjects (matched by sex and age). The mean chemokines levels found in GD, controls, euthyroid AT, MNG, or TNG were the following: CXCL9: 274 \pm 265, 76 \pm 33, 132 \pm 78, 87 \pm 48, and 112 \pm 56 pg/mL; CXCL11: 140 \pm 92, 64 \pm 20, 108 \pm 48, 76 \pm 33, 91 \pm 41 pg/mL, respectively ($p < 0.05$, ANOVA). These levels were more elevated in GD patients with respect to those in other subjects. Chemokines levels were significantly higher in hyperthyroid GD patients than hypothyroid, or euthyroid ones. Furthermore, these levels were higher in GD patients with untreated hyperthyroidism than euthyroid, or hyperthyroid, GD subjects treated

with MMI. Similar chemokines levels were measured in newly diagnosed hyperthyroid GD (not treated), vs. untreated patients with relapsed hyperthyroidism. In conclusion the serum chemokines levels were associated with the active phase of GD (newly diagnosed and relapsing) and their decrease in treated patients with GD may be related to the immunomodulatory effects of MMI [67].

A further study investigated about the relationship between the pathogenesis of AITD and functional polymorphisms in genes encoding some chemokines [68]. This study genotyped the following polymorphisms: IL8 -251 T/A, Monocyte Chemoattractant Protein1 (MCP1)-2518G/A, Regulated upon Activation, Normal T cell Expressed and presumably Secreted (RANTES)-403G/A, -28C/G, MIG rs2276886G/A, IP10 -1596C/T and IL16 -295 T/C. The study enrolled: 131 HT patients [of whom 54 who developed moderate to severe hypothyroidism before 50 years of age and were treated daily with thyroxine (severe HT) and 46 untreated euthyroid patients with HT, who were over 50 years of age (mild HT)]; 149 GD patients, of whom 53 with GD in remission, 59 who had been treated with MMI for at least five years and were still positive for anti-thyrotropin receptor antibody (TRAb) (intractable GD); 99 healthy controls. In AITD patients MIG rs2276886 A allele and IL8 -251TT genotype were more frequent; unlike the RANTES -403AA and -28GG genotypes which were less frequent. MIG rs2276886 AG genotype was less common in patients with intractable GD, while the MCP1 -2518GG genotype was more frequent in HT patients [68]. It's interesting that in GD patients with -28CG and GG genotypes the age at onset was higher in comparison to GD patients having RANTES -28CC genotype. This study firstly reported the association between the intractability of GD and the MIG rs2276886 AG genotype [68].

Other Cytokines/Chemokines (Table 1)

IL-37

The anti-inflammatory cytokine IL-37 belongs to the IL-1 family, and it is able to reduce local and systemic inflammation. Its role in GD patients is still unclear [69]. A study evaluated the expression of IL-37, IL-6, IL-17 and TNF- α mRNA in peripheral blood mononuclear cells (PBMCs) in 40 GD patients, and the serum levels of IL-37, IL-6, IL-17 and TNF- α . It was shown that IL-37, IL-6, IL-17 and TNF- α both in serum and PBMCs were significantly higher in GD patients in comparison to control subjects. Circulating IL-37 levels were strictly correlated with IL-17, IL-6, TNF- α , TSH, TRAb, free T3 (FT3) and free T4 (FT4). In the active phase of GD, IL-37 mRNA and serum protein levels were increased with respect to those in patients with inactive disease or healthy controls. In PBMCs in GD, IL-37 repressed the production of IL-6, IL-17 and TNF- α . These data suggested that IL-37 inhibits the production of proinflammatory cytokines in GD, having a protective role against inflammation. For this reason, IL-37 could be considered as a new research target for the pathogenesis and therapy of GD [69].

