Title: Cellular and Molecular Basis of Thyroid Autoimmunity

Short title: Cellular and Molecular Basis of AITD

Authors: Joanna Bogusławska¹, Marlena Godlewska¹, Ewa Gajda¹, Agnieszka Piekielko-Witkowska¹*

Affiliation: Centre of Postgraduate Medical Education, Department of Biochemistry and Molecular Biology, ul. Marymoncka 99/103, 01-813 Warsaw, Poland

*Corresponding author: Agnieszka Piekielko-Witkowska, apiekielko@cmkp.edu.pl

Keywords: thyroid autoimmunity, autoimmune thyroid disease, AITD, Hashimoto thyroiditis, Graves’ disease, thyroid antigens, non-coding RNAs, miRNAs, circRNAs, IncRNAs, microbiome

Word count of the full article, excluding references and figure legends: 6184
Abstract

Autoimmune thyroid disease (AITD) is the most common human autoimmune disease. The two major clinical manifestations of AITD are Graves’ disease (GD) and Hashimoto’s thyroiditis (HT). AITD is characterized by lymphocytic infiltration of the thyroid gland, leading either to follicular cell damage, thyroid gland destruction, and development of hypothyroidism (in HT) or thyroid hyperplasia, induced by thyroid antibodies which activate TSHR on thyrocytes, leading to hyperthyroidism.

The aim of this review is to present up-to-date picture of the molecular and cellular mechanisms that underlie the pathology of AITD. Based on studies involving patients, animal AITD models, and thyroid cell lines, we discuss the key events leading to the loss of immune tolerance to thyroid autoantigens as well as the signaling cascades leading to the destruction of thyroid gland. Special focus is given on the interplay between the environmental and genetic factors, as well as non-coding RNAs (ncRNAs) and microbiome contributing to AITD development. In particular, we describe mechanistic models by which single nucleotide polymorphisms (SNPs) in genes involved in immune regulation and thyroid function, such as CD40, TSHR, FLT3, and PTPN22 underlie AITD predisposition. The clinical significance of novel diagnostic and prognostic biomarkers based on ncRNAs, and microbiome composition is also underscored. Finally, we discuss the possible significance of probiotic supplementation on thyroid function in AITD.
1. Introduction

Autoimmune thyroid disease (AITD) is defined as a dysregulation of the immune system leading to autoimmune attack on the thyroid gland. It is the most common autoimmune disease affecting humans (McLachlan and Rapoport, 2014). The two major clinical manifestations of AITD are Graves’ disease (GD) and Hashimoto’s thyroiditis (HT). The additional, less prevalent AITDs include postpartum thyroiditis, drug induced thyroiditis, thyroiditis associated with polyglandular autoimmune syndromes (Tomer and Huber, 2009). Both HT and GD are characterized by lymphocytic infiltration of the thyroid gland, which, however, differently affects thyroid function. In the case of HT, the resulting inflammation leads to the follicular cell damage, thyroid gland destruction, and development of hypothyroidism. In contrast, GD is mainly associated with hyperthyroidism, resulting from the presence of thyroid stimulating antibodies which activate TSHR on thyrocytes, leading to thyroid hyperplasia. Only a minor number of GD patients develop hypothyroidism due to the TSHR-blocking antibodies (Tomer and Huber, 2009, McLachlan and Rapoport, 2014). Of note, hypothyroidism in GD patients can be observed as a short-term effect of the blocking autoantibodies, as well as a longer effect due to eventual autoimmune thyroid destruction (Wood and Ingbar, 1979).

The knowledge of the molecular AITD background comes from patient observations, animal AITD models, as well as in vitro experiments performed on thyroid cell lines. The mouse AITD models are achieved through classical immunization of susceptible mice with the thyroid autoantigens or by more advanced techniques such as use of adenovirus vector carrying the human sequences of key thyroid autoantigens. The topic of mouse AITD models is very broad and due to manuscript text limitations, we cannot discuss it here in detail. The reader can further explore this subject in the previously published excellent review articles (Eckstein et al., 2020, McLachlan and Rapoport, 2014).

The aim of this review is to present the up-to-date picture of the molecular and cellular mechanisms that underlie the pathology of AITD. Our intention is to inspire basic researchers to initiate more-in-
depth studies on the molecular biology of AITD. We also hope that our article will complement the clinical view of HT and GD, recently presented in several outstanding papers (Wiersinga, 2017, Davies et al., 2020, Wiersinga, 2018, Lane et al., 2020).

2. Thyroid antigens in AITD

The autoantigens targeted during autoimmune attack in AITD are proteins expressed by thyroid tissue, indispensable for its physiological function. The key features of thyroid autoantigens are presented below.

2.1. Thyrotropin receptor (TSHR)

TSHR is indispensable for thyrotropin signal transduction, thyroid hormones (TH) production and proliferation of follicular epithelial cells. TSHR, primarily expressed on the basolateral membrane of thyrocytes (Figure 1), belongs to the G protein-coupled receptors. TSHR consists of the extracellular leucine-rich repeat domain (LRD) that is linked by a hinge region (HinR) to the transmembrane-spanning domain. The single polypeptide chain TSHR precursor undergoes intramolecular cleavage within the hinge region. Due to the post-translational processing the TSHR comprises an extracellular heavily glycosylated A-subunit and a transmembrane and intracellular B-subunit coupled by disulphide bridges. Autoantibodies to TSHR are directly involved in the pathophysiology of AITD and their measurement is recommended for early diagnosis and management of patients with GD (Kahaly et al., 2020a). Classical biochemical features of hyperthyroid Graves’ disease, including elevated TH levels and undetectable TSH, arise from the action of TSHR-stimulating antibodies (TSAb), which act as TSHR agonists by stimulating thyroid growth and TH production in an unregulated manner. Contrary, TSHR-blocking antibodies (TBAb) acts as antagonists, which block the action of the TSH, leading to the HT hypothyroidism. Finally, neutral antibodies to TSHR, which are also detected in GD sera, bind to the receptor without any influence its activity (Morshed et al., 2010). The complexity of the TSHR’s tertiary and quaternary structure contributes to the difficulty in delineating the epitopes recognized by TSHR-
specific autoantibodies. It seems that all TSAb and most TBAb bind to conformational epitopes formed by residues that are sequentially discontinuous but close together in three-dimensional space whereas, neutral antibodies to TSHR recognize linear epitopes localized predominantly within HinR. The crystal structure of TSHR LRD bound to blocking (M22) or stimulating (K1-70) antibodies revealed that the exact antigenic sites of TSHR-specific TBAb and TSAb are strongly overlapping and directed almost exclusively to A-subunit (Sanders et al., 2007, Sanders et al., 2011).

2.2. Thyroid peroxidase (TPO)

TPO is a glycosylated heme-containing homodimer of two 107-kDa transmembrane subunits located in the apical membrane of thyrocytes facing the follicular lumen (Figure 1). It catalyzes iodination of tyrosyl residues in thyroglobulin and further coupling of iodotyrosine residues to form iodothyronines attached to Tg (Godlewska and Banga, 2019). TPO is made up of a large N-terminal extracellular ectodomain followed by short transmembrane and cytoplasmic regions. Ectodomain exposed to the lumen is composed of three domains: a heme-containing and catalytically active myeloperoxidase (MPO)-like domain, a complement control protein (CCP)-like domain, and an epidermal growth factor (EGF)-like domain (Le et al., 2015). TPO is also a major thyroid antigen, to which both humoral and cellular immune response is directed. TPO autoantibodies (TPOAb) occur in almost all HT patients and approximately 75% of individuals with GD, whereas their positivity in euthyroid patients may be the risk factor of future thyroid disorders (Godlewska et al., 2019). They may be involved in autoimmune thyroid cell death via antibody-dependent cytotoxicity cells (ADCC) and C3 complement-mediated cytotoxicity (Czarnocka et al., 2014). Moreover, TPOAb also influence the diversity of the pathogenic T-cell epitope repertoire. The majority of TPOAb recognize overlapping conformation-dependent epitopes, called immunodominant region A (IDR-A) and B (IDR-B), located in the MPO-like domain and to lesser extent in the CCP-like domain (Godlewska et al., 2012, Dubska et al., 2006). Specific patterns of TPOAb recognition are stable in an individual over time and genetically inherited in families (Czarnocka et al., 2014, Jaume et al., 1996). Recently reported data shed new light on interaction...
between TPO and autoantibodies (Le et al., 2015, Williams et al., 2020). Unexpectedly, it seems that monomeric TPO is preferentially recognized by autoantibodies, whereas in dimeric mature TPO the binding sites are hidden. Additionally, the antigen-antibody interaction is connected with conformational changes in TPO that bring together previously far apart residues into a continuous epitope. This may explain the previously contradictory epitope mapping data reported by various research teams, that IDR-A and IDR-B include residues theoretically too far apart to be involved by single autoantibody complementarity-determining regions (CDRs) based on previous modeling (Le et al., 2015, Williams et al., 2020).

2.3. Thyroglobulin (Tg)

Tg is the largest and most abundant autoantigen in the thyroid gland. It is a soluble glycoprotein homodimer composed of two subunits of ~330 kDa each. Tg is the matrix for TH synthesis and is the form in which hormones are stored in the gland (Figure 1). After endocytosis from the colloid to cytoplasmic lysosomes for subsequent proteolysis, free TH (T3 and T4) are released from Tg molecule (Di Jeso and Arvan, 2016). The N-terminal part of thyroglobulin contains a large number of cysteine-rich domains, which are spaced by linker domains and connected to a C-terminal domain of high homology to choline-esterase (ChEL) (Di Jeso and Arvan, 2016). Recent study (Coscia et al., 2020) revealed the first atomic structure of full-length Tg and identified its hormone-forming tyrosine residues. Interestingly, only four out of 67 tyrosine residues in each monomer are hormonogenic (Coscia et al., 2020). Anti-Tg antibodies (TgAb) may be present at high concentrations in AITD, nevertheless they are also found in some clinically euthyroid individuals. Therefore, they are less predictive of overt thyroid dysfunction than TPOAb. A pathogenic role for TgAb has been postulated via ADCC but not through complement fixation (Czarnocka et al., 2014). In AITD, the prevalent TgAb species recognize native (rather than denatured) antigen. TgAb bind to a number of overlapping epitopic domains located mainly in the central region and C-terminal end of Tg and no intramolecular...
epitope spreading is observed (similar to TPOAb). Whereas, TgAb in sera from healthy subjects do have a different epitopic pattern (McLachlan and Rapoport, 2017).

2.4. Other thyroid autoantigens

The immune response to iodide transporters, sodium iodide symporter (NIS) and pendrin, has been widely discussed (e.g. (Frohlich and Wahl, 2017, Eleftheriadou et al., 2020)). Taking into account all the available data, including the recent study of Eleftheriadou and co-workers (Eleftheriadou et al., 2020), it seems that only NIS antibodies (NISAb) may play a relevant role in AITD. NISAb positivity is increased in AITD, especially in GD patients, whereas their presence in healthy donors is rare (Eleftheriadou et al., 2020). NIS is a ~90-kDa glycoprotein that mediates the active transport of iodide along the basolateral membrane of thyrocyte (Figure 1). The protein is composed of 13 transmembrane domains, with the extracellular N-terminus, and the C-terminal cytosolic tail. Epitopes for NISAb are likely located in extramembranous regions of the protein and at least partly overlap. Compared to NISAb, the diagnostic value of antibodies directed against pendrin, which is a apical membrane-bound iodide transporter, seems to be low (Eleftheriadou et al., 2020). The characteristics of thyroid antigens are summarized in Table 1.

3. The molecular and cellular mechanisms involved in AITD

3.1. Loss of immune tolerance

The key mechanism behind AITD development is the loss of immune tolerance to autoantigens. Immune tolerance is the result of processes localized in thymic and extrathymic tissues (referred as a central and peripheral tolerance, respectively) (Figure 2). Bone marrow-derived lymphocyte precursors migrate to the thymus to undergo positive selection (in thymus cortex), with the following negative selection (in thymus medulla). The key transcriptional factors involved in the maintaining immune tolerance are Aire and Fezf2, which regulate the expression of tissue-restricted antigens (TRAs) in medullary thymic epithelial cells (mTEC). The mechanisms utilized by both transcription
regulators are different. Fezf2 is a classical transcription factor which directly binds to the transcription start sites of TRA genes. In contrast, Aire acts indirectly, by interacting with histone H3 and recruiting proteins which regulate chromatin structure and transcription. It is estimated that expression of more than 60% of TRA genes is controlled by Aire and/or Fezf2; however, the overlap between Fezf2- or Aire-regulated TRA genes is limited. In consequence, both functional Fezf2 and Aire are required for efficient suppression of autoimmune responses. Loss of any of the TRA transcriptional regulators leads to immune disturbances. *AIRE* mutations result in autoimmune polyendocrinopathy syndrome type 1 (APS-1), characterized by wide spectrum of autoimmunological dysfunctions affecting peripheral endocrine tissues, including parathyroid and adrenal cortex. In mice, loss of *Fezf2* leads to enhanced autoantibody secretion in serum and elevation of activated T cells in secondary lymphoid organs. So far, no human *FEZF2* mutations were linked with autoimmune disease (Takaba and Takayanagi, 2017).

mTEC present TRA peptides complexed with MHC to the T cells which enables elimination of autoreactive lymphocytes in the process of negative selection. The resulting T CD4+ cells undergo cytokine-regulated differentiation into various types of T helper cells (Th) and T regulatory lymphocytes (Tregs) (Takaba and Takayanagi, 2017, Simmonds, 2013, Pyzik et al., 2015). The autoreactive T cells which still remain following negative selection are removed in the additional processes which include anergy and Treg-mediated inhibition. The anergy is defined as a functional inactivation preventing the lymphocyte from activating an immune reaction against the antigen. The inhibition of T cells is triggered by CTLA-4 and PD-1 receptors which interact with antigen presenting cells (APC)-expressed CD80/86 and PD-L1/2, respectively (Walker and Abbas, 2002). The maturation and functioning of Tregs is regulated by Foxp3 transcription factor, which controls the expression of genes crucial for Treg functioning. Tregs inhibit the functioning of T CD4+ and T CD8+ cells by triggering several mechanisms, including secretion or sequestration of cytokines, and via direct interaction leading to attenuation of T cells proliferation (Shevach, 2009, Wiersinga, 2018) (Figure 2).
The loss of immune self-tolerance lies in the centre of AITD pathobiology (Figure 3). It may result from the loss of central tolerance (i.e. disturbed deletion of autoreactive T cells in the thymus), dysfunction of peripheral tolerance (i.e. impaired apoptosis of self-reactive T cells and inhibition of the activity of Tregs), and disturbed anergy.

3.2. The mechanisms of immune response in AITD

The key stage in AITD development is the thyroidal accumulation of APCs expressing MHC class II molecules. The infiltration of thyroid by APC (in particular, dendritic cells and macrophages) may be triggered by inflammation resulting from viral or bacterial infection or the exposure of thyroid cells to toxins. In addition, thyroid follicular cells of AITD patients abnormally express IFN-γ-induced MHC class II molecules which enable presentation of thyroid autoantigens, facilitating T cells activation (McLachlan and Rapoport, 2014). In the advanced thyroiditis, thyroid gland is infiltrated by B cells (representing up to 50% of the infiltrating immune cells), as well as cytotoxic T lymphocytes and CD4+ cells (Wiersinga, 2018). The interaction with APCs leads to the activation of T CD4+ cells which differentiate into Tregs and Th lymphocytes, including Th1, Th2, and Th17 cells. In HT, Th and cytotoxic T lymphocytes trigger the destruction of thyroid gland by inducing several mechanisms involving cytokines and/or cytotoxins (Figure 4). Notably, AITD is associated with attenuated Tregs which normally counteract proinflammatory Th17 activity. Proinflammatory Th17 activation and Tregs attenuation are considered as the two key mechanisms contributing to the loss of self-tolerance in autoimmune diseases (Wiersinga, 2018).

Apoptotic loss of thyrocytes in AITD can also be triggered by disruption of thyroxisome (a multiprotein complex including caveolin-1, TPO, and dual oxidase) at the apical thyrocyte membrane, induced by cytokines released by Th1 cells (IFN-γ, IL-1α) or by paracrine- or autocrine-mediated thyrocyte activation. IL-1β, of which secretion is increased in HT, activates the expression of Fas and FasL at the surface of thyrocytes which enables activation of apoptosis by thyrocyte-thyrocyte interactions (Wiersinga, 2018). Furthermore, IL-1β and TNF-α may contribute to upregulation of SphK1 sphingosine
kinase in HT, leading to the increased secretion of sphingosine-1-phosphate (S1P), which is a ligand of S1PR1, a G protein-coupled receptor on T CD4+ cells (Han et al., 2019). Notably, the expression of S1PR1 is increased in T CD4+ cells in autoimmune thyroiditis, triggering the activation of STAT3 signaling through S1PR1/mTOR/PSer\textsuperscript{727}STAT3 and S1PR1/JAK2/PTyr\textsuperscript{705}STAT3 cascades. In turn, STAT3 recurrently activates the expression of S1PR1, providing the positive feedback regulatory mechanism which ultimately leads to enhanced production of inflammatory cytokines including IL-17A, IL-21, IFN-γ, and IL-6 (Han et al., 2019).

In GD, T cells are activated following the presentation of TSHR peptides. Activated T cells trigger activation of B cells and plasma cells infiltrating the thyroid to produce autoantibodies directed against TSHR, thereby affecting the secretion of thyroid hormones. The functioning of B cells and plasma cells is also regulated by liver-produced IGF1 (insulin growth factor 1). The activated T and B cells infiltrating thyroid release pro-inflammatory cytokines, IL-2 and IL-17, which leads to further activation of the immune cells reactive to TSHR (Davies et al., 2020). IL-17 is secreted by Th cells which can be suppressed by Tregs. It was suggested that activation of inflammatory response in GD could result from depletion of Treg cells expressing CD4+CD25+ and Foxp3 transcription factor. The loss of Treg CD4+CD25+Foxp3+ cells disables suppression of Th1 and Th2 cells, responsible for cytotoxic and antibody-based immune responses, respectively (Davies et al., 2020).

In contrast to TPO and Tg, TSHR is widely expressed in extrathyroidal tissues and cells, including lymphocytes, adipose tissue and fibroblasts. In consequence, the presence of TSHR antibodies contributes to the extrathyroidal GD manifestations, such as Graves’ ophthalmopathy (GO), Graves’ dermopathy or GD-associated thymus hyperplasia (Davies et al., 2020, McLachlan and Rapoport, 2014). TSHR autoantigen is presented by macrophages and B cells recruited to the orbit, which leads to T cell activation (Wiersinga, 2017). In turn, activated T cells initiate immunological attack to the orbital fibroblasts expressing TSHR. In response to the cytokines released by Th cells, orbital fibroblasts produce and deposit large amounts of glycosaminoglycans such as hyaluronan. This in turn leads to...
the increase of osmotic pressure, followed by water uptake and swelling of the extraorbital muscles. The cytokines released by Th cells stimulate preadipocytes differentiation and adipogenesis contributing to the increased accumulation of orbital adipose tissue. Both fibroblasts and adipocytes express TSHR, which is activated by TSHRAb, thereby stimulating hyaluronan synthesis and adipogenesis. This process is additionally stimulated by IGF1-mediated stimulation of IGF1R expressed by fibroblasts and adipocytes as well as the crosstalk of TSHR and IGF1R signaling pathways which converge in synergistic stimulation of DNA synthesis, proliferation, and hyaluronan secretion (Wiersinga, 2017, Davies et al., 2020, Neumann et al., 2020). Similar molecular pathophysiology may underlie the Graves’ dermopathy (Davies et al., 2020).

The common mechanism involved in the initiation and acceleration of the inflammatory processes in AITD is the Th1-cytokine/chemokine axis (Fallahi et al., 2020). Th1 cells produce IFN-γ and TNF-α which stimulate thyrocytes (in HT and GD) and retroorbital cells in thyroid eye disease to secrete chemokines (CXCL10, CXCL9, CXCL11). The latter bind and activate CXCR3 receptor on Th1 cells, leading to enhanced IFN-γ and TNF-α release and creating a positive feedback circuit which accelerates recruitment and activation of inflammatory cells in the affected organs. This mechanism is reflected by the elevated levels of CXCL9, CXCL10, and CXCL11 in serum of HT patients. In GD, the CXCL10-mediated recruitment of Th1 cells plays a particular role in the early phase of the disease. In patients with active and relapsing GD high serum CXCL10 levels are found which decrease after treatment. Those observations suggested that although Th2 cells were initially considered as the predominant modulators of GD autoimmunity, the launch of the GD active phase and relapse are rather shaped by Th1 lymphocytes. Similar findings were reported regarding the active phase of thyroid eye disease, in which elevated serum CXCL10, CXCL9 and CXCL11 were found (Fallahi et al., 2020).

3.3. Genetic and environmental factors contributing to AITD

It is generally acknowledged that both genetic and environmental factors contribute to AITD development. The key environmental risk factors include smoking, iodine excess, deficiency of
selenium and vitamin D, stress, exposure to chemicals, as well as bacterial and viral infection or interferon-alpha treatment (McLachlan and Rapoport, 2014). The cellular responses to viral infection involve IFN-α which induces specific reprogramming of gene expression. In individuals with susceptible genetic background this mechanism may induce pathological autoimmune reactions (Stefan et al., 2011). In particular, the associations between chronic infection of hepatitis C virus (HCV) and AITD have been repeatedly reported. In general, HCV patients have significantly increased risk to develop hypothyroidism and present TgAb and TPOAb. The proposed mechanism of HCV-induced AITD includes HCV interaction with CD81 receptor on thyroid cells, and induction if IFN-γ-inducible chemokines which recruit Th1 lymphocytes. The latter secrete IFN-γ and TNF-α, which further stimulate thyrocytes to produce chemokines, resulting in self-feeding circuit accelerating thyroid inflammation and damage (Colaci et al., 2018). The link between genetic susceptibility and environmental factors is provided by epigenetic regulatory mechanisms. Both thyroid tissues and lymphocytes of AITD patients reveal widespread epigenetic modifications affecting chromatin condensation (i.e. DNA methylation and histone modifications) (Wang et al., 2017) as well as dysregulated expression of non-coding RNAs (see Chapter 4).

The most relevant data on the genetic AITD background come from studies involving patients and their families. The heritability of HT and GD has been estimated to 65% and 63%, respectively, with shared genetic effects contributing the 8% of AITD variance (Skov et al., 2021). The AITD-associated SNPs affect genes involved in immune responses (e.g. HLA, PTPN22, CTLA4, IL2RA), thyroid function (e.g. TSHR, FOXE1), as well as other processes (e.g. LPP, TRIB2) (Supplementary Table S1). Remarkably, no TPO polymorphisms were linked with AITD (McLachlan and Rapoport, 2014). Both GD and HT share several common genes (HLA, PTPN22, CTLA-4) in which SNPs are linked with increased AITD risk (Simmonds, 2013). The key genetic association linked with both GD and HT are HLA risk alleles. It is postulated that the HLA-DR SNPs affect the three dimensional structure of the pocket binding pathogenic peptides which are presented to T-cell receptors (Tomer and Huber, 2009). However, it is estimated that HLA variants represent less than 10% of the AITD genetic background. Furthermore,
recent population-based studies revealed that the shared genetic background of HT and GD is limited (Skov et al., 2021).