IL-21

Another study evaluated the mRNA and protein expression of inflammatory cytokines of *in vitro* PBMCs after the stimulation with recombinant human IL-21 (rhIL-21), to investigate the role of IL-21/IL-21 receptor (IL-21R) in the pathogenesis of AITD [70]. Serum IL-21 levels were higher in not treated GD and HT patients, and in PBMCs in HT, IL-21⁺CD3⁺CD8⁻ T cells were significantly higher in comparison to controls. The IL-21 mRNA in PBMCs was elevated in GD and HT patients, and a strong increase of IL-21 and IL-21R mRNA in thyroid tissues of HT patients was reported. The expression of IL-21R protein in HT thyroid cells and lymphocytes was shown by immunohistochemical staining. Cultured PBMCs from GD patients, in presence of rhIL-21, stimulated high IL-17A levels but reduced IL-4 production, whilst from HT patients, in presence of rhIL-21, stimulated an increased production

of IFN- γ . These results showed that in AITD, IL-21 could take part in the pathogenesis of the disease, as the expression of IL-21 and IL-21R was up-regulated, increasing an abnormal immune cascade [70].

CCL21

Another paper evaluated the relationship between CC-chemokine ligand 21 (CCL21) and GD development and the role of osteopontin (OPN) in the regulation of CCL21 production [71]. In GD, CCL21 has an important role in the circulation of CC-chemokine receptor 7 (CCR7)-expressing cells, while OPN stimulates the production of proinflammatory cytokines and chemokines, by NF- κ B and MAPK signaling pathways. Fourty newly diagnosed GD without previous treatment, 12 TRAb-negative GD patients, 15 euthyroid GD patients, and 25 healthy controls were enrolled. It has been shown that plasma CCL21 levels (determined in plasma and culture supernatants by enzyme-linked immunoassay) were higher in GD patients and normalized in TRAb-negative GD patients. Furthermore, CCL21 correlated with TRAb and plasma OPN. Moreover, recombinant OPN raised the expression of CCL21 time- and dose-dependently. The reported results suggested a clinical correlation between GD and plasma CCL21, and that CCL21 could be considered as a new marker in GD and a possible target for TRAb-positive GD therapy [71].

IL-23

The genetic association between rs11171806 (IL-23A gene polymorphism) and susceptibility to GD was investigated by 2 independent Chinese cohorts [72]. The Shanghai cohort included 712 unrelated GD patients and 705 controls, and the replication cohort from Xiamen Island consisted of 433 GD patients and 410 controls. Serum IL-23 levels were significantly higher in GD patients than in healthy subjects. Furthermore, in the subgroup analysis, higher concentrations of IL-23 were reported in the female gender and in patients of older age (≥ 40 years). It was also conducted an association study with the IL-23 gene polymorphism rs11171806. In Shanghai cohorts, the frequencies of rs11171806 alleles were markedly different between GD patients (G 95.7% and A 4.3%) and healthy controls (G 97.7% and A 2.3%) [$P = 2.6 \times 10^{-3}$, OR = 1.93 (95% CI: 1.25–2.97)], and in Xiamen cohorts, the proportion of individuals carrying the A allele of rs11171806 was high both in GD patients and in controls, in a similar manner [GD vs. control, 4.8% vs. 4.3%, OR = 2.15 (95% CI: 1.23–3.79), $P_{\text{allele}} = 6.3 \times 10^{-3}$]. These data indicated that in GD the IL-23A gene could be a genetic risk marker in Han Chinese population [72].

IL-6

The association between IL-6 -174 G/C polymorphism and GD have been investigated by various studies, with discordant results. A meta-analysis was conducted to evaluate such association, and included 4 case-control studies with 554 GD cases and 1201 healthy controls [73]. In the combined analysis, a significant association between the IL-6 -174 G/C polymorphism and the risk for GD in dominant model (OR = 1.39, 95% CI: 1.07–1.80), recessive model (OR = 2.75, 95% CI: 1.01–7.55) and homozygote contrast model (OR = 3.25, 95% CI: 1.1–9.58) was shown [73].

TNF- α

Another meta-analysis was performed on rs1800629 and rs361525 in the TNF- α gene from all the eligible case-control studies to investigate whether TNF- α is able to influence the susceptibility to GD [74]. Ten case-control studies involving 2790 GD patients and 3472 healthy controls were included. A significant association was shown between the rs1800629 polymorphism and GD in the homozygous model (AA compared with GG: OR = 1.97, 95% CI = 1.27–3.06,

$P = 0.002$) and recessive model (AA compared with GA + GG: OR = 1.62, 95% CI = 1.04–2.50, $P = 0.03$). GD susceptibility was significantly detected in European population in all genetic models after ethnicity stratification, while no significant association existed in Asian population. The single-nucleotide polymorphism (SNP) rs361525 did not show a significant association with GD in any genetic model before and after ethnicity stratification. The results suggested that only the promoter SNP rs1800629 within the TNF- α gene is associated with increased risk for developing GD, in particular in European population [74].