The mechanisms by which most SNPs in the AITD-linked genes contribute to thyroid autoimmunity are mostly unknown, with a few notable exceptions (Figure 5). The AITD-related SNPs can influence translation efficiency, transcriptional repression or alternative splicing of the affected genes (CD40, TSHR, FLT3). Regarding the failure of immune tolerance to TSHR, two possible mechanisms have been proposed. Brand et al. showed that GD-predisposing SNPs localized in intron 1 of TSHR (rs179247-AA and rs12101255-TT) are associated with decreased expression ratio of the full-length TSHR (fTSHR) relative to the two TSHR splice variants, ST4 and ST5. The authors suggested that translation of the short ST4 and ST5 transcripts might result in soluble polypeptides which could possibly induce autoimmune response to TSHR (Brand et al., 2009). This hypothesis was later undermined by the study of Pujol-Borrell/Colobran group who analyzed larger cohort of patients and showed that expression of ST4 and ST5 is not affected by rs179247 variants (neither protective nor predisposing). Furthermore, the rs179247-protective genotype was associated with higher thymic expression of TSHR, without affecting its expression in the thyroid. According to that study, TSHR protein in the thymus may be expressed both as a membrane-bound receptor and as soluble ST4 and ST5 peptides, and that the failure of TSHR tolerance could be the lack of presentation of fTSHR by APCs in thymic medulla (Marin-Sanchez et al., 2019). The detailed mechanism by which SNPs located in intron 1 of TSHR gene may affect the expression of thyrotropin receptor was elucidated by Stefan et al. (Stefan et al., 2011) (Figure 5). The environmental factors (e.g. viral infection or iodine excess) provide additional signals which modify the functioning of the affected genes involved in AITD pathology (e.g. by triggering inflammation-induced signaling cascades or epigenetic reprogramming). The final effects of these mechanisms, leading to AITD development, include B cell activation and secretion of thyroid-specific autoantibodies, loss of immune tolerance or expansion of dendritic cells (Figure 5) (Huber et al., 2012, Stefan et al., 2011, Saevarsdottir et al., 2020).
The 1858C/T SNP of *PTPN22* is found in European population patients with AITD and other autoimmune diseases (Vang et al., 2018). *PTPN22* encodes LYP (Lymphoid tyrosine phosphatase) which dephosphorylates Src family of kinases, leading to the attenuation of TCR (T cell receptor) and BCR (B cell receptor). The proposed models by which 1858C/T SNP variant may contribute to AITD development include impaired regulation of B and T cells functioning (Figure 6).

As all autoimmune diseases, AITD affects females much more frequently than males. This over-responsiveness of the female immune system is at least partially linked with X chromosome-encoded genes involved in the immune regulation (e.g. *FOXP3*, *CD40L*) and encoding interleukin receptors (*IL3RA*, *IL9R*, *IL13RA1*, *IL13RA2*). The hypotheses explaining the female predisposition to autoimmune diseases include skewed X chromosome inactivation, reactivation of genes on silenced X chromosome, and X chromosome monosomy in peripheral lymphocytes (Box 1) (Frohlich and Wahl, 2017). According to the recent twin study, although the same genetic factors contribute to HT development in both sexes, their impact on male HT is much stronger than in females. The authors suggested that this could be explained by greater variance of environmental factors in females (Skov et al., 2021).

**4. The role of non-coding RNA in AITD**

Non-coding RNAs (ncRNAs) are defined as untranslated RNA molecules that regulate gene expression. ncRNAs are categorized depending on their length into long non-coding RNAs (lncRNAs) and short non-coding RNAs (e.g. microRNAs, piRNAs). Basing on their function, ncRNAs are classified into infrastructural (e.g. snRNA, snoRNAs, rRNAs) and regulatory ncRNAs (e.g. microRNAs, lncRNAs, piRNAs, siRNAs) (Yin et al., 2020). Regardless of their structural differences, all these molecules are capable of regulation of gene expression by several distinct mechanisms (Figure 7).

The growing interest in the role of non-coding RNAs in the pathogenesis of AITD comes from their critical role in the regulation of immune responses. Most studies exploring the role of ncRNAs in AITD are focused on miRNAs. Indeed, expression of miRNAs is broadly altered in blood, serum, plasma and thyroid tissues of AITD patients (Supplementary Table S2). The key miRNAs, repeatedly reported as
linked with AITD pathology, include miR-16, miR-21, miR-22, miR-125a, miR-142, miR-146a, miR-146b, miR-155, miR-200a, miR-326, miR-375, miR-431*, and miR-451 (Supplementary Table S2). The suggested clinical relevance of miRNAs comes mainly from their diagnostic potential. Serum/plasma miRNA expression profiles differentiate healthy individuals from GD patients (e.g. multi-miRNAs–based biomarkers such as miR-762/miR-144-3p or miR-210/miR-155/miR-146) and HT patients (e.g. biomarkers consisting of miR-205/miR-20a-3p/miR-375/miR-296/miR-451/miR-500a). miRNAs expression often associates with clinical parameters, such as TPOAb, correlating with miR-21-5p and miR-142-3p in GD patients, or TgAb and TPOAb correlating with miR-326 in HT patients (Taheri et al., 2020, Liu et al., 2020). Regarding the prognostic significance, higher miR-21-5p expression associates with a worse prognosis for GD patients, while disturbed expression of miR-155 in serum of GD patients correlates with the extent of goitre (Taheri et al., 2020, Zheng et al., 2018). It must be noted however, that although clinically promising, most of these studies require careful validation on independent, large cohorts of AITD patients and healthy control individuals.

miRNAs can influence immune response by regulating the functioning of dendritic cells and various populations of T cells. For instance, miR-146a suppresses DC apoptosis and cytokine production by targeting of IRAK and TRAF6, the regulators of NF-κB signaling cascade (Taheri et al., 2020). Inhibition of miR-125a-5p expression reduced the proportion of Th1 cells and the expression of IFN-γ in T CD4+ cells (Taheri et al., 2020). Increased expression of miR-142-3p in GD patients results in inhibition of negative regulation of T CD4+C25+ cells proliferation by Tregs (Martinez-Hernandez et al., 2018). Moreover, decreased miR-200a expression in T CD8+ cells of HT patients possibly results in a more significant production of proinflammatory Th1 cytokines, contributing to the destruction of thyroid cells (Bernecker et al., 2012). In HT patients, peripheral blood mononuclear cells (PBMCs)-elevated miR-326 modified IL-23/IL-23R/Th17 pathway, thereby promoting differentiation of Th17 cells via regulation of ADAM17 (Taheri et al., 2020).
The studies on other types of ncRNAs in AITD are more limited. Regarding IncRNAs, impaired expression of n335641, TCONS-00022357-xloc-010919 and n337845 was found in B cells of GD patients. Computational analysis revealed that these IncRNAs might regulate B cells by changing the expression of TCL1A and SH2D1A, two regulators of B cell proliferation and survival (Jiang et al., 2020). IncRNAs were also postulated as a diagnostic biomarkers distinguishing GD and HT patients from healthy controls (Taheri et al., 2020). So far, only one study delineated the role of circRNAs in AITD. Xiong et al. reported altered expression of 627 circRNAs in PBMCs of HT patients. In particular, they demonstrated increased expression of circ_0000075, circ_0012152, circ_0089172, with ROC curve AUC ranging from 0.715 to 0.673, suggestive of their potential diagnostic significance. Furthermore, the study revealed that circ_0089172 acts by sponging of miR-125a-3p, a regulator of IL-23R expression (miRNA sponges are RNA molecules which bind and sequester miRNA, thereby decreasing their inhibitory effect on the expression of target genes) (Xiong et al., 2019).

To summarize, despite extensive work investigating the expression and role of ncRNA in AITD, this topic is still in its infancy. Importantly, the biological significance of altered ncRNAs in AITD remains an open question. Many discoveries also need to be further verified in larger clinical studies. Nevertheless, it is evident that studies focusing on the role of ncRNAs in AITD can increase the understanding of molecular basis of thyroid autoimmunity, and might contribute to the development of new diagnostic and therapeutic strategies.

5. The role of microbiome in AITD

Human organism is inhabited by over $10^{14}$ microorganisms (both commensal and pathogenic), collectively referred to as microbiota (Box 2). The most diverse human microbiome is found in the gastrointestinal tract (GIT), which is estimated to be colonized by over 60% of all microorganisms inhabiting human body. The composition of the intestinal microflora is dominated by anaerobic bacteria such as Bacteroides, Bifidobacterium, Eubacterium, Fusobacterium, Ruminococcus and, to a
The presence of viruses (including Caudovirales, Podoviridae or Microviridae) and fungi (the most common: Candida, Cladosporium, Cryptococcus and Saccharomyces) is also observed. Bacterial flora varies depending on the GIT section: the stomach is almost sterile, while the colon has the highest bacteria load. Moreover, it changes during a human’s life, and its shape is influenced by the composition of the mother’s microflora, type of birth, diet and other environmental and lifestyle factors (Zheng et al., 2020).

The gut microbiome plays several essential physiological functions: it prevents intestine colonization by pathogenic bacteria, facilitates fermentation/degradation of food debris and production of nutrients, and contributes to the development and functioning of gut-associated lymphoid tissue (GALT). In particular, the gut microbiome is essential for proper functioning of the immune system. Germ-free mice have reduced Th CD4+ and CD8+, increased Th2 lymphocytes and attenuated differentiation of Th17 and Treg cells, paralleled by thinner intestinal epithelium mucus layer, reduced intestinal lymphatic tissue and underdeveloped lymphoid organs (Round and Mazmanian, 2009). The interplay between microbiota and immune regulation involves a complicated network of interactions between specific bacteria, products of their metabolism which target immune cells, as well as cytokines and metabolites which shape the immune homeostasis. The schematic mechanisms involved in microbiota-immune system interactions are presented in Figure 8. The reader is referred to the recently published more detailed review articles focusing on this topic (Zheng et al., 2020, Masetti and Ludgate, 2020).

Microbiota can influence the functioning of thyroid hormones, by several mechanisms, including the uptake of micronutrients essential for thyroid hormones biosynthesis, such as iodine, selenium, and iron. By binding and deconjugating TH metabolites (sulfates and glucuronides), gut bacteria may serve as T4/T3 reservoir. Indeed, supplementation of hypothyroid patients with VSL#3® probiotics (a mixture
of four strains of *Lactobacillus spp.*, three *Bifidobacterium spp.*, and *Streptococcus thermophilus*) prevents fluctuations of thyroid hormone level (Frohlich and Wahl, 2019).

The relationship between microbiota dysbiosis and the development of autoimmune diseases such as inflammatory bowel disease, multiple sclerosis or rheumatoid arthritis is well documented. However, the role of gut microbiome in AITD development is still poorly understood. The first link between microbiota and AITD was reported in 1988, when Penhale and Young showed that the transfer of microbiome from healthy rats to germ-free animals increases the likelihood of AITD development (Penhale and Young, 1988). In mice, the composition of gut microbiota contributes to GD susceptibility (Moshkelgosha et al., 2018).

The studies on the microbiome of AITD patients clearly show that the composition of the gut microbiota can distinguish between healthy controls, HT, GD, and GO patients, correlate with the stage of disease, level of thyroid autoantibodies and response to therapy (Virili et al., 2021) ([Supplementary Table S3](#)).

The mechanisms by which microbiome influences AITD development may originate from molecular mimicry resulting from the similarity between microbial and human antigens. The example of such mimicry is the homology between the amino acid sequence of TPO and Tg, and protein antigens of *Bifidobacteria* and *Lactobacillus spp.* (Kiseleva et al., 2011). However, this hypothesis is undermined by studies showing that supplementation of mice with *Lactobacillus rhamnosus* HN001 and *Bifidobacterium lactis* HN019 does not induce the risk of HT development (Zhou and Gill, 2005). What is particularly important, probiotics affect the clinical parameters of patients treated with LT4. In hypothyroid patients treated with LT4, synbiotic supplementation for 8 weeks resulted in decrease of TSH concentration, LT4 dose and FT3/TSH ratio (Talebi et al., 2020). Moreover, the results of a large clinical study INDIGO, clearly showed the influence of LAB4 probiotics on gut microbiota composition of GD patients and a temporary reduction in the serum level of IgG and IgA antibodies (Masetti and Ludgate, 2020). Given these results, we can assume that determining the composition of gut microflora
and subsequent use of the appropriate probiotics can contribute to more effective treatment of AITD.

The abovementioned studies clearly demonstrate that there is a link between AITD and microbiome. However, the functional consequences of microbiome alterations still require more extensive research, in particular involving patients from different populations.

6. Conclusions and perspectives

It is generally acknowledged that AITD is the effect of environmental factors acting on genetically susceptible background with epigenetic mechanisms as mediators. There is strong evidence that polymorphic variants of genes involved in immune regulation or encoding thyroid autoantigens can be linked with AITD predisposition. However, despite multiple studies on molecular AITD background, several important questions still remain to be answered. The mechanisms by which SNPs contribute to AITD development are largely underexplored and require further investigation. It is still unknown what triggers the cascade of processes which lead to the loss of immune tolerance. It remains to be established which of the antigens is recognized as the first in the immune cascade which leads to the dysfunction of thyroid gland. The functional consequences of gut dysbiosis on AITD development and progression remain largely underexplored. In particular, the possible modification of AITD progression by probiotic supplementation remains an open question. Further studies on the molecular and cellular AITD background may help in development of effective novel therapeutic options as illustrated by recently introduced clinical trials on monoclonal antibodies in treatment of GD patients (Kahaly et al., 2020b, Lane et al., 2020).

7. Acknowledgements

We sincerely apologize to all authors whose work regretfully could not be cited due to manuscript space limitations.

The authors are financially supported by 501-1-025-01-20 CMKP grant.
Conflict of interest

AP-W is a member of European Thyroid Journal Editorial Board; she was not involved in the review process of the manuscript. The other authors declare no conflict of interest.
References

BERNECKER, C., LENZ, L., OSTAPCUZ, M. S., SCHINNER, S., WILLENBERG, H., EHlers, M., VORDENBAUMEN, S., FELDKAMP, J. & SCHOTT, M. 2012. MicroRNAs miR-146a1, miR-155_2, and miR-200a1 are regulated in autoimmune thyroid diseases. Thyroid, 22, 1294-5.


CZARNOCKA, B., ESCHLER, D. C., GODLEWSKA, M. & TOMER, Y. 2012. MicroRNAs miR-146a1, miR-155_2, and miR-200a1 are regulated in autoimmune thyroid diseases. Thyroid, 22, 1294-5.


Figure and Table Legends

Table 1. Characteristics of thyroid antigens.

Figure 1. Localization and physiological function of thyroid antigens in thyrocytes. The sodium iodide symporter (NIS) transports I\(^{-}\) and Na\(^{+}\) through the basolateral plasma membrane of a thyroid epithelial cell. The Na\(^{+}/K\(^{+}\) ATPase pump maintains the sodium diffusion gradient required for NIS function. Pendrin participates in the apical iodide efflux into the colloid of thyroid follicle. Thyroid peroxidase (TPO) catalyzes iodination of tyrosines in thyroglobulin (Tg), which attaches one or two iodine to form moniodotyrosine (MIT) or diiodotyrosine (DIT), respectively. TPO catalyzes also coupling of iodotyrosine residues to form triiodothyronine (T3) and thyroxine (T4) attached to Tg, whereas the dual oxidase (DUOX) supplies hydrogen peroxide (H\(_2\)O\(_2\)) for thyroid hormones (TH) biosynthesis.

Release of thyroid hormones requires engulfing colloid material (endocytosis) to form intracellular endosomes (not shown) that merge with lysosomes to form an endolysosome. TH liberated from the Tg scaffold are subsequently secreted into the blood vessels. Binding of thyrotropin (TSH; or thyroid stimulating antibody) to TSH receptor (TSHR) activates intracellular signaling by the cyclic adenosine monophosphate (cAMP) pathway leading to thyrocytes growth, differentiation, as well as production and release of TH.

Figure 2. The mechanisms of selection, differentiation and activation of T lymphocytes. A. The positive selection of T cells (called thymocytes) expressing both CD4 and CD8 molecules in thymus cortex. Random recombination of genes encoding TCR peptides in T cell precursors leads to the expression of multiple TCR variants. TCR receptors interact with antigens presented by cortical thymic epithelial cells (cTECs) in complex with MHC (major histocompatibility complex) molecules. This interaction triggers the process of lymphocyte differentiation, leading to the generation of T cells expressing either CD4 or CD8 glycoprotein receptors on their surface. B. The unwanted effect of TCR genes’ recombination is the production of receptor variants which recognize epitopes normally expressed by healthy cells (autoantigens). T cells which express autoreactive TCR variants are...
eliminated during the process of negative selection. The T CD4+ or T CD8+ cells resulting from positive
selection migrate to the thymus medulla and interact with autopeptides complexed with MHC
expressed at the surface of medullary thymic epithelial cells (mTECs) and dendritic cells (DC). This
interaction triggers the process of apoptotic deletion of autoreactive T cells. Negative selection is
enabled by the ability of mTECs to express peptides (tissue restricted antigens, TRAs) which are specific
for different tissues of the organism (Takaba and Takayanagi, 2017, Simmonds, 2013, Pyzik et al.,
2015). The expression of TRAs in mTEC is regulated by transcription factors Aire and Fezf2. C.

Differentiation of T CD4+ cells. Following the process of central selection, T cells which do not react
with autoantigens migrate to the lymphoid organs (spleen, lymph nodes) and then spread to all
peripheral tissues. Depending on the signaling triggered by various cytokines, T CD4+ cells can
differentiate to various types of T helper cells (Th) and Tregs (Pyzik et al., 2015). D. The mechanisms
regulating activation and anergy of T lymphocytes. Activation of T lymphocytes: TCR expressed on T
lymphocytes recognizes antigen complexed with MHC expressed by APC. T cell activation is triggered
only in the presence of additional stimulation resulting from the interaction of CD28 and CD80/86
receptor. Anergy: In the absence of co-stimulatory signal T cell undergoes anergy. The signals inhibiting
T cell activity are generated by interaction between CTLA-4 and PD-1 receptors (on T cell surface) and
their respective ligands (CD80/86 and PD-L1/2), expressed by APC. Inhibition: Tregs inhibit the
functioning of T CD4+ and T CD8+ cells by triggering several mechanisms, including secretion of
cytokines (IL-10, TGF-β, and IL-35) leading to inhibition of T cell proliferation, sequestration of IL-2,
which triggers T cells’ apoptosis, as well as secretion of granzymes which exert cytotoxic effect on T
cells. Tregs can also act directly by the molecules expressed on their cell surface which interact with T
cell surface receptors leading to proliferation attenuation (Shevach, 2009).

Figure 3. The mechanisms contributing to AITD pathobiology. The mechanisms triggering the cascade
of events leading to AITD involve the interplay between environmental factors (e.g. viral infection),
epigenetic/genetic predispositions and microbiome of which dysfunction contribute to the loss of
immune tolerance, activation of autoreactive lymphocytes and inflammation, leading to the damage
of thyrocytes and clinical AITD.

Figure 4. The key mechanisms leading to the destruction of thyroid gland in HT. APC activate T CD4+
lymphocytes which triggers their differentiation into T helper cells (Th1, Th2, and Th17). Th1
lymphocytes secreting mainly IL-12, TNF-α, and INF-γ activate cytotoxic lymphocytes (Tc) and
macrophages which directly target and destroy thyroid follicular cells. The cytokines released by Th1
activate Tc, triggering apoptosis of thyrocytes induced by cytotoxins (perforin, granzymes, granulysine)
or FasL-Fas interaction. Thyroid glands of HT patients express high levels of Fas on the surface of
follicular cells. Activated Tc express FasL, which interacts with Fas on thyrocytes, triggering pro-
apoptotic signaling cascade. Th2 cells stimulate B cells leading to formation of plasma cells which
produce antibodies directed against thyroid autoantigens which bind thyroid autoantigens and induce
thyrocyte apoptosis (mediated by antibody-dependent cytotoxicity or complement activation).
Proinflammatory Th17 lymphocytes secrete IL-17 which stimulates macrophages, fibroblasts and
epithelial cells to produce cytokines triggering apoptosis of thyrocytes. The suppressive actions of
Tregs are attenuated in AITD preventing counteraction of proinflammatory Th17 activity (Wiersinga,

Figure 5. The interference between environmental and genetic factors contributing to AITD
development. A. The GD-associated C allele of CD40 rs1883832 C/T SNP affects Kozak sequence
resulting in increased translation efficiency of CD40 receptor. CD40 is expressed on immune cells (e.g.
B lymphocytes) and thyrocytes. The environmental factors (e.g. viral infection or iodine excess) lead to
the local inflammation, resulting in T cell-mediated CD40 activation, triggering cascades activating the
expression of cytokines, including IL-6. The latter acts on B cells, leading to their activation and
differentiation into plasma cells which secrete large amounts of thyroid-specific antibodies (Jacobson
et al., 2005, Huber et al., 2012). B. The GD-associated rs12101261 TSHR SNP affects the binding site of
PLZF (promyelocytic leukemia zinc finger protein) transcription factor which acts as a TSHR repressor. T allele of rs12101261 determines stronger binding of PLZF, thereby contributing to more efficient repression of TSHR transcription. IFN-α (e.g. induced by viral infection) induces enrichment of histone H3 lysine 4-monomethylated (H3K4me1) at the site of rs12101261 and augments PLZF-mediated TSHR repression. This in turn leads to decreased intrathyMIC TSHR expression which facilitates loss of immune tolerance by T lymphocytes underexposed to the thyroid autoantigen (Stefan et al., 2011). C. FLT3 encodes fms-related tyrosine kinase 3 which acts as a receptor involved in functioning of dendritic cells and responses to viral infections. The HT-linked rs76428106-C allele of FLT3 creates a cryptic splice site which introduces the premature stop codon, potentially leading to the synthesis of the receptor devoid of the kinase domain. The expression of the rs76428106-C variant is associated with >20% decrease of the wild type FLT3 and nearly two-fold increase of the FLT3 ligand. The resulting increase of the FLT3 ligand level leads to overactivation of the full-length receptor, contributing to the preferential expansion of plasmocytoid dendritic cells which in response to viral infection produce large amounts of interferon. The same study also confirmed that this variants was predisposing to other autoimmune diseases (i.e. systemic lupus erythematosus, rheumatoid-factor/anti-CCP-positive rheumatoid arthritis and coeliac disease) (Saevarsdottir et al., 2020).

Figure 6. The mechanisms by which PTPN22 polymorphism may contribute to AITD development. PTPN22 encodes Lyp (lymphoid tyrosine phosphatase) which dephosphorylates Src family of kinases. Wild type Lyp affects the functioning of B cells (left panel) and T cells (right panel) by inhibiting the signaling triggered by BCR and TCR receptors, respectively. A 1858C/T SNP leads to R620W substitution, resulting in LypR620W variant which attenuates BCR and TCR signaling more strongly than the wild type counterpart. It is proposed that dysfunction of Lyp can lead to inefficient deletion of autoreactive B cells. The wild type Lyp is recruited to plasma membrane where it interacts with Csk (C-terminal Src kinase). Following TCR stimulation, Lyp dissociates from Csk and is recruited to lipid rafts to dephosphorylate its substrates. R620W substitution impairs Csk binding, leading enhanced...
LypR620W recruitment to lipid rafts and stronger inhibition of TCR signaling. In T cells, apart from inhibition of TCR cascade, LypR620W enhances the signaling triggered by CD28, resulting in Akt/mTOR activation. These alterations lead to the attenuation of the suppressive Treg actions which cannot prevent Th1 responsiveness, leading to the increased production of IFN-\(\gamma\). On the other hand, the responsiveness of Th17 lymphocytes is reduced, as reflected by decreased IL-17 production. LypR620W mutant contributes to the enhanced adhesion, migration, and homing of T cells by augmenting the signaling induced by LFA-1 (Lymphocyte function-associated antigen–1), an integrin receptor regulating the adhesion and migration of T cells. The efficient attenuation of LFA-1 signaling is mediated by Lyp-Csk complexes. Since LypR620W does not interact with Csk, it cannot inhibit LFA-1 signaling (Vang et al., 2013, Vang et al., 2012, Vang et al., 2018, Burn et al., 2016).

**Figure 7. The mechanisms of ncRNA-mediated regulation of gene expression.** (1.) LncRNA can affect gene expression by influencing chromatin regulation or the functioning of transcription factors. (2.) LncRNA and (3.) miRNA affect mRNA translation and/or promote mRNA degradation. (4.) LncRNA can act as scaffolds recruiting and binding proteins. (5.) LncRNA can influence posttranslational protein modifications such as phosphorylation. (6.) LncRNA and (7.) circRNA act as sponges binding miRNAs and precluding their regulatory effects.