Conclusion

Th1-dependent chemokines and their receptor, CXCR3, contribute to the pathogenesis of GD, and are secreted by thyrocytes upon the stimulation with IFN- γ . In tissues, recruited Th1 lymphocytes may be responsible for enhanced IFN- γ and TNF- α production, which in turn stimulates Th1 chemokines secretion from these cells, leading to an amplification feedback loop, that can further perpetuate the autoimmune process.

Chemokines produced in the inflamed tissues enter into the circulation, and high levels of IFN- γ induced chemokines present in peripheral fluids can be considered a marker for the host immune response. The determination of high levels of Th1 chemokines in peripheral liquids can be considered as a marker of a Th1 orientated immune response, and circulating levels of these chemokines are associated with the active phase of GD in both newly diagnosed, and relapsing hyperthyroid patients.

MMI reduces the secretion of Th1 chemokines by isolated thyrocytes, and serum levels, and promotes a transition from Th1 to Th2 dominance in patients in GD active phase. PPAR- γ and PPAR- α activators are able to modulate the IFN- γ induced Th1 chemokines secretion *in vitro*, in GD thyrocytes.

Other cytokines, such as IL-37, IL-21, CCL21 and IL-23A, have been investigated in order to evaluate their role in the pathogenesis of GD.

Additional researches are needed to evaluate the interactions between chemokines and cytokines in the pathogenesis of GD, and to investigate whether Th1 chemokines could be considered a novel therapeutic target in this autoimmune disorder.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jcte.2019.100196>.