**Figure 8. The effects of dysbiosis on immune regulation and autoimmunity.** Healthy microbiota produce compounds that acts as ligands of TLR and NLR receptors expressed on immune cells, including macrophages and dendritic cells which release cytokines and metabolites such as TGF-\(\beta\), retinoic acid, interleukins. The cytokines act on target cells, including Th17 lymphocytes and Tregs, ensuring the balance between pro-inflammatory and anti-inflammatory mechanisms. Dysbiosis leads to disturbed regulation of this signaling cascade, shifting the balance towards pro-inflammatory Th17, contributing to inflammation and autoimmunity (Masetti and Ludgate, 2020, Zheng et al., 2020).
<table>
<thead>
<tr>
<th></th>
<th>Tg</th>
<th>TSHR</th>
<th>TPO</th>
<th>NIS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Molecular weight</strong></td>
<td>Homodimer of ~330 kDa monomers</td>
<td>Dimer/oligomer of ~90 kDa monomers</td>
<td>Homodimer of ~107-kDa monomers</td>
<td>Dimer/oligomer of ~90 kDa monomers</td>
</tr>
<tr>
<td><strong>Localization in thyrocyte</strong></td>
<td>Follicular lumen</td>
<td>Basolateral membrane</td>
<td>Apical membrane</td>
<td>Basolateral membrane</td>
</tr>
<tr>
<td><strong>Function</strong></td>
<td>Matrix for the sequestration of iodine and synthesis of TH</td>
<td>Major stimulator of thyroid cell growth, differentiation and function</td>
<td>Iodination and coupling of the hormonogenic tyrosines in Tg</td>
<td>Active transport of iodide</td>
</tr>
<tr>
<td><strong>Post-translational modifications</strong></td>
<td>Iodination, phosphorylation, glycosylation, carboxymethylation, sulfation, dimerization</td>
<td>Intramolecular cleavage, palmitoylation, dimerization/oligomerization</td>
<td>N-terminal trimming, heme incorporation, glycosylation, dimerization</td>
<td>Glycosylation, dimerization/oligomerization</td>
</tr>
<tr>
<td><strong>Epitope localisation</strong></td>
<td>Predominantly central region and C-terminus</td>
<td>Predominantly A-subunit</td>
<td>Predominantly MPO-like domain and to lesser extent CCP-like domain</td>
<td>Predominantly extramembranous regions</td>
</tr>
</tbody>
</table>

CCP, complement control protein; GD, Graves’ disease; HT, Hashimoto's thyroiditis; MPO, myeloperoxidase; NIS, sodium iodide symporter; Tg, thyroglobulin; TH, thyroid hormones; TPO, thyroid peroxidase; TSHR, thyrotropin receptor
Figure 1. Localization and physiological function of thyroid antigens in thyrocytes. The sodium iodide symporter (NIS) transports I⁻ and Na⁺ through the basolateral plasma membrane of a thyroid epithelial cell. The Na⁺/K⁺ ATPase pump maintains the sodium diffusion gradient required for NIS function. Pendrin participates in the apical iodide efflux into the colloid of thyroid follicle. Thyroid peroxidase (TPO) catalyzes iodination of tyrosines in thyroglobulin (Tg), which attaches one or two iodine to form moniodothyrosine (MIT) or diiodotyrosine (DIT), respectively. TPO catalyzes also coupling of iodotyrosine residues to form triiodothyronine (T₃) and thyroxine (T₄) attached to Tg, whereas the dual oxidase (DUOX) supplies hydrogen peroxide (H₂O₂) for thyroid hormones (TH) biosynthesis. Release of thyroid hormones requires engulfing colloid material (endocytosis) to form intracellular endosomes (not shown) that merge with lysosomes to form an endolysosome. TH liberated from the Tg scaffold are subsequently secreted into the blood vessels. Binding of thyrotropin (TSH; or thyroid stimulating antibody) to TSH receptor (TSHR) activates intracellular signaling by the cyclic adenosine monophosphate (cAMP) pathway leading to thyrocytes growth, differentiation, as well as production and release of TH.
Figure 2. The mechanisms of selection, differentiation and activation of T lymphocytes. A. The positive selection of T cells (called thymocytes) expressing both CD4 and CD8 molecules in thymus cortex. Random recombination of genes encoding TCR peptides in T cell precursors leads to the expression of multiple TCR variants. TCR receptors interact with antigens presented by cortical thymic epithelial cells (cTECs) in complex with MHC (major histocompatibility complex) molecules. This interaction triggers the process of lymphocyte differentiation, leading to the generation of T cells expressing either CD4 or CD8 glycoprotein receptors on their surface. B. The unwanted effect of TCR genes’ recombination is the production of receptor variants which recognize epitopes normally expressed by healthy cells (autoantigens). T cells which express autoreactive TCR variants are eliminated during the process of negative selection. The T CD4+ or T CD8+ cells resulting from positive selection migrate to the thymus medulla and interact with autopeptides complexed with MHC expressed at the surface of medullary thymic epithelial cells (mTECs) and dendritic cells (DC). This interaction triggers the process of apoptotic deletion of autoreactive T cells. Negative selection is enabled by the ability of mTECs to express peptides which are specific for different tissues of the organism (Takaba and Takayanagi, 2017, Simmonds, 2013, Pyzik et al., 2015). C. Differentiation of T CD4+ cells. Following the process of central selection, T cells which do not react with autoantigens migrate to the lymphoid organs (spleen, lymph nodes) and then spread to all peripheral tissues. Depending on the signaling triggered by various cytokines, T CD4+ cells can differentiate to various types of T helper cells.
(Th) and Tregs (Pyzik et al., 2015). D. The mechanisms regulating activation and anergy of T lymphocytes.

Activation of T lymphocytes: TCR expressed on T lymphocytes recognizes antigen complexed with MHC expressed by APC. T cell activation is triggered only in the presence of additional stimulation resulting from the interaction of CD28 and CD80/86 receptor. Anergy: In the absence of co-stimulatory signal T cell undergoes anergy. The signals inhibiting T cell activity are generated by interaction between CTLA-4 and PD-1 receptors (on T cell surface) and their respective ligands (CD80/86 and PD-L1/2), expressed by APC. Inhibition: Tregs inhibit the functioning of T CD4+ and T CD8+ cells by triggering several mechanisms, including secretion of cytokines (IL-10, TGF-β, and IL-35) leading to inhibition of T cell proliferation, sequestration of IL-2, which triggers T cells' apoptosis, as well as secretion of granzymes which exert cytotoxic effect on T cells. Tregs can also act directly by the molecules expressed on their cell surface which interact with T cell surface receptors leading to proliferation attenuation (Shevach, 2009).
Figure 3. The mechanisms contributing to AITD pathobiology. The mechanisms triggering the cascade of events leading to AITD involve the interplay between environmental factors (e.g. viral infection), epigenetic/genetic predispositions and microbiome of which dysfunction contribute to the loss of immune tolerance, activation of autoreactive lymphocytes and inflammation, leading to the damage of thyrocytes and clinical AITD.
Figure 4. The key mechanisms leading to the destruction of thyroid gland in HT. APC activate T CD4+ lymphocytes which triggers their differentiation into T helper cells (Th1, Th2, and Th17). Th1 lymphocytes secreting mainly IL-12, TNF-α, and INF-γ activate cytotoxic lymphocytes (Tc) and macrophages which directly target and destroy thyroid follicular cells. The cytokines released by Th1 activate Tc, triggering apoptosis of thyrocytes induced by cytotoxins (perforin, granzymes, granulysine) or FasL-Fas interaction.

Thyroid glands of HT patients express high levels of Fas on the surface of follicular cells. Activated Tc express FasL, which interacts with Fas on thyocytes, triggering pro-apoptotic signaling cascade. Th2 cells stimulate B cells leading to formation of plasma cells which produce antibodies directed against thyroid autoantigens which bind thyroid autoantigens and induce thyrocyte apoptosis (mediated by antibody-dependent cytotoxicity or complement activation). Proinflammatory Th17 lymphocytes secrete IL-17 which stimulates macrophages, fibroblasts and epithelial cells to produce cytokines triggering apoptosis of thyrocytes. The suppressive actions of Tregs are attenuated in AITD preventing counteraction of proinflammatory Th17 activity (Wiersinga, 2018, Frohlich and Wahl, 2017).
Figure 5. The interference between environmental and genetic factors contributing to AITD development. A. The GD-associated C allele of CD40 rs1883832 C/T SNP affects Kozak sequence resulting in increased translation efficiency of CD40 receptor. CD40 is expressed on immune cells (e.g. B lymphocytes) and thyrocytes. The environmental factors (e.g. viral infection or iodine excess) lead to the local inflammation, resulting in T cell-mediated CD40 activation, triggering cascades activating the expression of cytokines, including IL-6. The latter acts on B cells, leading to their activation and differentiation into plasma cells which secrete large amounts of thyroid-specific antibodies (Jacobson et al., 2005, Huber et al., 2012). B. The GD-associated rs12101261 TSHR SNP affects the binding site of PLZF (promyelocytic leukemia zinc finger protein) transcription factor which acts as a TSHR repressor. T allele of rs12101261 determines stronger binding of PLZF, thereby contributing to more efficient repression of TSHR transcription. IFN-α (e.g. induced by viral infection) induces enrichment of histone H3 lysine 4-monomethylated (H3K4me1) at the site of rs12101261 and augments PLZF-mediated TSHR repression. This in turn leads to decreased intrathyrmic TSHR expression which facilitates loss of immune tolerance by T lymphocytes underexposed to the thyroid autoantigen (Stefan et al., 2011). C. FLT3 encodes fms-related tyrosine kinase 3 which acts as a receptor involved in functioning of dendritic cells and responses to viral infections. The HT-linked rs76428106-C allele of FLT3 creates a cryptic splice site which introduces the premature stop codon, potentially leading to the synthesis of the receptor devoid of the kinase domain. The expression of the rs76428106-C variant is associated with >20% decrease of the wild type FLT3 and nearly two-fold increase of the FLT3 ligand. The resulting increase of the FLT3 ligand level leads to overactivation of the full-length receptor, contributing to the preferential expansion of plasmocytoid dendritic cells which in response to viral infection produce large amounts of interferon. The same study also confirmed that this variants was predisposing to other autoimmune diseases (i.e. systemic lupus erythematosus, rheumatoid-factor/anti-CCP-positive rheumatoid arthritis and coeliac disease) (Saevarsdottir et al., 2020).
Figure 6. The mechanisms by which PTPN22 polymorphism may contribute to AITD development. PTPN22 encodes Lyp (lymphoid tyrosine phosphatase) which dephosphorylates Src family of kinases. Wild type Lyp affects the functioning of B cells (left panel) and T cells (right panel) by inhibiting the signaling triggered by BCR and TCR receptors, respectively. A 1858C/T SNP leads to R620W substitution, resulting in LypR620W variant which attenuates BCR and TCR signaling more strongly than the wild type counterpart. It is proposed that dysfunction of Lyp can lead to inefficient deletion of autoreactive B cells. The wild type Lyp is recruited to plasma membrane where it interacts with Csk (C-terminal Src kinase). Following TCR stimulation, Lyp dissociates from Csk and is recruited to lipid rafts to dephosphorylate its substrates. R620W substitution impairs Csk binding, leading enhanced LypR620W recruitment to lipid rafts and stronger inhibition of TCR signaling. In T cells, apart from inhibition of TCR cascade, LypR620W enhances the signaling triggered by CD28, resulting in Akt/mTOR activation. These alterations lead to the attenuation of the suppressive Treg actions which cannot prevent Th1 responsiveness, leading to the increased production of IFN-γ. On the other hand, the responsiveness of Th17 lymphocytes is reduced, as reflected by decreased IL-17 production. LypR620W mutant contributes to the enhanced adhesion, migration, and homing of T cells by augmenting the signaling induced by LFA-1 (Lymphocyte function-associated antigen-1), an integrin receptor regulating the adhesion and migration of T cells. The efficient attenuation of LFA-1 signaling is mediated by Lyp-Csk complexes. Since LypR620W does not interact with Csk, it cannot inhibit LFA-1 signaling (Vang et al., 2013, Vang et al., 2012, Vang et al., 2018, Burn et al., 2016).
Figure 7. The mechanisms of ncRNA-mediated regulation of gene expression. (1.) LncRNA can affect gene expression by influencing chromatin regulation or the functioning of transcription factors. (2.) LncRNA and (3.) miRNA affect mRNA translation and/or promote mRNA degradation. (4.) LncRNA can act as scaffolds recruiting and binding proteins. (5.) LncRNA can influence posttranslational protein modifications such as phosphorylation. (6.) LncRNA and (7.) circRNA act as sponges binding miRNAs and precluding their regulatory effects.
Figure 8. The effects of dysbiosis on immune regulation and autoimmunity. Healthy microbiota produce compounds that acts as ligands of TLR and NLR receptors expressed on immune cells, including macrophages and dendritic cells which release cytokines and metabolites such as TGF-β, retinoic acid, interleukins. The cytokines act on target cells, including Th17 lymphocytes and Tregs, ensuring the balance between pro-inflammatory and anti-inflammatory mechanisms. Dysbiosis leads to disturbed regulation of this signaling cascade, shifting the balance towards pro-inflammatory Th17, contributing to inflammation and autoimmunity (Masetti and Ludgate, 2020, Zheng et al., 2020).
**Box 1. The possible mechanisms behind female predisposition to AITD** (Frohlich and Wahl, 2017).

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Effect on immune system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune modulatory effects of estrogens</td>
<td>Estrogens acting on specific receptors expressed in lymphocytes lead, among others, to: 1) enhanced production of antibodies and autoantibodies by B cells; 2) decrease of T CD4+ cells in thymus, and increase of extrathymic T cell lymphopoiesis, resulting in disturbed negative T cell selection; 3) enhanced expression of perforin, IL-10, and TGF-β by Tregs.</td>
</tr>
<tr>
<td>Skewed X chromosome inactivation</td>
<td>Selective expression of mother-derived or father-derived chromosome in different tissues may lead to T-cells-restricted expression of genes encoded by X chromosome derived from one parent, while other cells and organs (e.g. the thymus) may express genes encoded by the chromosome inherited from another parent. Consequently, T lymphocytes recognize peptides expressed by the other cells as antigens.</td>
</tr>
<tr>
<td>Reactivation of genes on silenced X chromosome</td>
<td>Restored functioning of genes involved in immune regulation, which are normally silenced, may lead to over-responsiveness of the immune system.</td>
</tr>
<tr>
<td>Chromosomal monosomy</td>
<td>Loss of one of the chromosomes in peripheral B and/or T cells may lead to deficiency of genes suppressing over-responsiveness of the immune system.</td>
</tr>
<tr>
<td>Fetal microchimerism</td>
<td>Fetal cells (including cytotoxic T cells) migrate to mother and trigger immune response.</td>
</tr>
</tbody>
</table>
Box 2. Key terms used in microbiome studies.

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-diversity</td>
<td>diversity of microorganisms’ composition in one sample or environment</td>
</tr>
<tr>
<td>β-diversity</td>
<td>diversity of microorganisms’ composition between two samples or environments</td>
</tr>
<tr>
<td>Dysbiosis</td>
<td>loss of microflora homeostasis due to a change in its composition and function</td>
</tr>
<tr>
<td>Faecal microbiota transplantation</td>
<td>a procedure involving the transplantation of faecal bacteria from a healthy individual to the recipient</td>
</tr>
<tr>
<td>Microbiome</td>
<td>the genome of all microorganisms living in a given environment</td>
</tr>
<tr>
<td>Microbiota</td>
<td>a group of organisms inhabiting a given environment</td>
</tr>
<tr>
<td>Microflora</td>
<td>all microorganisms inhabiting a given environment, e.g. human digestive tract</td>
</tr>
<tr>
<td>Prebiotics</td>
<td>nutrients that stimulate the growth of microorganisms</td>
</tr>
<tr>
<td>Probiotics</td>
<td>live microorganisms with a potential pro-health benefits mainly by modulating gut microbiota and stimulating immune system</td>
</tr>
<tr>
<td>Shannon index</td>
<td>index which determines the probability that two individuals (e.g. bacteria species) drawn from the same samples will belong to different species</td>
</tr>
<tr>
<td>Synbiotic</td>
<td>dietary supplement containing both probiotics and prebiotics</td>
</tr>
</tbody>
</table>
**Supplementary Table S1. Key genetic loci associated with AITD.** The table shows genes or non-coding regions at which SNPs predisposing to AITD were found. HT: Hashimoto thyroiditis, GD: Graves’ disease. *Gene locations verified by the authors of this study by accessing https://www.ncbi.nlm.nih.gov/snp/ on March 12th 2021.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>Effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABO</td>
<td>rs505922</td>
<td>Increased predisposition to GD</td>
<td>(Zhao et al., 2013, Zhang et al., 2020)</td>
</tr>
<tr>
<td>BACH2</td>
<td>rs2474619</td>
<td>Increased predisposition to HT and GD</td>
<td>(Zhang et al., 2020)</td>
</tr>
<tr>
<td>C14orf177/VRK1</td>
<td>rs1456988</td>
<td>Increased predisposition to GD</td>
<td>(Zhang et al., 2020)</td>
</tr>
<tr>
<td>C1QTNF6-RAC2</td>
<td>rs229527 and rs2284038</td>
<td>Increased predisposition to GD</td>
<td>(Zhao et al., 2013, Zhang et al., 2020)</td>
</tr>
<tr>
<td>CD40</td>
<td>rs1883832</td>
<td>Increased predisposition to GD</td>
<td>(Kurylowicz et al., 2005, Zhang et al., 2020)</td>
</tr>
<tr>
<td>CTLA4</td>
<td>rs1024161</td>
<td>Increased predisposition to GD and HT</td>
<td>(Chu et al., 2011, Zhang et al., 2020)</td>
</tr>
<tr>
<td>CTLA4</td>
<td>rs231775</td>
<td>Increased predisposition to GD and HT</td>
<td>(Chen et al., 2018, Fathima et al., 2019)</td>
</tr>
<tr>
<td>ESR2</td>
<td>rs4986938</td>
<td>Increased predisposition to GD</td>
<td>(Kisiel et al., 2008)</td>
</tr>
<tr>
<td>FAM76B/SESN3</td>
<td>rs12575636</td>
<td>Increased predisposition to HT and GD</td>
<td>(Zhang et al., 2020)</td>
</tr>
<tr>
<td>FCRL3</td>
<td>rs17676303</td>
<td>Increased predisposition to GD and GD</td>
<td>(Khong et al., 2016)</td>
</tr>
<tr>
<td>FCRL3</td>
<td>rs7528684</td>
<td>Increased predisposition to GD</td>
<td>(Zhang et al., 2020)</td>
</tr>
<tr>
<td>FOXP3</td>
<td>rs3761548 and rs3761549</td>
<td>Increased predisposition to GD (especially in Asian)</td>
<td>(Li et al., 2020)</td>
</tr>
<tr>
<td>GPR174/ITM2A</td>
<td>rs5912838</td>
<td>Increased predisposition to HT and GD</td>
<td>(Zhao et al., 2013, Zhang et al., 2020)</td>
</tr>
<tr>
<td>HLA-B</td>
<td>rs1521</td>
<td>Increased predisposition to HT and GD</td>
<td>(Zhang et al., 2020, Zhao et al., 2013)</td>
</tr>
<tr>
<td>HLA-DPB1</td>
<td>rs2281388</td>
<td>Increased predisposition to GD</td>
<td>(Zhang et al., 2020)</td>
</tr>
<tr>
<td>IL-17F</td>
<td>rs9463772</td>
<td>Increased predisposition to GD</td>
<td>(Guo et al., 2013)</td>
</tr>
<tr>
<td>IL-23A</td>
<td>rs11171806</td>
<td>Increased predisposition to GD</td>
<td>(Jia et al., 2015)</td>
</tr>
<tr>
<td>IL6</td>
<td>rs1800795</td>
<td>Increased predisposition to HT</td>
<td>(Duraes et al., 2014)</td>
</tr>
<tr>
<td>Gene</td>
<td>SNP(s)</td>
<td>Increased predisposition to HT and GD</td>
<td>Reference(s)</td>
</tr>
<tr>
<td>------------</td>
<td>-------------------------------</td>
<td>--------------------------------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>LPP</td>
<td>rs13093110</td>
<td>Increased predisposition to HT and GD</td>
<td>(Zhang et al., 2020)</td>
</tr>
<tr>
<td>PD-L1</td>
<td>rs822339, rs2282055, and rs1411262</td>
<td>Increased predisposition to GD</td>
<td>(Mitchell et al., 2009)</td>
</tr>
<tr>
<td>PTPN22/LYP</td>
<td>rs2476601</td>
<td>Increased predisposition to GD</td>
<td>(Velaga et al., 2004, Wawrusiewicz-Kurylonek et al., 2019)</td>
</tr>
<tr>
<td>RHOH/CHRNA9</td>
<td>rs6832151</td>
<td>Increased predisposition to HT and GD</td>
<td>(Zhang et al., 2020)</td>
</tr>
<tr>
<td>RNASET2</td>
<td>rs9355610</td>
<td>Increased predisposition to HT and GD</td>
<td>(Zhang et al., 2020)</td>
</tr>
<tr>
<td>SLAMF6</td>
<td>rs1265883</td>
<td>Increased predisposition to HT and GD</td>
<td>(Zhang et al., 2020, Zhao et al., 2013)</td>
</tr>
<tr>
<td>TG</td>
<td>rs2294025 and rs4736437</td>
<td>Increased predisposition to GD</td>
<td>(Chu et al., 2011, Zhao et al., 2013, Zhang et al., 2020)</td>
</tr>
<tr>
<td>TNFA</td>
<td>rs1800629, rs1800630, and rs1799964</td>
<td>Increased predisposition to GD</td>
<td>(Li et al., 2008)</td>
</tr>
<tr>
<td>TRIB2</td>
<td>rs1881145</td>
<td>Increased predisposition to HT and GD</td>
<td>(Zhang et al., 2020)</td>
</tr>
<tr>
<td>TSHR</td>
<td>rs179247 and rs12101255</td>
<td>Increased predisposition to GD</td>
<td>(Brand et al., 2009, Xiong et al., 2016)</td>
</tr>
<tr>
<td>TSHR</td>
<td>rs12101261</td>
<td>Increased predisposition to GD</td>
<td>(Brand et al., 2009, Zhang et al., 2020)</td>
</tr>
<tr>
<td>TSHR</td>
<td>rs3783938</td>
<td>Increased predisposition to GD</td>
<td>(Liu et al., 2012)</td>
</tr>
<tr>
<td>VDR</td>
<td>TT subtype of the TaqI polymorphism</td>
<td>Increased predisposition to GD</td>
<td>(Veneti et al., 2019)</td>
</tr>
</tbody>
</table>

References:


**Supplementary Table S2.** Disturbances of non-coding RNAs (ncRNA) expression and functions in autoimmune thyroid disease (AITD). AUC: area under the curve, FFPE: formalin-fixed paraffin-embedded, GD: Graves’ disease, GO: Graves’ ophthalmopathy, HHV: human herpesvirus, HT: Hashimoto’s thyroiditis, lncRNAs: long non-coding RNAs, PBMCs: peripheral blood mononuclear cells, TC: thyroid cancer, TgAb: thyroglobulin autoantibodies, Th: T helper cells, TPOAb: TPO autoantibodies, Treg: T regulatory lymphocyte, TRAb: TSHR autoantibodies, TSLP: thymic stromal lymphopoietin, PTC: papillary thyroid cancer.
<table>
<thead>
<tr>
<th>microRNAs</th>
<th>Expression</th>
<th>Samples</th>
<th>Functions/Association with clinical parameters</th>
<th>Target genes, signalling pathways</th>
<th>Disease</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-16</td>
<td>Downregulated in Treg cells while upregulated in serum of GD patients, and in T cells infected with HHV.</td>
<td>Treg cells of GD patients and healthy controls; serum from HT patients, GD patients and healthy controls; human thyroid and T cell lines infected in vitro with HHV-6A, -6B and -7.</td>
<td>SIN3A; NF-κB pathway.</td>
<td>GD, HT</td>
<td>(Wang et al., 2014, Yamada et al., 2014, Caselli et al., 2017)</td>
<td></td>
</tr>
<tr>
<td>miR-21-5p</td>
<td>Upregulated in AITD thyroid and serum samples.</td>
<td>Fresh-frozen thyroid tissues and serum samples from AITD patients and healthy controls.</td>
<td>The combination of miR-21-5p and TRAb expression distinguished GD patients from healthy controls (AUC: 0.92, sensitivity: 91.7%, specificity: 87.5%). The expression of miR-21-5p positively correlated with the level of TPOAb, TgAb and TRAb. Higher expression of miR-21-5p was associated with worse GD patients’ prognosis.</td>
<td>miR-21-5p by inhibition of Smad7 regulated the Th1/Th2 balance and stimulates Th17 differentiation.</td>
<td>HT, GD, GO</td>
<td>(Martinez-Hernandez et al., 2018, Martinez-Hernandez et al., 2019)</td>
</tr>
<tr>
<td>miR-22</td>
<td>Upregulated in thyroid tissue of GD patients as well as in serum of GD and GO patients.</td>
<td>Thyroid specimens from GD patients and healthy controls; serum samples from HT, GO and healthy controls.</td>
<td>EDA; GATM; MLLT4.</td>
<td>GD, GO, HT</td>
<td>(Qin et al., 2015, Yamada et al., 2014)</td>
<td></td>
</tr>
<tr>
<td>miR-125a</td>
<td>Downregulated in serum of GD patients; downregulated/upregulated (depending on the publication) in PBMCs of HT patients; upregulated in serum from thyroiditis mice.</td>
<td>Blood samples from GD (intractable, in remission, and uncategorized) patients, HT (severe HT, mild HT and uncategorized) patients, as well as healthy controls; PBMCs from GD patients, HT patients and healthy controls; serum from thyroiditis mice and control animals.</td>
<td>rs12976445 C/T polymorphism occurred more frequently in patients with HT than healthy controls and in intractable GD patients compared with GD patients and GD patients in remission. Downregulation of this miRNA possibly indirectly facilitates the differentiation of Th17 cells, leading to HT development and GD intractability. The expression of miR-125a-3p inversely correlated with the level of TgAb. The expression of miR-125a-5p correlated positively with the percentage of circulating Th1 cells and the level of TPOAb. Disease biomarker distinguishes between HT patients and healthy controls (AUC: 0.74, sensitivity: 72.9%, specificity: 68%).</td>
<td>RANTES; IL-6; TGF-β; IL-23R; JAK-STAT signalling pathway; MAF. Inhibition of miR-125a-5p expression reduced the proportion of Th1 cells and the expression of IFN-γ in CD4+ T cells. In thyroiditis mice overexpression of miR-125a reduced autophagy of mouse macrophage cells, increased the apoptotic rate and the expression of TNF-α, IL-1β, IL-6 and IL-18.</td>
<td>GD, HT, thyroiditis mice (Inoue et al., 2014, Peng et al., 2015b, Caselli et al., 2017)</td>
<td></td>
</tr>
<tr>
<td>miR-142-3p</td>
<td>Upregulated in serum from untreated GD patients compared with patients in remission and healthy controls, in AITD thyroid and serum samples, and in thyroid tissue</td>
<td>Serum from GD patients (untreated and in remission) and healthy controls; thyroid tissue samples from AITD (HT, GD samples.</td>
<td>The level of miR-142-3p correlated positively with TPOAb and TgAb serum level in AITD patients.</td>
<td>CLDN1; Increased expression of miR-142-3p inhibited the negative regulation of CD4+C25+ T cells proliferation by Tregs.</td>
<td>HT, GD, GO, PTC (Chen et al., 2018, Martinez-Hernandez et al., 2018, Zhu et al., 2016)</td>
<td></td>
</tr>
<tr>
<td>miR-146a</td>
<td>Downregulated/upregulated (depending on the publication) in serum/plasma of AITD patients and intractable GD patients as well as Treg cells of GD. Upregulated in: PBMCs of HT patients, GD patients and patients with mild HT compared with controls, thyroid samples of GD patients as well as of HT patients (primary HT or accompanying PTC or nodular goitre), and in microvesicles isolated from the blood of GD and HT patients.</td>
<td>Serum/plasma, Treg cells from GD patients and healthy controls; plasma and PBMCs from GD patients (intractable GD, GD in remission, and uncategorized GD), HT (severe HT, mild HT, uncategorized HT), and healthy controls; thyroid gland fine-needle aspiration biopsies from GD patients, HT patients, and healthy controls; fresh-frozen thyroid tissues from AITD patients (HT, GD without GO, GD with GO) and healthy controls.</td>
<td>The expression of miR-146b-3p positively correlated with the level of TRAb. Disease biomarkers (together with miR-210 and miR-155) distinguish between GD patients and healthy controls (AUC: 0.98, sensitivity: 91.3%, specificity: 93.8% with 92.4% diagnostic efficiency).</td>
<td>Microvesicles isolated from the blood of GD and HT patients inhibited Treg cells differentiation and the induction of Th17 cells probably through regulation of IL-8 expression via miR-146a.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-146b</td>
<td>Upregulated in AITD fresh-frozen thyroid samples, PBMCs isolated from HT patients, Fresh-frozen thyroid tissues, serum samples and PBMCs</td>
<td></td>
<td>The expression of these miRNAs negatively correlated with the</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
thyroid tissues of patients with TC and HT compared with TC or HT separately.

from AITD patients (HT, GD without GO, GD with GO), and healthy controls; tissue samples of paraneoplastic and thyroid cancers from TC patients, TC with HT patients and HT patients.

expression of ENO4, INTU, KIF27, PACR6, and ITK36 genes, associated with cilia organization.

Martinez-Hernandez et al., 2019, Li et al., 2019, Liu et al., 2020b)

miR-155

Downregulated in: serum of GD patients, CD8+ T cells from HT patients, plasma of GD patients compared with HT, thyroid samples of HT patients, and in human thyroid cells infected with HHV. Upregulated in: plasma of patients with severe HT, Treg cells of GD patients, microvesicles isolated from blood of GD and HT patients, as well as in AITD thyroid samples.

Serum and Treg cells from GD patients and healthy controls; plasma and PBMCs from GD patients (intractable, in remission, and uncategorized), HT patients (severe, mild, uncategorized) and healthy controls; thyroid gland fine-needle aspiration biopsies from GD, HT patients, and healthy controls; CD4+ and CD8+ T cells from HT and GD patients as well as healthy controls; fresh-frozen thyroid tissues and serum samples from AITD patients (HT, GD without GO, GD with GO) and healthy controls; human thyroid and T cell lines

Disturbed expression of miR-155 in serum of GD patients was linked with the extent of goitre; disease biomarkers (together with miR-210 and miR-146a) distinguish between GD patients and healthy controls (AUC: 0.98, sensitivity: 91.3%, specificity: 93.8% with 92.4% diagnostic efficiency); HHV-6A infection leads to disturbances in the expression of AITD-associated miRNAs and, in consequence might stimulate AITD development.

Downregulation of this miRNA in CD8+ T cells might lead to pathological identification of thyroid-specific antigens by these cells. Microvesicles isolated from the blood of GD and HT patients inhibited Treg cells differentiation and the induction of Th17 cells probably through regulation of SMAD4 expression via miR-155.

GD, HT, GO

<table>
<thead>
<tr>
<th>miR-200a</th>
<th>Upregulated in thyroid samples of HT patients; downregulated in CD4+ and CD8+ T cells from HT and GD patients.</th>
<th>Thyroid gland fine-needle aspiration biopsies, PBMCs as well as CD4+ and CD8+ T cells from GD, HT patients and healthy controls.</th>
<th>Downregulation of these miRNAs in CD8+ T cells of HT patients possibly results in a more significant production of proinflammatory Th1 cytokines and destroy thyroid cells. In contrast, a decrease of their expression in CD8+ T cells might contribute to improper recognition of thyroid-specific antigens and AITDs development.</th>
<th>GD, HT</th>
<th>(Bernecker et al., 2014, Bernecker et al., 2012)</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-326</td>
<td>Upregulated in PBMCs from HT patients as well as in thyroid tissue samples from mice with autoimmune thyroiditis.</td>
<td>PBMCs from HT patients and healthy controls; thyroid samples and Th17 cells from mice with autoimmune thyroiditis and healthy animals.</td>
<td>The expression of miR-326 positively correlated with the serum level of TgAb and TPOAb of HT patients.</td>
<td>miR-326 by regulation of ADAM17 expression affected IL-23/IL-23R/Th17 pathway, which leads to stimulation of Th17 cells differentiation. miR-326 by inhibition of ETS-1 expression increases the level of Th17 cells.</td>
<td>HT, thyroiditis mice</td>
</tr>
<tr>
<td>miR-375</td>
<td>Upregulated in serum and plasma of GD and HT patients.</td>
<td>Serum from HT patients, GD patients and healthy controls; plasma samples from HT patients and healthy controls.</td>
<td>Disease biomarker (together with miR-205, miR-20a-3p, miR-296, miR-451, and miR-500a) distinguish between HT patients and healthy controls (AUC: 0.75, sensitivity: 0.75, specificity: 0.66). The expression of miR-375a correlated with lower Regulates TSLP expression, playing key role in the stimulation of CD4+ T cells differentiation into Th2 and Th17 cells.</td>
<td>GD, HT</td>
<td>(Yamada et al., 2014, Zhao et al., 2018)</td>
</tr>
<tr>
<td>miR-431*</td>
<td>Downregulated in PBMCs from initial (untreated) GD patients compared with GD in remission and healthy controls.</td>
<td>PBMCs from GD (initial and in remission) patients and healthy controls; CD4+ and CXCR5+ T cells from PBMCs of GD patients and healthy controls.</td>
<td>Treatment of PBMCs delivered from healthy subjects with T3 leads to downregulation of these miRNA.</td>
<td>GD</td>
<td>(Liu et al., 2012, Chen et al., 2015)</td>
</tr>
<tr>
<td>miR-451</td>
<td>Upregulated in serum and plasma of GD and HT patients, and in T cells infected with HHV.</td>
<td>Serum from HT, GD patients and healthy controls; plasma samples from HT patients and healthy controls; human thyroid and T cell lines infected in vitro with HHV-6A, -6B and -7.</td>
<td>Disease biomarkers (together with miR-205, miR-20a-3p, miR-296, miR-500a) distinguish between HT patients and healthy controls (AUC: 0.75, sensitivity: 0.75, specificity: 0.66). The expression of miR-451 correlated with higher TSH level.</td>
<td>Cell death pathway</td>
<td>GD, HT</td>
</tr>
<tr>
<td>n335641; TCONS-00022357-XLOC-010919</td>
<td>Upregulated in PBMCs from blood of GD patients.</td>
<td>PBMCs from GD patients and healthy controls.</td>
<td>These lncRNAs via regulation of TCL1A and SH2D1A expression might regulate B cells proliferation and survival and thus participate in GD development.</td>
<td>TCL1A</td>
<td>GD</td>
</tr>
<tr>
<td>n337845</td>
<td>Downregulated in PBMCs from blood of GD patients.</td>
<td>PBMCs from GD patients and healthy controls.</td>
<td>The expression of HMlincRNA1474 correlated positively with JUNB expression while the level of AK021954 and AB075506 with NRCAM expression.</td>
<td>SH2D1A</td>
<td>GD</td>
</tr>
<tr>
<td>AB075506; AL832122; AK055670; AF318328; AK021954</td>
<td>Upregulated in the CD4+ T cells of initial GD patients.</td>
<td>PBMCs from GD patients (untreated, euthyroid as well as in remission) and healthy controls.</td>
<td>The expression of HMlincRNA1474 correlated positively with JUNB expression while the level of AK021954 and AB075506 with NRCAM expression.</td>
<td></td>
<td>GD</td>
</tr>
<tr>
<td>HMlincRNA1474; TCONS-00012608;</td>
<td>Downregulated in the CD4+ T cells of initial GD patients.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table continued...
<table>
<thead>
<tr>
<th>Gene/Genomic Region</th>
<th>Expression Status</th>
<th>Samples</th>
<th>Association/Pathway Details</th>
<th>HT Disease Biomarkers</th>
</tr>
</thead>
<tbody>
<tr>
<td>AK126108</td>
<td></td>
<td></td>
<td>TRAb. Additionally, the expression of AK021954 and AB075506 correlated negatively with TSH level. Moreover, the level of TCONS-00012608 was negatively associated with the level of FT3, FT4 and TRAb. Diagnostic biomarkers distinguish between GD patients and healthy controls: AUC for AK021954: 0.81, for AB075506: 0.76, and for HMlincRNA1474: 0.81.</td>
<td>(Peng et al., 2015a)</td>
</tr>
<tr>
<td>IFNG-AS1</td>
<td>Upregulated in PBMCs and thyroid tissue samples from HT patients.</td>
<td>Blood and thyroid tissue samples from HT patients and healthy controls.</td>
<td>The expression of IFNG-AS1 correlated positively with the percentage of Th1 cells and the level of TgAb as well as TPOAb. Downregulation of IFNG-AS1 expression resulted in a decline of percentage of IFN-γ+ cells.</td>
<td>(Peng et al., 2015a)</td>
</tr>
<tr>
<td>XLOC_I2_006631; LOC729737</td>
<td>Upregulated in PBMCs from HT patients.</td>
<td>PBMCs from HT patients and healthy controls.</td>
<td>The expression of XLOC_I2_006631 correlated positively with TPOAb level. Disease biomarkers distinguish between HT patients and healthy controls. (XLOC_I2_006631: AUC: 0.85, sensitivity: 88.9%, specificity: 75%; LOC729737: AUC: 0.83, sensitivity: 74.1%,</td>
<td>(Peng et al., 2020)</td>
</tr>
<tr>
<td>circRNAs</td>
<td>Blood samples from HT patients and healthy controls.</td>
<td>The expression of circ_0089172 correlated positively with the serum level of TPOAb. Disease biomarkers distinguish between HT patients and healthy controls (AUC for circ_0089172: 0.67, circ_0012152: 0.70, and circ_0000075: 0.72).</td>
<td>IL-23R; circ_0089172 play the role of miR-125a-3p sponge. Downregulation of circ_0089172 expression leads to overexpression of miR-125a-3p and decline in IL-23R expression.</td>
<td>HT</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>----------------------------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>BC041964</td>
<td>Downregulated in PBMCs from HT patients.</td>
<td>specificity: 89.3%).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BC041964</td>
<td>Downregulated in PBMCs from HT patients.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>circ_0089172;</td>
<td>Upregulated in PBMCs of HT patients.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>circ_0000075</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>circ_0007777;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>circ_0012152</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BC041964</td>
<td>Downregulated in PBMCs from HT patients.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

References:


BERNECKER, C., LENZ, L., OSTAPCZUK, M. S., SCHINNER, S., WILLENBERG, H., EHLERS, M., VORDENBAEUMEN, S., FELDKAMP, J. & SCHOTT, M. 2012. MicroRNAs miR-146a1, miR-155_2, and miR-200a1 are regulated in autoimmune thyroid diseases. Thyroid, 22, 1294-5.