References

- [1] Erik D Schraga. Hyperthyroidism, thyroid storm, and graves disease. <https://emedicine.medscape.com/article/767130-overview>.
- [2] Bahn Chair RS, Burch HB, Cooper DS, Garber JR, Greenlee MC, Klein I, et al. Hyperthyroidism and other causes of thyrotoxicosis: management guidelines of the American Thyroid Association and American Association of Clinical Endocrinologists. *Thyroid* 2011;2011(6):593–646. <https://doi.org/10.1089/thy.2010.0417>.
- [3] Hyperthyroidism. <https://www.niddk.nih.gov/health-information/endocrine-diseases/hyperthyroidism>.
- [4] Devereaux D, Tewelde SZ. Hyperthyroidism and thyrotoxicosis. *Emerg Med Clin North Am* 2014;32:277–92. <https://doi.org/10.1016/j.emc.2013.12.001>.
- [5] Brent Gregory A. Clinical practice. Graves' disease. *N Engl J Med* 2008;358:2594–605. <https://doi.org/10.1056/NEJMc0801880>.
- [6] Lytton SD, Kahaly GJ. Bioassays for TSH-receptor autoantibodies: an update. *Autoimmun Rev* 2010;10:116–22. <https://doi.org/10.1016/j.autrev.2010.08.018>.
- [7] Ferrari SM, Fallahi P, Ruffilli I, Elia G, Ragusa F, Benvenga S, et al. The association of other autoimmune diseases in patients with Graves' disease (with or without ophthalmopathy): review of the literature and report of a large series. *Autoimmun Rev* 2019;18:287–92. <https://doi.org/10.1016/j.autrev.2018.10.001>.
- [8] Brix TH, Hegedus L. Twin studies as a model for exploring the aetiology of autoimmune thyroid disease. *Clin Endocrinol (Oxf)* 2012;76:457–64. <https://doi.org/10.1111/j.1365-2265.2011.04318.x>.
- [9] Davies TF, Latif R, Yin X. New genetic insights from autoimmune thyroid disease. *J Thyroid Res* 2012;2012:623852. <https://doi.org/10.1155/2012/623852>.
- [10] Jacobson EM, Huber A, Tomer Y. The HLA gene complex in thyroid autoimmunity: from epidemiology to etiology. *J Autoimmun* 2008;30:58–62. <https://doi.org/10.1016/j.jaut.2007.11.010>.
- [11] Vita R, Lapa D, Trimarchi F, Vita G, Fallahi P, Antonelli A, et al. Certain HLA alleles are associated with stress-triggered Graves' disease and influence its course. *Endocrine* 2017;55:93–100. <https://doi.org/10.1007/s12020-016-0909-6>.
- [12] Simmonds MJ, Howson JM, Heward JM, Cordell HJ, Foxall H, Carr-Smith J, et al. Regression mapping of association between the human leukocyte antigen region and Graves disease. *Am J Hum Genet* 2005;76:157–63. <https://doi.org/10.1086/426947>.
- [13] Vita R, Lapa D, Vita G, Trimarchi F, Benvenga S. A patient with stress-related onset and exacerbations of Graves disease. *Nat Clin Pract Endocrinol Metab* 2009;5:55–61. <https://doi.org/10.1038/ncpendmet1006>.
- [14] Vita R, Lapa D, Trimarchi F, Benvenga S. Stress triggers the onset and the recurrences of hyperthyroidism in patients with Graves' disease. *Endocrine* 2015;48:254–63. <https://doi.org/10.1007/s12020-014-0289-8>.
- [15] Effraimidis G, Wiersinga WM. Mechanisms in endocrinology: autoimmune thyroid disease: old and new players. *Eur J Endocrinol* 2014;170:R241–52. <https://doi.org/10.1530/EJE-14-0047>.
- [16] Perricone C, Versini M, Ben-Ami D, Gertel S, Watad A, Segel MJ, et al. Smoke and autoimmunity: The fire behind the disease. *Autoimmun Rev* 2016;15:354–74. <https://doi.org/10.1016/j.autrev.2016.01.001>.
- [17] Fallahi P, Ferrari SM, Vita R, Benvenga S, Antonelli A. The role of human parvovirus B19 and hepatitis C virus in the development of thyroid disorders. *Rev Endocr Metab Disord* 2016;17:529–35. <https://doi.org/10.1007/s11154-016-9361-4>.
- [18] Antonelli A, Ferrari SM, Ruffilli I, Fallahi P. Cytokines and HCV-related autoimmune disorders. *Immunol Res* 2014;60:311–9. <https://doi.org/10.1007/s12026-014-8569-1>.
- [19] Rapoport B, McLachlan SM. Graves' hyperthyroidism is antibody-mediated but is predominantly a Th1-type cytokine disease. *J Clin Endocrinol Metab* 2014;99:4060–1. <https://doi.org/10.1210/jc.2014-3011>.
- [20] Antonelli A, Ferrari SM, Corrado A, Di Domenicantonio A, Fallahi P. Autoimmune Thyroid Disorders. *Autoimmun Rev* 2015;14:174–80. <https://doi.org/10.1016/j.autrev.2014.10.016>.
- [21] John Lackie. Cytokine. In: A Dictionary of Biomedicine. Oxford University Press. 2010.
- [22] Cytokine. In: Stedman's Medical Dictionary, 28th ed. Wolters Kluwer Health, Lippincott, Williams & Wilkins.2006.
- [23] Mélik-Parsadaniantz S, Rostène W. Chemokines and neuromodulation. *J Neuroimmunol* 2008;198:62–8. <https://doi.org/10.1016/j.jneuroim.2008.04.022>.
- [24] Lasagni L, Francalanci M, Annunziato F, Lazzeri E, Giannini S, Cosmi L, et al. An alternatively spliced variant of CXCR3 mediates the inhibition of endothelial cell growth induced by IP-10, Mig, and I-TAC, and acts as functional receptor for platelet factor 4. *J Exp Med* 2003;197:1537–49. <https://doi.org/10.1084/jem.20021897>.
- [25] Smit MJ, Verdijk P, van der Raaij-Helmer EM, Navis M, Hensbergen PJ, Leurs R, et al. CXCR3-mediated chemotaxis of human T cells is regulated by a Gi- and phospholipase C-dependent pathway and not via activation of MEK/p44/p42 MAPK nor Akt/Pi-3 kinase. *Blood* 2003;102:1959–65. <https://doi.org/10.1182/blood-2002-12-3945>.
- [26] Tokunaga R, Zhang W, Naseem M, Puccini A, Berger MD, Soni S, et al. CXCL9, CXCL10, CXCL11/CXCR3 axis for immune activation – a target for novel cancer therapy. *Cancer Treat Rev* 2018;63:40–7. <https://doi.org/10.1016/j.ctrv.2017.11.007>.
- [27] Antonelli A, Ferrari SM, Giuggioli D, Ferrannini E, Ferri C, Fallahi P. Chemokine (C-X-C motif) ligand (CXCL)10 in autoimmune diseases. *Autoimmun Rev* 2014;13:272–80. <https://doi.org/10.1016/j.autrev.2013.10.010>.
- [28] Liu C, Papewalis C, Domberg J, Scherbaum WA, Schott M. Chemokines and autoimmune thyroid diseases. *Horm Metab Res* 2008;40:361–8. <https://doi.org/10.1055/s-2008-1073153>.
- [29] Antonelli A, Ferrari SM, Frascerra S, Galetta F, Franzoni F, Corrado A, et al. Circulating chemokine (CXC motif) ligand (CXCL)9 is increased in aggressive chronic autoimmune thyroiditis, in association with CXCL10. *Cytokine* 2011;55:288–93. <https://doi.org/10.1016/j.cyto.2011.04.022>.
- [30] Antonelli A, Ferrari SM, Frascerra S, Di Domenicantonio A, Nicolini A, Ferrari P, et al. Increase of circulating CXCL9 and CXCL11 associated with euthyroid or subclinically hypothyroid autoimmune thyroiditis. *J Clin Endocrinol Metab* 2011;96:1859–63. <https://doi.org/10.1210/jc.2010-2905>.
- [31] Antonelli A, Ferri C, Fallahi P, Ferrari SM, Frascerra S, Sebastiani M, et al. High values of CXCL10 serum levels in patients with hepatitis C associated mixed cryoglobulinemia in presence or absence of autoimmune thyroiditis. *Cytokine* 2008;42:137–43. <https://doi.org/10.1016/j.cyto.2008.01.002>.
- [32] Antonelli A, Ferrari SM, Fallahi P, Ghire E, Crescioli C, Romagnani P, et al. Interferon-alpha, -beta and -gamma induce CXCL9 and CXCL10 secretion by human thyrocytes: modulation by peroxisome proliferator-activated receptor-gamma agonists. *Cytokine* 2010;50:260–7. <https://doi.org/10.1016/j.cyto.2010.01.009>.
- [33] Fallahi P, Ferrari SM, Ruffilli I, Elia G, Bircotti M, Vita R, et al. The association of other autoimmune diseases in patients with autoimmune thyroiditis: review of the literature and report of a large series of patients. *Autoimmun Rev* 2016;15:1125–8. <https://doi.org/10.1016/j.autrev.2016.09.009>.
- [34] Bagnasco M, Minciullo PL, Saraceno GS, Gangemi S, Benvenga S. Urticaria and thyroid autoimmunity. *Thyroid* 2011;21:401–10. <https://doi.org/10.1089/thy.2010.0103>.
- [35] Fallahi P, Ferrari SM, Elia G, Ragusa F, Paparo SR, Caruso C, et al. Myo-inositol in autoimmune thyroiditis, and hypothyroidism. *Rev Endocr Metab Disord* 2018;19:349–54. <https://doi.org/10.1007/s11154-018-9477-9>.

- [36] Ferrari SM, Elia G, Ragusa F, Paparo SR, Caruso C, Benvenga S, et al. The protective effect of myo-inositol on human thyrocytes. *Rev Endocr Metab Disord* 2018;19:355–62. <https://doi.org/10.1007/s11154-018-9476-x>.
- [37] Antonelli A, Rotondi M, Fallahi P, Romagnani P, Ferrari SM, Barani L, et al. Increase of interferon-gamma-inducible CXC chemokine CXCL10 serum levels in patients with active Graves' disease, and modulation by methimazole therapy. *Clin Endocrinol (Oxf)* 2006;64:189–95. <https://doi.org/10.1111/j.1365-2265.2006.02447.x>.
- [38] Antonelli A, Ferrari SM, Frascerra S, Pupilli C, Mancusi C, Metelli MR, et al. CXCL9 and CXCL11 chemokines modulation by peroxisome proliferator-activated receptor- α agonists secretion in Graves' and normal thyrocytes. *J Clin Endocrinol Metab* 2010;95:E413–20. <https://doi.org/10.1210/jc.2010-0923>.
- [39] Antonelli A, Ferrari SM, Fallahi P, Piaggi S, Paolicchi A, Franceschini SS, et al. Cytokines (interferon- γ - and tumor necrosis factor- α)-induced nuclear factor- κ B activation and chemokine (C-X-C motif) ligand 10 release in Graves disease and ophthalmopathy are modulated by pioglitazone. *Metabolism* 2011;60:277–83. <https://doi.org/10.1016/j.metabol.2010.02.002>.
- [40] Antonelli A, Ferrari SM, Corrado A, Ferrannini E, Fallahi P. CXCR3, CXCL10 and type 1 diabetes. *Cytokine Growth Factor Rev* 2014;25:57–65. <https://doi.org/10.1016/j.cytogfr.2014.01.006>.
- [41] Lacotte S, Brun S, Muller S, Dumortier H. CXCR3, inflammation, and autoimmune diseases. *Ann N Y Acad Sci* 2009;1173:310–7. <https://doi.org/10.1111/j.1749-6632.2009.04813.x>.
- [42] Lee EY, Lee ZH, Song YW. The interaction between CXCL10 and cytokines in chronic inflammatory arthritis. *Autoimmun Rev* 2013;12:554–7. <https://doi.org/10.1016/j.autrev.2012.10.001>.
- [43] Antonelli A, Ferri C, Fallahi P, Colaci M, Giuggioli D, Ferrari SM, et al. Th1 and Th2 chemokine serum levels in systemic sclerosis in the presence or absence of autoimmune thyroiditis. *J Rheumatol* 2008;35:1809–11.
- [44] Su R, Nguyen ML, Agarwal MR, Kirby C, Nguyen CP, Ramstein J, et al. Interferon-inducible chemokines reflect severity and progression in sarcoidosis. *Respir Res* 2013;14:121. <https://doi.org/10.1186/1465-9921-14-121>.
- [45] Antonelli A, Fallahi P, Delle Sedie A, Ferrari SM, Maccheroni M, Bombardieri S, et al. High values of Th1 (CXCL10) and Th2 (CCL2) chemokines in patients with psoriatic arthritis. *Clin Exp Rheumatol* 2009;27:22–7.
- [46] Antonelli A, Fallahi P, Delle Sedie A, Ferrari SM, Maccheroni M, Bombardieri S, et al. High values of alpha (CXCL10) and beta (CCL2) circulating chemokines in patients with psoriatic arthritis, in presence or absence of autoimmune thyroiditis. *Autoimmunity* 2008;41:537–42. <https://doi.org/10.1080/08916930802170401>.
- [47] Antonelli A, Ferri C, Fallahi P, Ferrari SM, Sebastiani M, Ferrarini D, et al. High values of CXCL10 serum levels in mixed cryoglobulinemia associated with hepatitis C infection. *Am J Gastroenterol* 2008;103:2488–94. <https://doi.org/10.1111/j.1572-0241.2008.02040.x>.
- [48] Ferrari SM, Fallahi P, Mancusi C, et al. HCV-related autoimmune disorders in HCV chronic infection. *Clin Ter* 2013;164:e305–12. <https://doi.org/10.7417/CT.2013.1594>.
- [49] Fallahi P, Ferri C, Ferrari SM, Corrado A, Sansonno D, Antonelli A. Cytokines and HCV-related disorders. *Clin Dev Immunol* 2012;2012:468107. <https://doi.org/10.1155/2012/468107>.
- [50] Zignego AL, Gragnani L, Piluso A, Sebastiani M, Giuggioli D, Fallahi P, et al. Virus-driven autoimmunity and lymphoproliferation: the example of HCV infection. *Expert Rev Clin Immunol* 2015;11:15–31. <https://doi.org/10.1586/1744666X.2015.997214>.
- [51] Ferri C, Sebastiani M, Giuggioli D, et al. Hepatitis C virus syndrome: a constellation of organ- and non-organ specific autoimmune disorders, B-cell non-Hodgkin's lymphoma, and cancer. *World J Hepatol* 2015;7:327–43. <https://doi.org/10.4254/wjh.v7.i3.327>.
- [52] Antonelli A, Ferrari SM, Fallahi P, Frascerra S, Piaggi S, Gelmini S, et al. Dysregulation of secretion of CXC alpha-chemokine CXCL10 in papillary thyroid cancer: modulation by peroxisome proliferator-activated receptor-gamma agonists. *Endocr Relat Cancer* 2009;16:1299–311. <https://doi.org/10.1677/ERC-08-0337>.
- [53] Salvi M, Girasole G, Pedrazzoni M, Passeri M, Giuliani N, Minelli R, et al. Increased serum concentrations of interleukin-6 (IL-6) and soluble IL-6 receptor in patients with Graves'disease. *J Clin Endocrinol Metab* 1996;81:2976–9. <https://doi.org/10.1210/jcem.81.8.8768861>.
- [54] Salvi M, Pedrazzoni M, Girasole G, Giuliani N, Minelli R, Wall JR, et al. Serum concentrations of proinflammatory cytokines in Graves' disease: effect of treatment, thyroid function, ophthalmopathy and cigarette smoking. *Eur J Endocrinol* 2000;143:197–202.
- [55] Miyauchi S, Matsuura B, Onji M. Increased levels of serum interleukin-18 in Graves' disease. *Thyroid* 2000;10:815–9. <https://doi.org/10.1089/thy.2000.10.815>.
- [56] Celik I, Akalin S, Erbaş T. Serum levels of interleukin 6 and tumor necrosis factor-alpha in hyperthyroid patients before and after propylthiouracil treatment. *Eur J Endocrinol* 1995;132:668–72.
- [57] Lakatos P, Földes J, Horvath C, Kiss L, Tatrai A, Takacs I, et al. Serum interleukin-6 and bone metabolism in patients with thyroid function disorders. *J Clin Endocrinol Metab* 1997;82:78–81. <https://doi.org/10.1210/jcem.82.1.3641>.
- [58] Siddiqi A, Monson JP, Wood DF, Besser GM, Burrin JM. Serum cytokines in thyrotoxicosis. *J Clin Endocrinol Metab* 1999;84:435–9. <https://doi.org/10.1210/jcem.84.2.5436>.
- [59] Antonelli A, Rotondi M, Fallahi P, Romagnani P, Ferrari SM, Ferrannini E, et al. Age-dependent changes in CXC chemokine ligand 10 serum levels in euthyroid subjects. *J Interferon Cytokine Res* 2005;25:547–52. <https://doi.org/10.1089/jir.2005.25.547>.
- [60] García-López MA, Sancho D, Sánchez-Madrid F, Marazuela M. Thyrocytes from autoimmune thyroid disorders produce the chemokines IP-10 and Mig and attract CXCR3+ lymphocytes. *J Clin Endocrinol Metab* 2001;86:5008–16. <https://doi.org/10.1210/jcem.86.10.7953>.
- [61] Romagnani P, Rotondi M, Lazzeri E, Lasagni L, Francalanci M, Buonamano A, et al. Expression of IP-10/CXCL10 and MIG/CXCL9 in the thyroid and increased levels of IP-10/CXCL10 in the serum of patients with recent-onset Graves' disease. *Am J Pathol* 2002;161:195–206. [https://doi.org/10.1016/S0002-9440\(10\)64171-5](https://doi.org/10.1016/S0002-9440(10)64171-5).
- [62] Antonelli A, Rotondi M, Ferrari SM, Fallahi P, Romagnani P, Franceschini SS, et al. Interferon-gamma-inducible alpha-chemokine CXCL10 involvement in Graves' ophthalmopathy: modulation by peroxisome proliferator-activated receptor-gamma agonists. *J Clin Endocrinol Metab* 2006;91:614–20. <https://doi.org/10.1210/jc.2005-1689>.
- [63] Domberg J, Liu C, Papewalis C, Pflieger C, Xu K, Willenberg HS, et al. Circulating chemokines in patients with autoimmune thyroid diseases. *Horm Metab Res* 2008;40:416–21. <https://doi.org/10.1055/s-2008-1073151>.
- [64] Antonelli A, Ferrari SM, Fallahi P, Frascerra S, Santini E, Franceschini SS, et al. Monokine induced by interferon gamma (IFN γ) (CXCL9) and IFN γ inducible T-cell alpha-chemoattractant (CXCL11) involvement in Graves' disease and ophthalmopathy: modulation by peroxisome proliferator-activated receptor-gamma agonists. *J Clin Endocrinol Metab* 2009;94:1803–9. <https://doi.org/10.1210/jc.2008-2450>.
- [65] Ferrari SM, Fallahi P, Vita R, Antonelli A, Benvenga S. Peroxisome proliferator-activated receptor- γ in thyroid autoimmunity. *PPAR Res* 2015;2015:232818. <https://doi.org/10.1155/2015/232818>.
- [66] Antonelli A, Ferrari SM, Frascerra S, Corrado A, Pupilli C, Bernini G, et al. Peroxisome proliferator-activated receptor α agonists modulate Th1 and Th2 chemokine secretion in normal thyrocytes and Graves' disease. *Exp Cell Res* 2011;317:1527–33. <https://doi.org/10.1016/j.yexcr.2011.04.007>.
- [67] Antonelli A, Ferrari SM, Corrado A, Ferrannini E, Fallahi P. Increase of interferon- γ inducible CXCL9 and CXCL11 serum levels in patients with active Graves' disease and modulation by methimazole therapy. *Thyroid* 2013;23:1461–9. <https://doi.org/10.1089/thy.2012.0485>.
- [68] Akahane M, Watanabe M, Inoue N, Miyahara Y, Arakawa Y, Inoue Y, et al. Association of the polymorphisms of chemokine genes (IL8, RANTES, MIG, IP10, MCP1 and IL16) with the pathogenesis of autoimmune thyroid diseases. *Autoimmunity* 2016;49:312–9. <https://doi.org/10.3109/08916934.2015.1134507>.
- [69] Li Y, Wang Z, Yu T, Chen B, Zhang J, Huang K. Increased expression of IL-37 in patients with Graves' disease and its contribution to suppression of proinflammatory cytokines production in peripheral blood mononuclear cells. *PLoS ONE* 2014;9:e107183. <https://doi.org/10.1371/journal.pone.0107183>.
- [70] Guan LJ, Wang X, Meng S, Shi LF, Jiang WL, Xiao L, et al. Increased IL-21/IL-21R expression and its proinflammatory effects in autoimmune thyroid disease. *Cytokine* 2015;72:160–5. <https://doi.org/10.1016/j.cyto.2014.11.005>.
- [71] Qi Y, Li X, Zhang Q, Huang F, Lin D, Zhou Y, et al. Increased chemokine (C-C motif) ligand 21 expression and its correlation with osteopontin in Graves' disease. *Endocrine* 2015;50:123–9. <https://doi.org/10.1007/s12020-015-0552-7>.
- [72] Jia H, Tao F, Liu C, Guo T, Zhu W, Wang S, et al. Both interleukin-23A polymorphism and serum interleukin-23 expression are associated with Graves' disease risk. *Cell Immunol* 2015;294:39–43. <https://doi.org/10.1016/j.cellimm.2015.01.015>.
- [73] Imani D, Rezaei R, Razi B, Alizadeh S, Mahmoudi M. Association between IL6-174 G/C polymorphism and Graves' disease: a systematic review and meta-analysis. *Acta Med Iran* 2017;55:665–71.
- [74] Tu Y, Fan G, Zeng T, Cai X, Kong W. Association of TNF- α promoter polymorphism and Graves' disease: an updated systematic review and meta-analysis. *Biosci Rep* 2018;38. <https://doi.org/10.1042/BSR20180143>.