Copyright © 2021 the authors


Copyright © 2021 the authors


Supplementary Table S3. The changes in composition of gut microbiota in AITD patients. Regarding GD, most studies report higher proportion of Bacteroidetes and lower of Firmicutes (at the phyla level) whereas higher number of *Bacteroides*, Lactobacillus and *Prevotella* (at the genus level) (Ishaq et al., 2018, Jiang et al., 2021). The abundance of genera such as *Blautia*, *Eubacterium helii* group, *Lactobacillus* and *Dorea* distinguished GD patients from healthy controls, suggestive of the potential diagnostic significance. The number of *Blautia* correlated positively, while *Dorea* correlated negatively with serum level of TPOAb, indicating clinical significance of these findings (Jiang et al., 2021). Similarly, the abundance of Proteobacteria, Tenericutes and Synergistetes correlated negatively with FT3, FT4, TPOAb and TRAb levels in GD patients (Su et al., 2020). These changes may have biological significance, since Ishaq et al. showed that supernatant of medium used for culturing of *B. fragilis* (of which proportion is reduced in GD patients) increased the percentage of CD4+ CD25+ FOXP3+ Treg cells and the level of IL-10, while suppressed the percentage of CD4+ IL17+ Th17 cells and the level of IL-17A in PBMCs from healthy controls (Su et al., 2020). The reciprocal links between thyroid function and microbiome are further supported by studies GD patients treated with antithyroid drugs, PTU or methimazole (MMI), which significantly changed the structure of gut microbiota (Sun et al., 2020). In HT patients, key microbiota alterations include increased proportion of gut Firmicutes and reduced number of Bacteroidetes. At the genus level, the abundance of *Faecalibacterium* and *Prevotella* was increased, whereas the number of *Lechnospiraceae*, *Fusicatenibacter* and additional 13 genera were associated with TPOAb level (Zhao et al., 2018), while the proportion of *Clostridium coccoides* correlated with TSH level and the time of HT disease duration. The composition of the intestinal microflora also correlates with the clinical parameters of HT patients and response to LT4 treatments. Specifically, the number of *Lechnospiraceae*, *Fusicatenibacter* and additional 13 genera were associated with TPOAb level (Zhao et al., 2018), while the proportion of *Clostridium coccoides* correlated with TSH level and the time of HT disease duration. The abundance of *Bacteroides*, *Faecalibacterium*, *Prevotella* and additional 7 genera was suggested as a promising biomarker distinguishing HT patients from healthy controls (Zhao et al., 2018).

<table>
<thead>
<tr>
<th>Composition of gut microbiota.</th>
<th>Samples; population.</th>
<th>Association between microbiota and clinical data.</th>
<th>Disease</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial richness: (SOBs, ACE and Chao1 indexes) reduced in GD patients.</td>
<td>Faecal and blood samples from GD (GD with HT, GD without HT) untreated patients as well as from GD patients (HG – group before treatment; TG-group after treatment with methimazole for 3-5 months) and healthy controls.</td>
<td>Analysis of abundance of Bacteroides, <em>Blautia</em>, <em>Eubacterium helii</em> group, <em>Anaerostipes</em>, <em>Lactobacillus</em>, <em>Dorea</em>, Peptostreptococcaceae, <em>Collinsella</em> and <em>Ruminococcus_torques</em> group might serve as diagnostic biomarkers distinguish GD patients from healthy controls (AUC: 0.8109). The number of <em>Blautia</em> correlated positively while <em>Bacteroides</em> negatively with the level of TPOAb and TRAb. Moreover, <em>Dorea</em> negatively correlated with the level of TPOAb. The number of Synergistetes correlated negatively with the level of TRAb, TGAb and TPOAb, while the number of <em>Lactobacillus</em> positively with the level of TRAb and TPOAb. The level of TRAb correlated negatively also with the number of <em>Phascolarctobacterium</em>. After treatment of GD patients the relative abundance of</td>
<td>GD</td>
<td>(Jiang et al., 2021, Ishaq et al., 2018, Chen et al., 2021, Su et al., 2020, Yan et al., 2020)</td>
</tr>
<tr>
<td>Bacterial α diversity: Shannon index reduced in GD.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial β diversity: (PLS-DA; Bray-Curtis index): Significant differences between microbiome of GD and healthy controls.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phyla level: Nitrospinae and Spirochaetes are unique for GD patients. Higher abundance of Bacteroidetes and Saccharibacteria while lower of Firmicutes, Synergistetes, Proteobacteria, Verrucomicrobiota, and Tenericutes.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genus level: <em>Negativicoccus</em>, <em>Bacillus</em>, <em>Succiniclastricum</em>, <em>Campylobacterales</em> and <em>Gastranaerophilales</em> are unique for GD.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>Bacterial richness: (ACE and Chao1 indexes)</td>
<td>Faecal samples form GD patients without GO, GO patients and healthy controls. Chinese population.</td>
<td>Bacterial richness: (ACE and Chao1 indexes)</td>
<td>Faecal samples from patients with severe and active GO and healthy controls. Chinese population.</td>
</tr>
<tr>
<td>----------</td>
<td>--------------------------------------------</td>
<td>----------------------------------------------------------------------------------</td>
<td>--------------------------------------------</td>
<td>----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Higher proportion of <em>Bacteroides, Lactobacillus, Prevotella, Veillonella</em> and <em>Phascolarctobacterium</em>, while lower of <em>Blautia, Anaerostipes, Collinella, Dorea, Alistipes, Ruminococcus, and Faecalibacterium</em> in GD. Higher proportion of <em>Blautia, Streptococcus, and Ruminococcus</em>, while lower of <em>Phascolarctobacterium</em> and <em>Lachnospira</em> in TG vs HG.</td>
<td><em>Ruminococcus</em> correlated positively, while <em>Phascolarctobacterium</em> negatively with the level of TRAb. The number of Proteobacteria, Tenericutes, Verrucomicrobia and Synergistetes correlated negatively with the serum levels of FT3, FT4, TPOAb and TRAb, while positively with TSH level. Contrary, Bacteroidetes and Saccharibacteria correlated positively with the level of FT3, FT4, TPOAb and TRAb, while negatively with the level of TSH.</td>
<td>Bacterial richness: (ACE and Chao1 indexes)</td>
<td>No differences.</td>
<td>Bacterial α diversity: Shannon index reduced in GD and GO patients. Bacterial β diversity: (PCoA) Significant differences between GD patients and healthy controls. Phyla level: Deinococcus-Thermus Chloroflexi decreased in GO compared with GD. Genus level: <em>Subdoligranulum</em> and <em>Bilophila</em> augmented while <em>Blautia, Anaerostipes, Dorea, Butyricicoccus, Romboutsia, Fusicatenibacter, Collinella, Intestinibacter</em>, and <em>Phascolarclobacterium</em> decreased in GO patients compared with GD.</td>
</tr>
</tbody>
</table>
**Phyla level:** Bacteroidetes are more enriched in AITD vs healthy controls. Firmicutes, Proteobacteria, and Actinobacteria are reduced in GO patients compared with healthy controls.  
**Genus level:** Higher proportion of *Prevotellaceae* while lower of *Blautia, Fusicatenibacter, Butyricicoccus, Anaerostipes*, and *Collinsella* in GO patients compared with healthy controls.

**Bacterial α diversity:**  
No differences or lower level (depending on publication) of Shannon index in AITD vs healthy controls, no differences between HT and GD.  
**Bacterial β diversity:** (PCoA) Significant differences in microbiome composition between GD and HT vs healthy controls.  
**Genus level:**  
*Fusobacterium* was more enriched while *Faecalibacterium* reduced in GD compared with HT and controls. Higher abundance of *Sutterella* in GD compared with controls. Decreased number of *Rikenellaceae* in GD compared with HT.

<table>
<thead>
<tr>
<th>Bacterial α diversity: Reduced Shannon index in HT vs controls, and in hypothyroid group vs euthyroid.</th>
<th>Faecal and blood samples from HT patients (euthyroid and hypothyroid) and healthy controls. Chinese and Brazil populations.</th>
<th>The number of <em>Lechnospiraceae, Fusicatenibacter, Anaerostipes, Eubacterium hallii_group, Blautia</em> and additional 10 genera correlated positively with the level of TPOAb while the number of <em>Prevotella_9, Bacteroides, Phascolactobacterium</em> and <em>Paraprevotella</em> negatively with the levels of these antibodies. Moreover, the number of <em>Fusicatenibacter</em> correlated negatively while <em>Alloprevotella</em> positively with the level of FT4, and with <em>Romboutsia</em> correlated negatively with the level of TSH. The analysis of <em>Bacteroides, Faecalibacterium, Prevotella_9, Blautia,</em></th>
<th>HT</th>
<th>(Liu et al., 2020, Zhao et al., 2018, Cayres et al., 2021)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phyla level:</strong></td>
<td>Faecal and blood samples from GD, HT patients and healthy controls. Spain and Egyptian populations.</td>
<td>The number of Alistipes, Ruminococcaceae and Enterobacteriaceae correlated positively while <em>Faecalibacterium</em> negatively with the level of TPOAb. Moreover, the number of <em>Lactobacillaceae, Lactobacillus</em> and <em>Pasteurellaceae</em> correlated positively while <em>Paecalibacterium</em> negatively with the level of TSIAb (thyroid stimulating immunoglobulin antibody). The abundance of Bacteroidetes and Firmicutes correlated positively with the level of TRAb, while Bacteroidetes, Firmicutes and Prevotella correlated positively with TPOAb level.</td>
<td>GD, HT</td>
<td>(Cornejo-Pareja et al., 2020, El-Zawawy et al., 2021)</td>
</tr>
</tbody>
</table>
enriched Lechnospiraceae_incertae_sedis, Lactorifactor, Alistipes, and Subdoligranulum in euthyroid group vs controls. More enriched Phascolarctobacterium in hypothyroid group vs controls. Higher proportion of Blautia, Roseburia, Ruminococcus_torques_group, Romboutia, Dorea, and Fusicatenibacter while lower of Faecalibacterium, Bacteroides, Prevotella_9, and Lachnoclostridium in HT vs controls.

Eubacterium_hallii_group, Ruminococcus_torques_group, Streptococcus, Alloprevotella, Roseburia and Fusicatenibacter abundance distinguish HT patients from healthy controls (AUC:0.88).

The REUs of Clostridium_coccoides and Clostridium_coccoides-Eubacteria_rectale correlated positively with TSH level and with the time of HT disease duration. Moreover, the REUs of Roseburia species correlated negatively with FT4 levels. Additionally, the REUs of Lactobacillus differed between patients treated with LT4 and untreated.

References:


