Hashimoto's Thyroiditis

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Abstract

Hashimoto's thyroiditis is best defined as an organ-specific autoimmune disease, characterized by autoimmune-mediated destruction of the thyroid gland. Diagnostic criteria have changed dramatically since the first description in 1912; they now include the presence of antibodies against thyroid peroxidase (TPOAb) and thyroglobulin, hypoechogenicity on thyroid ultrasound, and often but not always hypothyroidism. Distinct pathologic phenotypes are recognized: goitrous and atrophic variants but also an IgG4-related variant, hashitoxicosis, juvenile thyroiditis, and

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silent or painless thyroiditis. With a prevalence of 10–12% in the general population, it is the most common autoimmune disease. The prevalence is higher in females than in males, increases with advancing age, and is highest in Whites and lowest in Blacks. The incidence of autoimmune hypothyroidism is about 350 cases/100,000/year for women and 60 cases/100,000/year for men in iodine-sufficient regions and 44 (females) and 12 (males) per 100,000 per year in iodine-deficient areas. Breakdown of self-tolerance against thyroid antigens may lead to thyroid autoimmunity. Loss of T_{reg} inhibitory actions and gain of Th17 proinflammatory actions (reflected by a shift to higher values of Th17/Th10 ratio in peripheral blood) play a crucial role in the loss of tolerance against thyroid antigens. Cytotoxic CD8+ T cells directed against TPO and Tg mediate thyroid gland destruction, either by the granule exocytosis pathway or apoptosis (programmed cell death). TPOAb and TgAb may cause antibody-dependent cell-mediated cytotoxicity (ADCC) via complement-mediated lysis of thyrocytes. Hashimoto's thyroiditis often runs in families as evident from a high sibling risk ratio of 28. Twin studies suggest genes contribute about 73% of the liability to the development of TPOAb and TgAb; environmental factors would thus contribute about 20–30%. Polymorphisms in *TSHR*, *Tg*, *HLA*, *CTLA-4*, *IL2RA*, and *FOXP3* have all been associated with Hashimoto's thyroiditis but account for only a small proportion of the heritability. Genome-wide association studies continue to detect novel genetic loci linked to TPOAb. Smoking and moderate alcohol consumption to a certain extent protect against Hashimoto's thyroiditis. Low selenium or vitamin D intake are presumably related to a higher prevalence of TPOAb, but presently there is no convincing evidence that selenium or vitamin D supplementation may lower TPOAb concentration. Infections may provoke Hashimoto's thyroiditis, but available epidemiological studies do not support a causative role.

Keywords
Hashimoto's thyroiditis · History · Diagnosis · Epidemiology · Immunopathogenesis · IgG4 · Genetic polymorphisms · Environment

Introduction

Autoimmune thyroid disease (AITD) can be defined as a complex disease characterized by an autoimmune response against thyroid antigens, which may develop against a certain genetic background and facilitated by exposure to particular environmental factors. Hashimoto's disease and Graves' disease are well-known examples of AITD: Hashimoto's disease is associated with antibodies against thyroid peroxidase (TPOAb) and hypothyroidism, whereas Graves' disease is associated with antibodies against thyroid-stimulating hormone receptor (stimulating TSHRAb) and hyperthyroidism (Aijan and Weetman 2015; Smith and Hegedus 2016). Thus, at first sight, there is a clear distinction between Hashimoto's and Graves' disease, TPOAb being the hallmark of Hashimoto's disease and TSHRAb being the hallmark of Graves' disease. The neat

dichotomy, however, is deceptive. TPOAb are also present in 70% of patients with Graves' disease, and hypothyroidism occurs in the long run in up to 20% of patients with Graves' hyperthyroidism who have entered remission after a course of antithyroid drugs; blocking TSHRAb contributed to the late development of hypothyroidism in one third of cases and chronic autoimmune thyroiditis in the remaining two thirds (Wood and Ingbar 1979; Hedley et al. 1989; Tamai et al. 1989). Conversely, some patients with Hashimoto's disease may have either stimulating or blocking TSHRAb (Konishi et al. 1983), and cases have been described in which Hashimoto's hypothyroidism converts into overt Graves' hyperthyroidism likely explained by a change from blocking to stimulating TSHRAb (Bell et al. 1985; McLachlan and Rapoport 2013). One may consider Hashimoto's hypothyroidism and Graves' hyperthyroidism as the extreme ends of a continuous spectrum of thyroid autoimmunity (Prummel and Wiersinga 2002; Mariotti 2012). It is tempting to speculate that the natural history of autoimmune thyroid disease is in principle that of Hashimoto's thyroiditis characterized by the occurrence of TPOAb and TgAb, with the slow development over years of subclinical and finally overt autoimmune hypothyroidism in some but not all patients (Effraimidis et al. 2011a). Development of Graves' hyperthyroidism in contrast is rather fast, happening in a few months; its occurrence might be considered the default of AITD, which usually follows the course of Hashimoto's thyroiditis. Hashimoto's thyroiditis can probably best be defined as an organ-specific autoimmune disease, characterized by autoimmune-mediated destruction of the thyroid gland. The diagnosis is nowadays suggested by a typical ultrasound pattern and the presence of TPOAb and/or TgAb (Radetti 2014). Indeed thyroid hypoechogenicity, among patients with goiter and circulating thyroid antibodies, identifies those with Hashimoto's thyroiditis who are prone to develop hypothyroidism (Fig. 1; Marcocci et al. 1991). Such characteristics are

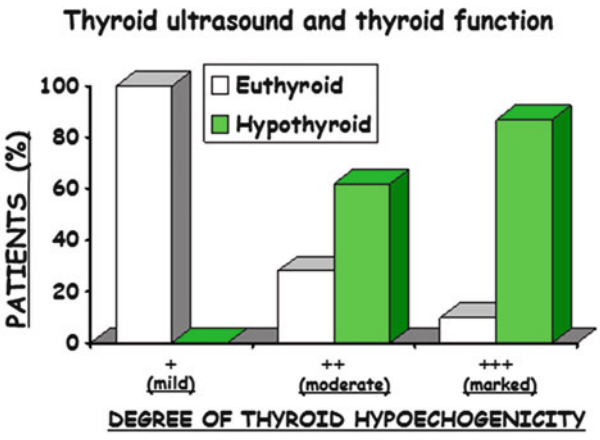

Fig. 1 Prevalence of hypothyroidism according to the degree of thyroid hypoechogenicity in patients with Hashimoto's thyroiditis and diffuse low thyroid echogenicity (Modified from Marcocci et al. 1991)

a world apart from the original description by Hashimoto himself, which mentions goitrous enlargement of the thyroid gland with lymphocytic infiltration ("struma lymphomatosa") (Hashimoto 1912). At the time of Hashimoto's paper, the very existence of thyroid antibodies was unknown (they were detected only in 1956 by Roitt et al.) (Roitt et al. 1956), and thyroid ultrasonography was gradually introduced in clinical practice not earlier than in the 1980s. The concept of Hashimoto's thyroiditis as a particular disease entity thus has evolved enormously over the past century. We will investigate the accuracy of the various diagnostic criteria for Hashimoto's thyroiditis. Applied criteria are from the disciplines of pathology (lymphocytic infiltrate?), clinical medicine (goiter?), immunology (thyroid antibodies?), radiology (thyroid hypoechogenicity?), and biochemistry (abnormal thyroid function?).

Diagnostic Criteria

Histopathology

Hashimoto's thyroiditis is typically a diffuse inflammation of the thyroid gland consisting of a combination of epithelial cell destruction, lymphoid cellular infiltration, and fibrosis. The thyroid cells are packed with mitochondria and have an acidophilic character; they are called Hűrthle or Askenazy cells. Clusters of macrophage-like cells can be seen within the follicles. The lymphoid infiltration in the interstitial tissue is accompanied by actual lymphoid follicles and germinal centers (Ben-Skowronek et al. 2011).

In the early 1960s, the consistency of the histopathological diagnosis of Hashimoto's disease was studied among pathologists (Masi et al. 1965). At that time uncertainty existed about the proper use of the terms Hashimoto's disease versus chronic thyroiditis: were the lesions designated by each term distinguishable from each other and were there specific criteria for each diagnosis? Randomly selected surgical specimens which had been indexed in the pathology department of the Johns Hopkins Hospital as either Hashimoto's disease, chronic thyroiditis, or other fibro-lymphocytic disorders were investigated in a blinded fashion by eight experts. They had to mark as absent, minimal, moderate, or severe each of the following histopathologic characteristics present in the original description of Hashimoto: lymphoid infiltration, lymphoid follicle formation, plasma cell infiltration, Hűrthle cell dysplasia, epithelial hyperplasia, epithelial desquamation, follicular atrophy, colloid nodules, and scarring and then make a diagnosis: Hashimoto's disease, chronic thyroiditis, or other disease. There was considerable difference between observers in their use of the terms Hashimoto's disease and chronic thyroiditis: both diagnoses were frequently made on the same slides by different observers. If, however, the diagnoses Hashimoto's disease and chronic thyroiditis were combined, the interobserver agreement increased appreciably to 85%. Hashimoto's disease was diagnosed preferentially on the more fully developed lesions showing marked alterations in all fields, whereas slides diagnosed as chronic thyroiditis tended to show focal alterations. The alterations were indistinguishable from each other under high-power magnification. The authors concluded that both terms indicated the same

disease entity, which they preferred to call Hashimoto's disease (Masi et al. 1965). But the label chronic lymphocytic thyroiditis seemed equally acceptable. The issue of focal thyroiditis has been given much consideration. Early studies recognized its frequent occurrence in macroscopically normal thyroids at necropsy, chiefly in middle-aged and elderly women; it was regarded as a precursor or a nonprogressive form of the diffuse chronic thyroiditis associated with primary myxedema (Williams and Doniach 1962). A quantitative study on the postmortem incidence of focal thyroiditis was done in all thyroids sectioned over 5 years (1948–1953) at the Hammersmith Hospital in London, UK. Thyroid sections were available in 40% of all necropsies; availability depended more on the person carrying out the examination than on the nature of the disease, and therefore it was thought the observed trends in sex and age distribution were generally reliable. The sole criterion applied for the existence of focal thyroiditis was the presence of a collection of lymphocytes or plasma cells easily visible with a low-power objective in 6-μ-thick sections. The presence of follicle breakdown or oxyphil cell change was not considered a prerequisite for the diagnosis of focal thyroiditis, although follicle breakdown was almost invariably and oxyphil change frequently present in those cases with more than 10 foci per square cm. Sections from 724 thyroids were examined. Focal thyroiditis (>10 foci/cm^2) was present in 6% of adult males and 22% of adult females, with a higher incidence in older women (Williams and Doniach 1962). An increase in the incidence and severity of focal thyroiditis was found in the presence of the autoimmune disease pernicious anemia, suggesting that focal thyroiditis points to weakening of immune tolerance. Whether all cases of focal thyroiditis belong to the disease entity of Hashimoto's disease is uncertain. On the one hand, the presence of focal thyroiditis in thyroid tissue obtained by biopsy and at autopsy from patients with no evidence of hypothyroidism during life is correlated to raised serum concentrations of TPOAb and TgAb (Vanderpump 2011). On the other hand, focal thyroiditis is not uncommon, and practically all thyrotoxic glands removed at operation show small lymphocytic foci. Does this mean that the thyroid is limited in its pattern of reaction and that several distinct processes lead to the same morphological end result? Such questions are still not answered in a satisfactory manner.

To celebrate the centennial of Hashimoto's original paper in 2012, the surgical pathology archives of the Johns Hopkins Hospital were searched for cases of Hashimoto's thyroiditis in the period 1889–2012 (Caturegli et al. 2013). Cases with lymphocytic infiltration of the thyroid gland with germinal center formation and Hűrthle cell metaplasia were accepted as Hashimoto's thyroiditis. However, the presence of a simple lymphocytic infiltration was not considered indicative of Hashimoto's thyroiditis; such lesions of focal nature without germinal centers and lack of Hűrthle cells are often referred to as chronic nonspecific thyroiditis. The first case labeled as Hashimoto appeared in 1942, 30 years after the original description. Prior to this date, there were three cases satisfying the diagnostic criteria (in 1928, 1935, and 1939). Between 1942 and 2012, a total of 867 cases of Hashimoto's thyroiditis were identified, 6% of all thyroid surgical specimens. Approximately half of the cases ($n = 462$) were isolated, meaning Hashimoto's thyroiditis was the sole pathologic finding. The remaining half ($n = 405$) were those in which Hashimoto's thyroiditis was found incidentally in association with other pathologies, mainly with

papillary thyroid carcinoma (but also with Hűrthle cell carcinoma and medullary thyroid carcinoma). Papillary thyroid carcinoma was found in 231 of the 867 cases (26.6%) of Hashimoto's thyroiditis (Caturegli et al. 2013). This prevalence is similar to that reported from Krakow (106 papillary cancers out of 452 cases of Hashimoto's thyroiditis, 23.5%); interestingly, this prevalence of 23.5% is threefold higher than the 7.5% prevalence of papillary thyroid carcinoma in non-Hashimoto's thyroiditis patients (Konturek et al. 2013). The number of annual Hashimoto cases at Johns Hopkins increased significantly over the period 1943–1967 and then remained constant between 1968 and 1992, before increasing again between 1993 and 2012 (Caturegli et al. 2013). The main contributor to the recent increase in incidence was Hashimoto's thyroiditis associated with papillary thyroid carcinoma. The annual increase in papillary thyroid carcinoma was accompanied not only by increases in cases associated with Hashimoto's thyroiditis but also by increases in cases associated with less prominent lymphocytic infiltration sometimes referred to as chronic nonspecific thyroiditis. The overall conclusion is that modern-day pathologic features of Hashimoto's thyroiditis are nearly identical to the ones originally reported by Hashimoto in 1912 (Caturegli et al. 2013).

Thyroid Antibodies

After the 1956 discovery of thyroid antibodies in the serum of patients with Hashimoto's disease (Roitt et al. 1956), measurement of TPOAb (originally known as thyroid microsomal antibodies) and TgAb gradually entered daily clinical practice. Consequently, as of the 1970s the presence of TPOAb and/or TgAb in serum was considered as positive proof for the existence of Hashimoto's thyroiditis. The original assays (e.g., applied in the Whickham Survey in 1972) used a semi-quantitative particle agglutination technique. But improved technology employing purified antigens and monoclonal antibodies led to quantitative immunoassays with higher sensitivity and specificity in the next decades. It resulted in assays capable to detect TPOAb and TgAb in the serum of every single healthy person (Zophel et al. 2003). This generated the quest for reliable reference intervals, as only elevated concentrations of thyroid antibodies in serum would permit the diagnosis of thyroid autoimmunity. The upper normal limit can be determined in various ways:

1. By a traditional nonparametric scale
2. By NACB (National Academy of Clinical Biochemistry) criteria, i.e., restricting blood sampling to males younger than 30 years without risk factors for thyroid autoimmunity and with TSH values between 0.5 and 2.5 mU/L (Demers and Spencer 2003)
3. By a model of "composite logarithmic Gaussian distributions" (Jensen et al. 2006)

The 97.5% upper limits (using an immunometric assay with detection limits of $<$1.0 kU/L for both TPOAb and TgAb) according to these three models were

284, 24, and 9.8 kU/L for TPOAb, respectively, and 84, 22, and 19 lU/L for TgAb. The decision value (defined as the concentration corresponding to 0.1% false positives) was 15 kU/L for TPOAb and 31 kU/L for TgAb (Jensen et al. 2006). For meaningful interpretation of results, the assay methodology and especially its upper normal limit should be known. Patients with hypothyroidism caused by either atrophic or goitrous autoimmune thyroiditis have rather high serum concentrations of TPOAb and TgAb, but these antibodies are also present – albeit usually at lower concentrations – in 70% of patients with Graves' hyperthyroidism. Serum TPOAb may not be detected in about 10% of individuals with ultrasound evidence of Hashimoto's thyroiditis (Biondi and Cooper 2008). Cases of seronegative Hashimoto's thyroiditis have been described in which thyroid autoantibody production was localized to the thyroid (Baker et al. 1988). Nevertheless, serum TPOAb and TgAb remain sensitive and specific markers for thyroid autoimmunity. Interestingly, antibodies against the TSHR have also been described in Hashimoto's thyroiditis. Blocking TSHRAb were detected already in the 1980s (Konishi et al. 1983; Endo et al. 1978), but their role in various conditions remained controversial. With the advent of more accurate assays for blocking and stimulating TSHRAb, it has become clear that patients can exhibit a mixture of both blocking and stimulating TSHRAb, the ratio of which may vary over time and influence the clinical presentation (Evans et al. 2010; Li et al. 2013a; Diana et al. 2016). Stimulating TSHRAb are present in all patients with Graves' hyperthyroidism with or without Graves' orbitopathy but also in 5.5% of patients with Hashimoto's thyroiditis (defined as euthyroid or hypothyroid patients with at least fivefold increased levels of TPOAb and heterogeneous hypoechoic pattern on thyroid ultrasound) and in 68.2% of patients with Hashimoto's thyroiditis and coexistent Graves' orbitopathy (Kahaly et al. 2016). Autoimmunity against the TSH receptor consequently is not limited to Graves' disease but is involved in Hashimoto's thyroiditis as well.

Goiter

Following wider application of TPOAb and TgAb assays, it became obvious that these antibodies were also present in subjects without goiter. Actually, the vast majority of patients with Hashimoto's thyroiditis have no goiter, and at the time of diagnosis only a minority of patients with overt autoimmune hypothyroidism have goiter (Laurberg et al. 1999). The prevalence of goiter in the setting of Hashimoto's thyroiditis is in the order of 5–10% according to a rough estimate.

Thyroid Function

In a study published in 1992, a correlation was sought between thyroid function and histology in 601 patients with chronic thyroiditis, as characterized by mononuclear cell infiltration (Mizukami et al. 1992). There were 137 patients (23%) with oxyphilic chronic thyroiditis (moderate to severe diffuse cell infiltration, follicular

epithelial changes almost in all cells, mild to severe fibrosis); their median age was 44 years and 85% were hypothyroid (latent in 38% and overt in 47%). In the group of 161 patients (27% of total) with mixed chronic thyroiditis (moderate diffuse cell infiltration, various follicular epithelial changes, and minimal to mild fibrosis), median age was 38 years; 39% had hypothyroidism (latent in 33.5% and overt in 5.5%), 38% were euthyroid and 23% hyperthyroid. Focal thyroiditis was observed in 149 patients (25%) with a median age of 37 years; hypothyroidism was present in 15% (all except one had latent hypothyroidism), euthyroidism in 83%, and hyperthyroidism in 2%. The greater the extent of cell infiltration in focal thyroiditis, the smaller the proportion of patients who were euthyroid and the greater the proportion of latent hypothyroid patients. Hyperplastic chronic thyroiditis (mild to moderate focal cell infiltration, mild to severe diffuse hyperplastic epithelial cell changes) occurred in 154 patients (26%) with a median age of 33 years; hypothyroidism was present in 10% (latent in 3% and overt in 7%), euthyroidism in 5%, and hyperthyroidism in 85%. Taken together, patients with the classic oxyphilic variant were the oldest and those with hyperplastic thyroiditis the youngest (44 vs. 33 years), whereas hypothyroidism was most prevalent in the oxyphilic variant (85%) and hyperthyroidism most in the hyperplastic variant (85%). Patients with focal thyroiditis had the highest proportion of euthyroidism (83%), and their mean age of 37 years lay in between those of the two other groups. Hashimoto's thyroiditis may thus occur in the presence of hypothyroidism, euthyroidism, or hyperthyroidism, and thyroid function by itself lacks sufficient sensitivity and specificity for the diagnosis of Hashimoto's thyroiditis.

Thyroid Ultrasonography

Marked diffuse or inhomogeneous hypoechogenicity or patchy echo pattern are typical sonographic signs of Hashimoto's thyroiditis (Bennedbaek and Hegedus 2007). Hypoechogenicity is a consequence of lymphocytic aggregations, which appear as very homogeneous tissue without reflecting surfaces (Radetti 2014). Thus, abnormal echogenicity is observed in all patients with Hashimoto's thyroiditis (Fig. 2). The high sensitivity of thyroid ultrasonography is further illustrated by reports that a hypoechoic ultrasound pattern or an irregular echo pattern may precede the occurrence of TPOAb in serum (Biondi and Cooper 2008). Specificity of hypoechogenicity is however more limited: e.g., a homogeneous and diffusely hypoechoic echo pattern is observed in Graves' disease.

Taken together, none of the abovementioned diagnostic criteria has 100% sensitivity and specificity for the diagnosis of Hashimoto's thyroiditis. Histopathology comes close, although the issue of focal thyroiditis has not been resolved satisfactorily. TPOAb and TgAb, if elevated, do indicate thyroid autoimmunity in general; they are more associated with Hashimoto's thyroiditis and hypothyroidism than with Graves' hyperthyroidism. The absence of goiter and hypothyroidism does not exclude the diagnosis of Hashimoto's thyroiditis. The finding of thyroid hypoechogenicity can be of great help. It follows that the combination of thyroid

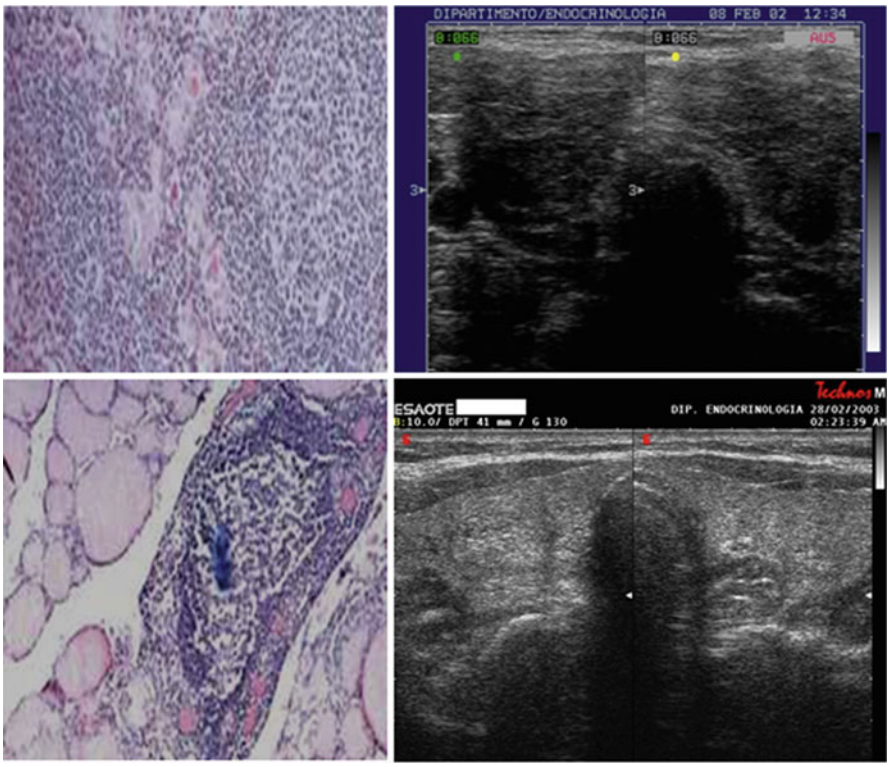

Fig. 2 Thyroid ultrasonography and cytology in two patients with Hashimoto's thyroiditis: note hypoechogenicity and lymphocytic infiltration in patient A with diffusely enlarged thyroid gland (*top panel*: TSH 1.8 mU/L, TPOAb >1,000 kU/L, TgAb 157 kU/L) and patient B with nodular goiter (*bottom panel*: TSH 0.5 mU/L, TPOAb 1,000 kU/L, TgAb 89 kU/L) (Courtesy of Prof. Paolo Vitti, Pisa, Italy)

ultrasound, thyroid function tests, and thyroid antibodies usually allows to make a reliable diagnosis of Hashimoto's thyroiditis. Cytology and histopathology are nowadays rarely required to ascertain the diagnosis.

Classification of Hashimoto's Thyroiditis and Its Variants

A number of variants of Hashimoto's thyroiditis have been described (Doniach et al. 1979; Livolsi 1994; Caturegli et al. 2014) and listed in Table 1:

Fibrous variant. The fibrosis is dominant (in contrast to what is seen in the classic variant and the IgG4-related variant), still remaining within the thyroid capsule (thus distinguishing it from Riedel's thyroiditis with its adhesion to the surrounding structures) (Caturegli et al. 2014). Pathologically, the thyroid architecture is destroyed; there is marked follicular atrophy, dense keloid-like fibrosis, and

Table 1 Characteristics of the various forms of Hashimoto's thyroiditis

	Juvenile	Hashitoxicosis	IgG4 related	Classic (goitrous)	Fibrous (atrophic)
Peak age at onset	10–18 year	40–60 year	40–50 year	40–60 year	60–70 year
F:M ratio	6:1	5:1	3:1	12:1	10:1
Thyroid function at presentation	Normal/subclinical hypo	Hyperthyroid	Hypothyroid	Mostly normal	Hypothyroid
Ultrasonography	Hypoechogenicity	Hypoechogenicity	Strong hypoechogenicity	Hypoechogenicity	Hypoechogenicity + nodularity
24 h RAI uptake	Variable	Increased	Unknown	Variable	Decreased
Fibrosis	No	No	Yes	Yes	Severe

Modified from Caturegli et al. 2014

prominent squamous metaplasia (Livolsi 1994). It occurs in about 10% of cases, often affecting elderly people with symptomatic goiters and hypothyroidism (Katz and Vickery 1974). The fibrous variant coincides mostly with the atrophic subtype. Both names, however, do not always indicate the same condition. For example, there are patients belonging to the fibrous variant, who present with a goiter and consequently cannot be regarded as having atrophic thyroiditis.

Goitrous and atrophic variants. Chronic autoimmune thyroiditis has a goitrous (classic) form often referred to as Hashimoto's disease (in agreement with the presence of a goiter in the patients described by Hashimoto himself in 1912) and an atrophic form sometimes referred to as Ord's disease (as first reported by W.M. Ord in 1888 in the famous Report on Myxedema from the Clinical Society of London, mentioning the thyroid as "in every case reduced in size, and described variously as durated, fibrous and structureless") (Dayan and Daniels 1996; Davies 2003). Goitrous autoimmune thyroiditis is characterized by diffuse lymphocytic infiltration with occasional germinal centers, thyroid follicles of reduced size containing sparse colloid, and fibrosis. Although the follicles are small, individual thyroid cells often appear enlarged and contain cytoplasma that is granular and pink (oxyphil change), known as Hűrthle or Askenazy cells (Dayan and Daniels 1996). In atrophic autoimmune thyroiditis, the thyroid gland is small, with lymphocytic infiltration and fibrous tissue replacing the thyroid parenchyma. It has been proposed that goitrous autoimmune thyroiditis and atrophic autoimmune thyroiditis (also known as primary or idiopathic myxedema) are two separate disease entities, the former associated with HLA-DR5 and the latter with HLA-DR3/B8 (Irvine et al. 1978; Doniach 1981). Atrophic thyroiditis, as compared to goitrous thyroiditis, is further associated with greater antibody-dependent cell-mediated cytotoxicity (Bogner et al. 1995) and a higher prevalence of TSH receptor-blocking antibodies (Takasu et al. 1987; Chiovato et al. 1990; Cho et al. 1995). It has been hypothesized that atrophic thyroiditis represents the end stage of goitrous thyroiditis. Actually, a number of studies argue against this hypothesis. The age at presentation is not different between either subtypes (Bogner et al. 1995; Carlé et al. 2009). Little histologic progression has been observed in patients with Hashimoto's thyroiditis undergoing second biopsies up to 20 years after the first (Vickery and Hamblin 1961; Hayashi et al. 1985). Goitrous lymphocytic thyroiditis changed little as a function of time in many patients regardless of whether thyroid hormone was administered (Hayashi et al. 1985). In a population-based study in Denmark, the prevalence of subclinical goitrous Hashimoto's disease was 0.62% (thyroid volume $>$14.9 ml) and of subclinical autoimmune atrophic thyroiditis was 0.24% (thyroid volume $<$6.6 ml); there was a strong association between large volume and TPOAb/TgAb but only in subjects with elevated TSH ($>$3.6 mU/L) (Bulow Pedersen et al. 2005). In another Danish population-based study, all patients with incident overt autoimmune hypothyroidism were prospectively identified (Carlé et al. 2009). Thyroid volume showed a Gaussian distribution in both males and females with no bimodal pattern. Thyroid volume was positively associated with TPOAb and TgAb concentrations and negatively with echogenicity and serum TSH. Thyroid volume was not related to the prevalence of

TSH receptor antibodies nor to duration of symptoms before diagnosis. The authors conclude that goitrous thyroiditis and atrophic autoimmune thyroiditis are only extremes of a continuous Gaussian distribution and do not represent separate disorders. However, patients with low or high thyroid volume differ with respect to several characteristics.

IgG4-related variant. IgG4-related disease is a newly recognized fibro-inflammatory condition characterized by a tendency to form tumefactive lesions at multiple sites, a peculiar histopathologic appearance, and often but not always elevated serum IgG4 concentrations (Deshpande et al. 2012a). Critical histopathological features are a dense lymphoplasmacytic infiltrate (predominantly T cells with scattered aggregates of B cells; plasma cells are essential and may predominate), a storiform pattern of fibrosis (irregularly whorled pattern), and obliterative phlebitis. The diagnosis of IgG4-related diseases requires tissue IgG4 immunostaining: $IgG4^+$ plasma cells that number >50 per high-power field might be specific but appropriate cutoffs vary per organ. The $IgG4^+/IgG^+$ plasma cell ratio might be a more accurate diagnostic tool than $IgG4^+$ plasma cell count, with a ratio of >40% as a comprehensive cutoff value in any organ. The serum IgG4 concentration is elevated in many patients with IgG4-related disease but is normal in up to 40% of patients with biopsy-proven IgG4-related disease. Neither elevated serum IgG4 nor elevated numbers of $IgG4^+$ plasma cells in tissue are specific for IgG4-related disease. Morphological features in the appropriate clinical context form the basis for the diagnosis of IgG4-related disease (Deshpande et al. 2012a). Riedel's thyroiditis is the prime example of an IgG4-related thyroid disease (Dahlgren et al. 2010). IgG4-related variants of Hashimoto's thyroiditis were first described in 2009 (Li et al. 2009). Among 70 Japanese patients, who had undergone thyroidectomy because of goitrous Hashimoto's thyroiditis, 19 (27%) had IgG4 thyroiditis (Li et al. 2010). Patients with IgG4 thyroiditis showed a higher grade of stromal fibrosis, lymphoplasmacytic infiltration, and follicular cell degeneration than non-IgG4 thyroiditis patients. IgG4 thyroiditis was further associated with a higher proportion of male gender, a shorter disease duration, more prevalent subclinical hypothyroidism, higher serum IgG4, higher concentration of TPOAb and TgAb, and more frequently low echogenicity at ultrasound examination. The prevalence of IgG4-related Hashimoto's thyroiditis was 36%, 23%, and 13% among all cases of Hashimoto's thyroiditis in subsequent studies (Tasli et al. 2014; Zhang et al. 2014; Jokisch et al. 2016). The IgG4-related variant in these studies was associated with younger age, relatively more males, more fibrosis, and greater tissue expression of TGF-β1. The fibrous variant of Hashimoto's thyroiditis was proposed to be a IgG4-related thyroid disease (Deshpande et al. 2012b). A recent German study observed a 96% prevalence of the fibrous variant among IgG4-related Hashimoto's thyroiditis, whereas the prevalence was 18% in non-IgG4-related Hashimoto's thyroiditis (Jokisch et al. 2016). So the majority of cases with the fibrous variant are not IgG4 related. The clinical relevance of identifying the IgG4-related variant is unclear, especially because the pathogenetic role of IgG4 – if any – has not been resolved. IgG4 (in contrast to IgG1) is

not causing damage to thyroid cells (Guo et al. 1997). However, it could be speculated that IgG4-related thyroiditis might benefit from steroids which could diminish progression of fibrosis (Minamino et al. 2016).

Hashitoxicosis. Thyroidal lymphocytic infiltration is similar to that in the classic (goitrous) form, but germinal center formation is rare or absent, follicular atrophy absent or actually replaced by hyperplasia, Hűrthle cell metaplasia less extensive, and fibrosis milder (Caturegli et al. 2014). Hashitoxicosis, described first by Fatourechi in 1971 (Fatourechi et al. 1971), has the clinical features of Graves' hyperthyroidism (elevated thyroidal radioiodine uptake and presence of stimulating TSHRAb) but the histopathology of Hashimoto's thyroiditis. The hyperthyroidism is transient and evolves into permanent hypothyroidism after 3–24 months (Wasniewska et al. 2012a). This sequence of events is frequently related to a change from stimulating to blocking TSHRAb (Nabhan et al. 2005).

Juvenile thyroiditis has the same pathology as hashitoxicosis. Most children have goiter but are usually asymptomatic. At time of diagnosis, 43% are euthyroid, and 24% have subclinical hypothyroidism, 21% overt hypothyroidism, 9% overt hyperthyroidism, and 3% subclinical hyperthyroidism (Demirbilek et al. 2007; Wasniewska et al. 2012b). The natural history is variable, with remissions and recurrences or evolution to permanent hypothyroidism.

Silent or painless thyroiditis and *postpartum thyroiditis* can be considered as still other variants of Hashimoto's thyroiditis. Thyroid biopsy shows follicular disruption and lymphocytic thyroiditis, while stromal fibrous and oxyphilic changes are rare. Dissimilarities with the classic form include the relative lack of oncocytic metaplasia, minimal to absent follicular atrophy, and mild or no fibrosis (Mizukami et al. 1988, 1993). Typically, these conditions have a triphasic pattern, starting with thyrotoxicosis, followed by hypothyroidism, and then recovery. The initial thyrotoxicosis is not caused by excessive production of thyroid hormones by the thyroid gland but rather by the release of preformed hormones from the thyroid follicles caused by destructive thyroiditis. Whereas most patients recover from the subsequent hypothyroid phase with restoration of the normal thyroid architecture, permanent hypothyroidism may occur later. The natural history of these conditions fits with the definition of Hashimoto's thyroiditis as an autoimmune-mediated destruction of the thyroid gland.

Classification system. There is no satisfactory or internationally accepted classification of Hashimoto's thyroiditis and its variants. Hashimoto's thyroiditis, chronic lymphocytic thyroiditis, and chronic autoimmune thyroiditis are used in the literature almost as synonyms, often without mentioning clear diagnostic criteria. Pathologists continue to employ terms in their histologic diagnoses of AITD that clinicians rarely use because they have little clinical relevance (Table 1; Davies and Amino 1993). One attempt for a clinically and scientifically useful and meaningful classification is presented in Table 2 (Davies 2003; Davies and Amino 1993). The scheme has not attracted much attention, and it is not being used. Nevertheless, a classification system which is agreed upon by the scientific community would be very helpful. It could form the basis for further exploration of differences in immunopathogenesis between the various variants, which up to now remain largely unknown.

Table 2 Proposed classification of human autoimmune thyroiditis

Type	Thyroid function	Thyroid antibodies
Type 1. Euthyroid autoimmune thyroiditis (Hashimoto's thyroiditis)		
1A. Goitrous	TSH normal	TPOAb and TgAb
1B. Non-goitrous		
Type 2. Hypothyroid autoimmune thyroiditis (Hashimoto's thyroiditis)		
2A. Goitrous	TSH increased FT4 decreased	TPOAb and TgAb TSHRAb (blocking) in non-goitrous subset
2B. Non-goitrous		
2C. Transient aggravation[a]	Initial TSH decreased later TSH increased	TPOAb and TgAb
Type 3. Autoimmune thyroiditis (Graves' disease)		
3A. Hyperthyroid	TSH decreased FT4 increased	TSHRAb (stimulating) usually TPOAb and TgAb
3B. Euthyroid	TSH normal FT4 normal	TSHRAb (stimulating) usually TPOAb and TgAb
3C. Hypothyroid[b]	TSH increased FT4 decreased	TSHRAb (stimulating) usually TPOAb and TgAb

Modified from Davies and Amino (1993) and Davies (2003)
[a]May start as transient destructive thyrotoxicosis, often followed by transient hypothyroidism (postpartum thyroiditis, painless or silent thyroiditis)
[b]Hypothyroidism with Graves' orbitopathy

Epidemiology

Hashimoto's thyroiditis was considered a rare condition in the first half of the twentieth century (Caturegli et al. 2013). This changed after the detection of thyroid antibodies in serum in 1956 and the introduction of simple immunoassays for these antibodies as of the 1970s. Nowadays, Hashimoto's thyroiditis appears to be very common and actually is the most prevalent autoimmune disease among all autoimmune diseases. Comparison of incidence and prevalence figures between studies is hampered by a number of factors (McLeod and Cooper 2012). First, disease definition may be different: diagnostic methods vary between eras, and disease definition is not standardized. Second, laboratory techniques vary among eras and may not be standardized even when similar techniques are used, and positive cutoff points are not standardized. Third, study populations are rarely comparable. Thus, the combination of genetic background and environmental exposures is certainly different, and there is no standardization for age, gender, and race among studies. Despite these inherent difficulties, available data allow a number of general conclusions about the epidemiology of Hashimoto's thyroiditis. Data of the most relevant studies have been summarized in a number of recent papers (Vanderpump 2011; McLeod and Cooper 2012; McGrogan et al. 2008; Endocrine Society 2015). Hashimoto's thyroiditis in the majority of these studies was diagnosed by the presence of TPOAb and/or TgAb, with or without autoimmune hypothyroidism. However, postpartum thyroiditis and painless/silent

thyroiditis were not included in most studies, although these conditions could be considered as still other variants of Hashimoto's thyroiditis in view of their autoimmune-mediated destructive type of thyroiditis.

Prevalence

The epidemiological studies allow firm conclusions with respect to the prevalence of Hashimoto's thyroiditis: (a) the prevalence is very high in the general population; (b) the prevalence is higher in females than in males; (c) the prevalence increases with advancing age; and (d) the prevalence is highest in Whites and lowest in Blacks, whereas the prevalence in Hispanics lies in between those of Whites and Blacks (Tables 3, 4, and 5). The reported prevalence of 10–12% for Hashimoto's thyroiditis in the general population is higher than for any other autoimmune disease. Prevalences vary between populations. One explanation for such differences is ambient iodine intake: prevalence of Hashimoto's thyroiditis is lower in iodine-deficient and higher in iodine-replete regions (Bulow Pedersen et al. 2002). In line with other autoimmune diseases, there is a strong *female preponderance* (Table 3). For instance, in the NHANES III study, the prevalence of TPOAb was 17.0% in women and 8.7% in men, and the prevalence of TgAb was 15.2% in women and 7.6% in men (Hollowell et al. 2002). The female to male ratio varies between 5:1 and 2:1. An equal sex ratio of 1:1 was only observed in APS1 (autoimmune polyglandular syndrome type 1), caused by a genomic mutation in *AIRE* (autoimmune regulator gene) (Nithiyananthan et al. 2000; Meyer et al. 2001). In a large collective of 686 children and adolescents with Hashimoto's disease, the female to male ratio was 2.8:1 (Demirbilek et al. 2007; Wasniewska et al. 2012b; Radetti et al. 2006). Available data suggest that the female preponderance becomes more marked in adulthood. The question why thyroid autoimmunity preferentially occurs in women remains incompletely resolved. Sex steroids might be involved as well as pregnancy. Recovering from the immune effects of pregnancy, a rebound reaction may activate the Th1-mediated pathway leading to cellular immunity and destruction of thyroid follicular cells (Weetman 2010). In susceptible subjects it could result in postpartum thyroiditis, a prevalent condition associated with the development of permanent autoimmune hypothyroidism (Azizi 2005; Stagnaro-Green et al. 2011). However, conflicting results are reported in the literature with regard to a possible association between parity, thyroid antibodies, and autoimmune thyroid disease (Walsh et al. 2005; Bulow Pedersen et al. 2006; Friedriech et al. 2008). A recent paper reports a dose-dependent association between development of autoimmune overt hypothyroidism and the number of live births and induced abortions but only in premenopausal women: odds ratios for hypothyroidism after 1, 2, or $\geq$3 live births were 1.72, 3.12, and 4.51, respectively, and after 1 or $\geq$2 induced abortions 1.02 and 2.70, respectively (Carlé et al. 2014). The prevalence increased with *advancing age* (Table 4). In the NHANES III study, the prevalence of TPOAb increased fivefold (from 4.8% to 23.9%) and of TgAb threefold (from 6.3% to 21.6%) between the second and ninth decades of life. The effect of age was even more prominent when

Table 3 Incidence and prevalence figures of Hashimoto's thyroiditis

Author and year	*Population*	*Method*	*Incidence per 100,000/year*[a]	*Prevalence*[b]
Furszyfer et al. (1970)	Olmsted Co, USA $n = 240$ cases, all F, Age 0–70^{+} Year 1935–1967	Tissue sample Clinical signs	F only 6.5 (1935–1944) 21.4 (1945–1954) 67.0 (1955–1964) 69.0 (1965–1967)	F, 10.5% M, 0.26%
Tunbridge et al. (1977)	Whickham, UK $n = 2779$ Age ≥20 Year 1972	Hemagglutination TgAb		F, 16.2% M, 4.3%
Sundbeck et al. (1991)	Gothenburg, Sweden $n = 514$ Age 70–79 Year 1971–1988	TSH	F, 243	
Galofre et al. 1994 (1994)	Vigo, Spain $n = 278{,}370$ Year 1990–1992	TSH, FT4 TPOAb and TgAb ultrasonography	F, 45.4 M, 2.2	
Vanderpump et al. (1995)	Whickham, UK $n = 1877$ 1972–1992	Serum TSH, FT4	F, 350 M, 60	F, 10.3% M, 2.7%
Hollowell et al. (2002)	USA population $n = 17{,}353$ Age ≥12 Year 1988–2004	Serum TSH, FT4 TPOAb and TgAb		F, 17.0% M, 8.7%
Leese et al. (2008)	Tayside, Scotland $n = 390{,}000$ 1994–2001	Serum TSH TPOAb and TgAb	F, 448 M, 92	
Zaletel et al. (2011)	Ljubljana, Slovenia $n = 1{,}000{,}000$ Adults 1999 and 2009	Serum TSH, FT4 TPOAb and TgAb ultrasonography	36.9 (1999) 68.8 (2009)[c]	
Carlé et al. (2006a)	Denmark $n = 538{,}734$ 1997–2000	Serum TSH, FT4 TPOAb and TgAb	F, 44.4 M, 11.9	
Lombardi et al. (2013)	Pescopagano, Italy 1995, $n = 1{,}411$ 2010, $n = 1{,}148$[c] All ages	Serum TSH, FT4 TPOAb and TgAb ultrasonography		1995 F,17.2%, M, 5.8% 2010[c] F,25.6%, M, 10.7%
McLeod et al. (2014)	US military 20,270,688 year Age 20–54 year 1997–2011	ICD-9-CM code 245.2	F, 26.3 M, 3.2	

F females, *M* males

[a]Limited to autoimmune hypothyroidism, see also text

[b]Based on frequency of TPOAb and TgAb

[c]After salt iodization as of 1999

Table 4 Gender-specific prevalence of TPOAb and TgAb as a function of age in the total NHANES III population (Hollowell et al. 2002)

	TPO antibodies %		Tg antibodies %	
Age group	Males	Females	Males	Females
12–19 year	2.9	6.7	5.2	7.3
20–29 year	5.7	11.3	5.2	9.2
30–39 year	9.5	14.2	7.8	14.5
40–49 year	11.2	18.0	7.4	16.4
50–59 year	11.0	20.7	8.8	18.6
60–69 year	11.7	27.3	10.3	22.4
70–79 year	13.2	29.0	14.1	22.3
80–89 year	12.3	30.2	11.3	27.0

studied in the first two decades. The prevalence of TPOAb and/or TgAb in an iodine-sufficient region of Spain was 0.6, 4.6, and 6.2% in the age groups 1–6, 6–12, and 12–16 years, respectively (Garcia-Garcia et al. 2012). The prevalence was associated with age (odds ratio 1.30) and female sex (odds ratio 2.78). The prevalence of thyroid antibodies in girls was 5.0% and in boys 2.3%; at prepubertal age these figures were 3.3% in girls and 1.5% in boys and at postpubertal age 8.1% and 3.9%, respectively. Another study reported a higher frequency of TPOAb in girls in Tanner stage II–IV than in girls in Tanner stage I (8.2% vs. 2.2%) (Kaloumenou et al. 2008). So puberty is one of the factors promoting thyroid autoimmunity. If the disease manifests itself already during childhood, one may hypothesize this happens preferentially in subjects harboring a rather high genetic susceptibility for autoimmune thyroid disease. Conversely, if one contracts the disease later in life, it is more likely that exposure to environmental insults play a more dominant role in the pathogenesis. Naturally, the environment will play a less prominent role in children because their young age prevents a long exposure time to environmental stressors. Lastly, the prevalence of Hashimoto's thyroiditis has been found to differ between *ethnic groups* (Table 5). Interestingly, the prevalence (and incidence) of Hashimoto's thyroiditis is highest in Whites and lowest in Blacks and Asian/Pacific Islanders, whereas the opposite is true for Graves' disease (highest incidence in Blacks and Asian/Pacific Islanders and lowest in Whites (McLeod et al. 2014). Little is known about the presumably genetic factors responsible for these ethnic differences.

Incidence

The medical-indexing and record-retrieval system at the Mayo Clinics in Rochester, MN, assured the identification of practically all Olmsted County residents in whom the diagnosis of significant illness had been made. Using this register, the incidence of Hashimoto's thyroiditis among female residents of Olmsted County increased significantly over a 33-year period, especially between 1935 and 1954, but not any longer between 1955 and 1967 (Table 2; Furszyfer et al. 1970). In the same period, the incidence of Graves' disease did not change (Furszyfer et al.

Table 5 Ethnic differences in the incidence and prevalence of Hashimoto's thyroiditis and incidence of Graves' disease

Ethnic group[a,b]	*Prevalence TPOAb*	*Prevalence TgAb*
White, non-Hispanic	14.3%	12.9%
Mexican American	10.9%	8.8%
Black, non-Hispanic	5.3%	3.0%
Ethnic group[c,d]	*Incidence rate ratio Hashimoto's thyroiditis*	*Incidence rate ratio Graves' disease*
White	1.00	1.00
Black	F, 0.33 (95% CI 0.21–0.51) M, 0.22 (95% CI 0.11–0.47)	F, 1.92 (95% CI 1.56–2.37) M, 2.53 (95% CI 2.01–3.18)
Asian/Pacific Islander	F, 0.31 (95% CI 0.17–0.56) M, 0.23 (95% CI 0.07–0.72)	F, 1.78 (95% CI 1.20–2.66) M, 3.36 (95% CI 2.57–4.40)

F females, *M* males
[a]Hollowell et al. (2002)
[b]Total NHANES III population
[c]McLeod et al. (2014)
[d]Incidence rate ratio of Hispanic group lies in between those of Whites and Blacks

1972). Between 1935 and 1944, the diagnosis of Hashimoto's thyroiditis was exclusively made by thyroidectomy, but between 1965 and 1967, the diagnosis was established in 21% by thyroidectomy, in 28% by needle biopsy, and in 51% by clinical features (like diffusely enlarged sometimes lobulated thyroid gland with rubbery feeling, low basal metabolic rate, normal or increased protein-bound iodine, low butanol-extractable iodine, high tanned red cell agglutination titer). Changes in applied diagnostic methods may explain to a large extent the observed rising incidence of Hashimoto's thyroiditis, especially between 1935 and 1955, but the authors speculate that increased ingestion of iodide triggers the disease and might be partly responsible for the increasing incidence up to 70/100,000/year in women in the 1960s (Furszyfer et al. 1970). The incidence rate of autoimmune hypothyroidism for women in the iodine-sufficient Spanish city of Vigo in the early 1990s was 45.4/100,000/year (Galofre et al. 1994). Much higher incidence rates of spontaneous overt hypothyroidism (presumably of autoimmune origin) were reported in the 1990s in a 20-year follow-up of the Whickham Survey in Northern England, the first (and rightly famous) population-based survey on thyroid autoimmunity (Vanderpump et al. 1995): 350 cases/100,000/year in women and 60 cases/100,000 per year in men. Odds ratios for development of spontaneous hypothyroidism in women were 14 [95% CI 9–24] for raised TSH at baseline, 13 [95% CI 8–19] for positive TPOAb and/or TgAb at baseline, and 38 [95% CI 22–65] for raised TSH and positive thyroid antibodies combined; corresponding values in men were consistently higher, with odds ratios of 44 [95% CI 19–104], 25 [95% CI 10–63], and 173 [95% CI 81–370], respectively. Various subsequent longitudinal studies have confirmed that a serum TSH >2.5 mU/L and the presence of TPOAb and/or TgAb increase the risk of developing overt hypothyroidism (Strieder et al. 2008; Walsh et al. 2010). Incidence rates of hypothyroidism in the

same order of magnitude (448/100,000/year for women and 92/100,000/year for men) are published for the population of Tayside in Scotland from 1994 to 2001 (Leese et al. 2008). The data are based on the Thyroid Epidemiology, Audit, and Research Study (TEARS), in which hypothyroidism is defined as continuous long-term replacement therapy because of an underactive thyroid, excluding past thyroid surgery or hyperthyroidism from the register; consequently it is assumed that almost all cases of incident hypothyroidism are due to autoimmune thyroid disease. An interesting study from Slovenia demonstrated a clear increase in the incidence of hypothyroid Hashimoto's thyroiditis after mandatory salt iodization in 1999 (Zaletel et al. 2011).The incidence of hypothyroid Hashimoto's thyroiditis increased from 36.9 before to 68.8 per 100,000 per year after the rise in iodine intake (RR 1.86, 95% CI 1.64–2.12). The increase of Hashimoto's thyroiditis (hypothyroid and euthyroid cases combined) was even more dramatic, from 73.2/100,000 in 1999 to 166.4/ 100,000 in 2009. Similar results were obtained in the small rural community of Pescopagano in Italy. Fifteen years after voluntary iodine prophylaxis, the prevalence of TPOAb and TgAb had increased significantly, and Hashimoto's thyroiditis (defined as hypo- or euthyroidism with raised thyroid antibodies or euthyroidism without detectable antibodies but hypoechogenicity at thyroid ultrasound) had become more frequent in 2010 than in 1995 (14.5% and 3.5%, respectively), both in females and in males (Lombardi et al. 2013). Much relevant information has been generated by the DanThyr program, the Danish joint iodine fortification program (Laurberg 2015). The sale of iodized salt was prohibited in Denmark until 1998, when a voluntary program was started of adding KI to all salt for human consumption. Due to the ineffectiveness of this voluntary program, it was changed after 2 years into a mandatory program: the level of iodization of household and bread salt was set to 13 ppm, that is, 13 μg iodine per g salt, which would provide a daily iodine intake of about 50 μg at an average salt intake of 4 g per day. The mandatory program was implemented during the period July 2000 to April 2001, and 4–5 years later the median urinary iodine concentration had increased toward 101 μg/L (Rasmussen et al. 2008). This value is just above the lower limit of 100 μg/L compatible with an adequate iodine intake as recommended by WHO/UNICEF/ICCIDD. Before the mandatory program, the unadjusted incidence rate of spontaneous hypothyroidism was 44.4 (females) and 11.9 (males) per 100,000 per year (Carlé et al. 2006a). Hypothyroidism in this study had most likely an autoimmune origin, as serum thyroid antibodies were measurable in practically all patients (Carlé et al. 2006b). The incidence of spontaneous hypothyroidism increased nearly exponentially with age, with a sharp increase above 50 years of age; half of the patients were 67.6 years or older. Of much interest is the higher standardized incidence rate in the mildly iodine-deficient region of Copenhagen than in the moderately iodine-deficient region of Aalborg: 35.0 and 23.1 per 100,000 per year, respectively, and standardized incidence rate ratio of 1.53 (95% CI 1.29–1.80). Thus spontaneous hypothyroidism was 53% more common in Copenhagen with higher – but still deficient – iodine intake. Median urinary iodine excretion values in this study were 61 μg/L (93 μg/24 h) in Copenhagen and 45 μg/L (62 μg/24 h) in Aalborg. After

implementation of the mandatory iodization program, the median urinary iodine concentrations increased to 108 μg/L in Copenhagen and 93 μg/L in Aalborg (Rasmussen et al. 2002). This was associated with an increased prevalence of mild (but not overt) hypothyroidism in both regions: figures before/after for the Copenhagen region were 4.45%/5.60% and for the Aalborg region 3.24%/5.40% (Vejbjerg et al. 2009). In line with the increased prevalence of hypothyroidism, a doubling in the use of thyroid hormone replacement was documented: in Copenhagen the incidence rate increased by 75% (incident users/100,000 person-years were 72.2 in 1997 and 126.6 in 2008) and in Aalborg by 87% (from 86.9 to 162.9, respectively) (Cerqueira et al. 2011). A subsequent longitudinal study within the DanThyr cohort reported a significant increase in serum TSH during an 11-year follow-up: from 1.3 mU/L in 1997–1998 to 1.5 mU/L in 2008–2010 (Bjergved et al. 2012). Urinary iodine excretion in this cohort had increased from 52 to 75 μg/L. The prevalence of TPOAb had increased from 16.1% to 23.9% and of TgAb from 12.1% to 21.5%. The DanThyr studies provide compelling evidence that increasing iodine intake is associated with higher incidence of Hashimoto's thyroiditis and autoimmune hypothyroidism.

The incidence of spontaneous hypothyroidism in iodine-deficient Denmark is about 50% lower than in iodine-sufficient Sweden. The incidence rate of spontaneous hypothyroidism in Sweden in women aged 38–66 years is 156/100,000 person-years and in Denmark in women aged 40–69 years 68.6/100,000 person-years (Carlé et al. 2006b; Nystrom et al. 1981); in women aged 70–79 years, the incidence rates were 243 in Sweden and 121 in Denmark (Sundbeck et al. 1991; Carlé et al. 2006b). The relevance of ambient iodine intake in a population for the frequency of thyroid diseases in that population has again been demonstrated nicely in large studies from China (Teng et al. 2006). Three regions with mildly deficient iodine intake, more than adequate iodine intake, and excessive iodine intake were compared, in 1999 at baseline and during a 5-year follow-up between 1999 and 2004. Both the prevalence and incidence of TPOAb, TgAb, and autoimmune thyroiditis (defined as TPOAb >100 kU/L with overt or subclinical hypothyroidism, called Hashimoto's thyroiditis in the presence of goiter but atrophic thyroiditis in the absence of goiter) were in general lowest in regions with mild iodine deficiency and higher in the regions with adequate or excessive iodine intake (Table 6; Teng et al. 2006). Other studies from China confirm that higher iodine intake increases the prevalence of hypothyroidism and autoimmune thyroiditis (Teng et al. 2011; Shan et al. 2016).

In conclusion, the incidence of Hashimoto's thyroiditis (like the prevalence) is higher in females, in old age, and in regions with high iodine intake. Although raising the iodine intake (by salt iodization) increases the incidence rate of Hashimoto's thyroiditis and autoimmune hypothyroidism, differences in ambient iodine intake are unlikely to be the sole explanation for differences in frequency of Hashimoto's thyroiditis between populations. Differences in genetic makeup (see Table 4 for ethnic differences) and environmental exposures, apart from iodine, must be involved as well. The present data cannot answer whether there is a secular trend toward a worldwide increasing incidence of Hashimoto's thyroiditis.

Table 6 Prevalence and cumulative incidence[a] of thyroid autoimmunity in three regions of China with different iodine intake (Modified from Teng et al. 2006)

	Mildly deficient iodine intake	*More than adequate iodine intake*	*Excessive iodine intake*
N in 1999 and 2004	1,103 and 884	1,584 and 1270	1,074 and 864
Urinary iodine[b] in 1999 and 2004	84–88 μg/L	243–214 μg/L	651–634 μg/L
TPOAb ≥50 kU/L	Prevalence 9.2% Incidence 2.8%	Prevalence 9.8% Incidence 4.1%	Prevalence10.5% Incidence 3.7%
TgAb ≥40 kU/L	Prevalence 9.0% Incidence 3.3%	Prevalence 9.0% Incidence 3.9%	Prevalence 9.4% Incidence 5.1%
Autoimmune thyroiditis[c]	Prevalence 0.5% Incidence 0.2%	Prevalence 1.7% Incidence 1.0%	Prevalence 2.8% Incidence 1.3%
Goitrous autoimmune hypothyroidism	Prevalence 0.4% Incidence 0%	Prevalence 1.0% Incidence 0.3%	Prevalence 1.5% Incidence 0.5%
Atrophic autoimmune hypothyroidism	Prevalence 0.1% Incidence 0.2%	Prevalence 0.7% Incidence 0.7%	Prevalence 1.3% Incidence 0.8%

[a]As % of persons followed-up for 5 years
[b]Median values
[c]Defined as TPOAb >100 kU/L with overt or subclinical hypothyroidism, subdivided in Hashimoto's thyroiditis (with goiter) and atrophic thyroiditis (without goiter)

Pathogenesis

Immunological synapse. The immunological synapse refers to the interaction between antigen-presenting cells (APCs) and T lymphocytes (T cells) (Weetman 2016). Macrophages (Mϕ), dendritic cells (DC), and also B lymphocytes (B cells) act as professional APCs. APCs take up antigens and process them into peptides of 12–18 amino acids (the epitopes of the antigen) which bind to major histocompatibility complex (MHC) class II molecules on the cell surface (MHC is termed human leukocyte antigen, HLA, in humans). The complex of HLA and antigenic epitope may be recognized by T-cell receptors (TCR) on helper T cells (identified by expression of the surface molecule CD4; CD stands for cluster of differentiation). Formation of the trimolecular complex HLA class II, antigenic epitope, and TCR activates CD4+ T cells, which involves expression of the interleukin-2 receptor (IL-2R) and autocrine stimulation by IL-2 release. Activation of T cells, however, requires, next to recognition of the antigen, costimulation. Formation of the trimolecular complex induces CD40 ligand on T cells, which binds to constitutively expressed CD40 on APCs; it results in induction of B7-1 (CD80) or B7-2 (CD81) molecules on APCs, which then binds to constitutively expressed CD28 on T cells. This induces CTLA-4 (cytotoxic T-lymphocyte-associated protein-4, now named CD152) on T cells, which reduces the interaction between APCs and T cells and terminates the immune response (Weetman 2016; McLachlan and Rapoport 2014). Activated CD4+ T cells may develop into type 1 helper T cells (Th1) or type 2 helper T cells (Th2). Th1 cells (typically producing INF-γ) promote cell destruction; Th2

cells (typically producing IL-4) promote antibody production. Whereas CD4 is the receptor for MHC class II molecules on APCs, CD8+ is the coreceptor for HLA class I molecules. CD8+ T cells are typically cytotoxic but were presumed to have suppressor functions in the past. Cytotoxicity in CD8+ T cells is directed against antigenic epitopes that are synthesized in the target cell (e.g., products of viral infection or malignant transformation) and presented by MHC class I molecules. B cells may turn into plasma cells secreting antibodies. Some B cells act as memory B cells or as APCs by their cell surface immunoglobulins that function as specific antigen receptor. These specific antigens are then internalized, processed, and presented to T cells (McLachlan and Rapoport 2014; Wiersinga 2014).

Tolerance to self-antigens. Mounting an immune response against exogenous antigens (e.g., from invading microorganisms) has obvious advantages, but presentation of endogenous "self"-antigens to T cells may result in autoimmunity. A number of complex regulatory mechanisms serve to prevent an immune response directed against self-antigens. Self-tolerance is enacted in the thymus (central tolerance) and in peripheral tissues (peripheral tolerance). Immature T cells from the bone marrow enter the thymus, where they undergo a process of selection and finally exit as CD4+ or CD8+ T cells depleted of high-affinity binding sites of self-peptides (McLachlan and Rapoport 2014). This central tolerance is accomplished by negative selection of autoreactive T cells in the thymic medulla. Self-reactive T cells emerge during the random recombination of gene segments that encode variable parts of the TCR for the antigen (Geenen et al. 2013). Thymic medullary epithelial cells express peptides from self-proteins, which in cooperation with dendritic cells are presented to immature T cells. T cells that recognize these self-peptides with high affinity are deleted. T cells that have moderate affinities for self-peptides are positively selected and leave the thymus to become mature T cells. In general, the higher the concentration of autoantigen in the thymus is, the greater the degree of self-tolerance will be (Wiersinga 2014).

Central tolerance may not eliminate all self-reactive T cells. Interaction of the TCR on naïve T cells with an MHC class II molecule plus epitope, in the absence of CD28 ligation with CD80, induces anergy, that is, the T cell is paralyzed and unable to respond. Similarly, engagement of CTLA4 results in T-cell anergy (Weetman 2016). Furthermore, self-antigen presentation in the thymus generates regulatory T cells (T_{reg}) that can inhibit in peripheral tissues those self-reactive T cells that escaped negative selection in the thymus (Geenen et al. 2013). T_{reg} can be classified as either natural regulatory T cells (nT_{reg}, constitutive, developing in the thymus) or inducible regulatory T cells (iT_{reg}, involved in the adaptive immune response) which are generated from naïve T cells in the periphery after antigenic stimulation (Marazuela et al. 2006). T_{reg} are characterized by the expression of CD4, CD25 (the interleukin-2 receptor α-chain), and the transcription factor FoxP3 (forkhead box P3 protein); they produce interleukin-10 (IL-10) and transforming growth factor-β (TGF-β). T_{reg} might act directly on effector CD4+ and CD8+ lymphocytes by cell-to-cell contact, hampering their activation and proliferation, or indirectly via secretion of IL-10 and TGF-β (Bossowski et al. 2016). Control of autoreactive T cells in the periphery may be considered as a secondary or "fail-safe" mechanism in

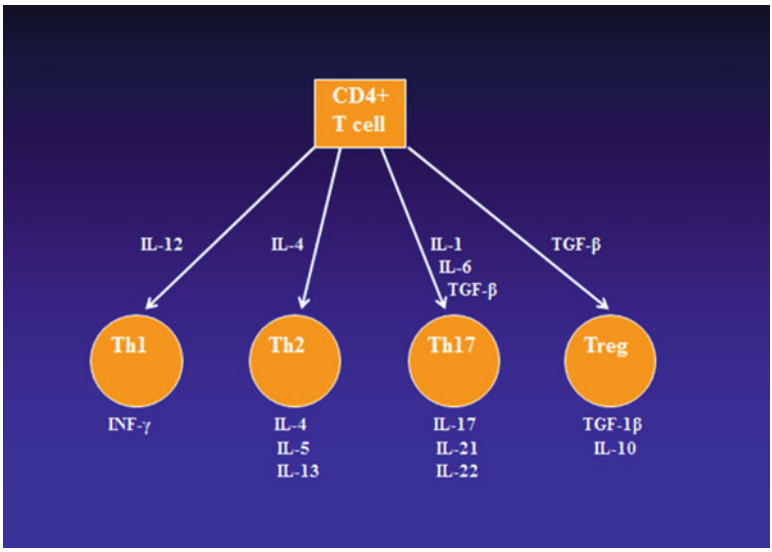

Fig. 3 Differentiation of CD4+ T cells. Indicated are cytokines that promote differentiation into one of the four T-cell subsets and the cytokines that are predominantly produced by each T-cell subset (Modified from Pyzik et al. 2015)

the prevention of autoimmune reactions. Recently, next to Th1, Th2, and T_{reg}, a fourth subset of T helper cells has been identified, designated Th17 cells generating interleukin-17 (IL-17) (Fig. 3; Pyzik et al. 2015). These cells are involved in the clearance of extracellular pathogens but have also been associated with several autoimmune diseases. IL-6 together with TGF-β promotes differentiation into Th17 cells, which are highly proinflammatory and may lead to severe autoimmune responses. T_{reg} are natural suppressors that control overactive cells. Thus a balance between Th17 and T_{reg} might be crucial for immune homeostasis (Bossowski et al. 2016).

Breakdown of self-tolerance. Breakdown of self-tolerance against thyroid antigens may lead to thyroid autoimmunity (Fig. 4; Weetman 2003). The three major thyroid antigens are thyroid peroxidase (TPO), thyroglobulin (Tg), and TSH receptor (TSHR). In general, immunogenicity of antigens is higher in case of (a) genetic polymorphisms, (b) a higher number of peptides available for binding to MHC molecules on APCs, (c) membrane-bound antigens, and (d) a high degree of glycosylation which facilitates antigen binding to cell surface mannose receptors on APCs. According to these features, the immunogenicity of Tg is higher than that of TPO and TSHR (McLachlan and Rapoport 2014; Wiersinga 2014). The interplay between thyroid antigens and immunocompetent cells determines the outcome of the immune response. In Hashimoto's thyroiditis, the massive lymphocytic infiltration in the thyroid gland is composed mostly (up to 50%) of B lymphocytes. There is also an abundance of CD8+ cytotoxic/suppressor T cells but less CD4+ T cells (Ben-Skowronek et al. 2011; Pyzik et al. 2015; Zha et al. 2014). Th1 cells activate

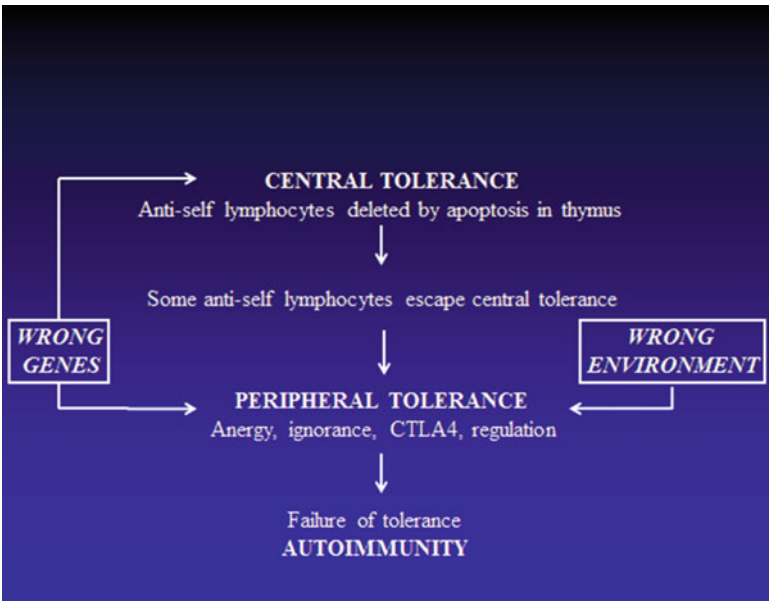

Fig. 4 Interaction between genes and environment in the development of autoimmune thyroid disease (Modified from Weetman 2003)

cytotoxic lymphocytes and macrophages, directly destroying thyroid follicular cells. Activation of Th2 cells results in stimulation of B cells and plasma cells, which produce antibodies against thyroid antigens. The number of T_{reg} in peripheral blood in patients with Hashimoto's thyroiditis, as compared to healthy controls, has been reported as increased, normal, or decreased; however, T_{reg} of Hashimoto patients are less capable of inhibiting proliferation of T cells (Pellegrini et al. 2012; Bossowski et al. 2013; Glick et al. 2013; Liu et al. 2014). Patients with Hashimoto's thyroiditis have enhanced levels of T cells synthesizing IL-17 and IL-22 in their peripheral blood; in addition, a stronger expression of IL-17 and IL-22 and of IL-23R+ cells is observed in their thyroid glands. In vitro differentiation of T lymphocytes into Th17 cells (induced by IL-23/IL-6) is also enhanced (Figueroa-Vega et al. 2010). It indicates increased differentiation of Th17 cells and enhanced synthesis of IL-17 in Hashimoto's thyroiditis. This was confirmed in another study demonstrating an increase of thyroid-infiltrating Th17 cells and IL-17 mRNA, which correlated with local fibrosis; serum IL-17 was also increased, inversely related to the degree of hypothyroidism (Li et al. 2013b). Whereas Th17 by their proinflammatory nature would be pathogenic, Th10 would play a protective role (IL-10 being produced by T_{reg}). Several studies describe a shift to higher values of the Th17/Th10 ratio in peripheral blood of patients with Hashimoto's thyroiditis. A skew to higher ratios would promote autoimmunity, and indeed a direct relationship has been found between Th17/Th10 ratios and TPOAb, TgAb, and TSH (Liu et al. 2014; Kristensen et al. 2014; Xue et al. 2015a, b). These findings strengthen the hypothesis that loss of

T_{reg} inhibitory actions and gain of Th17 proinflammatory actions play a crucial role in the loss of tolerance to thyroid antigens in AITD.

Further support for this proposition comes from a recent study on microvesicles. Circulating microvesicles are emerging as important contributors to the development of inflammatory and autoimmune diseases. Microvesicles are involved in intercellular communication; they can modulate immune reactions by transferring membrane and cytoplasmic components and genetic information (including microRNAs) between cells. In plasma samples from AITD patients, the proportion of platelet-derived microvesicles was increased, and that of leukocyte- and endothelial cell-derived microvesicles decreased compared with healthy controls (Rodriguez-Munoz et al. 2015). Functional assays showed that microvesicles from Hashimoto patients inhibit the in vitro differentiation of FoxP3+ T_{reg} and induce expression of Th17 pathogenic (IL-17+IFNγ$^-$) cells.

A reduced frequency of IL-10-producing regulatory B cells in peripheral blood of patients with Hashimoto's thyroiditis has not been observed (Kristensen et al. 2015). It has further been investigated whether defects in immunoregulatory mechanisms exist in circulating and thyroid dendritic cells (DCs) (Leskela et al. 2013). Tolerogenic DCs have an important role in the intrathymic deletion of autoreactive lymphocytes as well as in the maintenance of peripheral tolerance to self-antigens. This immunoregulatory function is exerted through different mechanisms, including the induction of anergy or programmed cell death of effector lymphocytes and the generation of regulatory T cells. The tolerogenic activity of DCs seems to be closely associated with the expression and function of different cell surface receptors, including the P-selectin glycosylated ligand-1 (PSGL-1), the inhibitory isoforms of immunoglobulin-like transcripts (ILTs or CD85), the ligands of the programmed death 1 receptor (PD-L1,2), and CD69. DCs synthesize various cytokines that activate and proliferate T_{reg} and effector lymphocytes. DCs also express indoleamine 2,3-dioxygenase (IDO) which through tryptophan (Trp) starvation and generation of Trp metabolites seems to participate in differentiation of T_{reg}. AITD patients (either with Graves' disease or Hashimoto's thyroiditis) have a diminished number of peripheral blood plasmacytoid DCs (but not of conventional immunogenic DCs), a defective expression of several immunoregulatory molecules (including ILT3, PSGL-1, CD69, and IDO), and a diminished generation of Trp metabolites, mainly in those with severe disease. In thyroid tissue of AITD patients, there are more plasmacytoid DCs, and expression of regulatory molecules like ILT3 and PSGL-1 is diminished (Leskela et al. 2013).

Effector mechanisms. Autoimmune-mediated destruction of the thyroid gland (the hallmark of Hashimoto's thyroiditis) is mediated by both cellular and humoral immune responses. Cytotoxic CD8+ T cells directed against both TPO and Tg mediate thyroid gland destruction (Ehlers et al. 2012). Cytotoxicity is directed against antigenic epitopes presented by MHC class I molecules on thyrocytes. Expression of MHC class I and adhesion molecules on thyrocytes in Hashimoto's thyroiditis enhance cytotoxicity by the binding of CD8+ cytotoxic T cells to thyrocytes. Destruction of target cells is by the granule exocytosis pathway (utilizing perforin and granzyme A and B) and by apoptosis or programmed cell death. Perforin-containing T cells have been detected in Hashimoto's thyroiditis and may contribute to thyroid cell destruction (Popko et al. 2015). There is increased

expression of the apoptotic molecule Fas on thyroid follicular cells from patients with Hashimoto's thyroiditis, which can be mediated by cytokines like IL-1 (Kotani et al. 1995; Baker 1999); expression of Fas renders thyrocytes liable to apoptosis by cytotoxic T cells expressing Fas ligand (Fas expression is generalized, whereas Fas ligand is restricted to cells of the immune system) (Weetman 2016). Indeed, upregulation of caspase-3 and downregulation of bcl-2 in the thyroid of patients with Hashimoto's disease support a pathogenetic role of apoptosis (Kaczmarek et al. 2011). Disruption of the thyroxisome (caveolin-1, thyroid peroxidase, and dual oxidase) at the apical membrane of the thyrocyte induced by Th1 cytokines (IFN-γ, IL-1α) may lead to uncontrolled oxidative stress and cell apoptosis (Marique et al. 2014). Apoptosis may also occur via TRAIL (TNF-related apoptosis-inducing ligand), which can be induced on thyrocytes by cytokines like TNF and IFN-γ (Pyzik et al. 2015). IL-1β may directly reduce expression of tight junction proteins, thus disturbing thyroid epithelium integrity and mediating thyroid follicular cell destruction in Hashimoto's thyroiditis (Rebuffat et al. 2013). Peripheral blood CD4+ and CD8+ T cells from children with Hashimoto's thyroiditis show lower surface expression of the cytotoxic lymphocyte antigen-4 (CTLA-4) than controls, which may enhance the immune response (Kucharska et al. 2013).

TPOAb and TgAb may cause antibody-dependent cell-mediated cytotoxicity (ADCC) via complement-mediated lysis of thyrocytes. The ability to fix complement depends on the IgG subclass. ADCC would cause more damage to the thyroid gland in comparison to T cells and cytokine-mediated apoptosis (Chiovato et al. 1993). TSHR blocking antibodies may also contribute to thyroid atrophy, albeit only in a small minority of Hashimoto's thyroiditis cases. Thyroid-infiltrating immunocompetent cells may release a wide variety of cytokines which could enhance the autoimmune response and further modulate thyroid hormone production and thyroid growth.

Genes and Environment

Breaking tolerance to self-antigens can happen in subjects who have the wrong genes and who are exposed to the wrong environment (Fig. 4; Weetman 2003). AITD often runs in families, which can be quantified by the sibling risk ratio (Brix et al. 1998). The sibling risk ratio (λs) is defined as the ratio of the risk for developing AITD in siblings of AITD patients to the frequency of AITD in the general population. The λs value for AITD is 16.9, for Graves' disease 11.6, and for Hashimoto's thyroiditis 28.0 (Villanueva et al. 2003). Family members may share the same genes but also the same environment. Twin studies are uniquely suitable to assess the relative contribution of genes in the development of AITD. A series of elegant twin studies in Denmark have concluded that genes contribute 79% (95% CI 38–90%) of the liability to developing Graves' hyperthyroidism and 73% (95% CI 46–89%) of the liability to developing TPOAb and/or TgAb (Brix et al. 2001; Hansen et al. 2006; Brix and Hegedus 2012). The implication is that the contribution of the environment to AITD would be more limited, in the order of 20–30%.

Genetic Factors

Polymorphisms in thyroid-specific genes (*TSHR, Tg*) and immunoregulatory genes (*HLA*, *CTLA-4*, *CD40*, *CD25*, *FOXP3)* have all been associated with Graves' disease but in general less so with Hashimoto's thyroiditis (Smith and Hegedus 2016; Effraimidis and Wiersinga 2014). Genetic variants may influence whether patients with AITD develop Graves' disease or Hashimoto's disease, sometimes in a gender-specific manner (Walsh et al. 2011; Campbell et al. 2015).

1. *Thyroid-specific genes TSHR and Tg.* Single-nucleotide polymorphisms (SNPs) in *TSHR* have been specifically associated with Graves' disease, but not with autoimmune hypothyroidism in a Caucasian population; however, more recently an association has been described between *TSHR* intron SNPs and Hashimoto's thyroiditis in a Chinese Han population (Liu et al. 2012). Functional analyses of *TSHR* intron 1 polymorphisms provide direct evidence of a link between central tolerance and these SNPs. The disease-predisposing genotype (TT) of SNP rs12101261 was associated with decreased thymic expression levels of TSHR mRNA (Stefan et al. 2014; Lee et al. 2015). Multiple SNPs in *Tg* have been associated with both Graves' and Hashimoto's disease; SNPs were located in exons in Caucasians and Indians and in introns in the Japanese (Jacobson and Tomer 2007; Ban et al. 2012; Patel et al. 2016).
2. *Immunoregulatory genes HLA*, *CTLA-4*, *and CD40* (involved in antigen presentation and T-cell activation) (Lee et al. 2015). Associations with *HLA class I and II* molecules have been recognized for a long time. *HLA-A*02:07* and *HLA-DRB4* confer susceptibility to Hashimoto's thyroiditis in Japanese subjects, whereas the haplotype *HLA-A*33:03-C*14:03-B*44:03-DRB1*13:02-DQB1*06:04-DPB1*04:01* conferred protection (Ueda et al. 2014). *HLA-B* appears to be a risk factor for Hashimoto's thyroiditis in Han Chinese (Huang et al. 2012). Only Tg peptides were found to be bound to HLA-DR within thyroid glands of Graves' patients, suggesting that presentation of Tg peptides by HLA-DR to T cells may be the initial trigger of AITD (Lee et al. 2015; Muixi et al. 2008). Two polymorphisms in *CTLA-4* (+49 A/G and CT60) have been linked to Hashimoto's thyroiditis in Taiwanese and Indian people (Patel et al. 2016; Ting et al. 2016). Polymorphisms in *CD40* are associated with Graves' disease, but not with Hashimoto's thyroiditis (Li et al. 2012).
3. *Immunoregulatory genes CD25 and FOXP3* (involved in the establishment of peripheral tolerance) (Lee et al. 2015). *IL2RA* and *FOXP3* encode markers for T_{reg}. *CD25* is marker for the interleukin-2 receptor-α chain present predominantly on CD25+ T cells, a susceptibility locus for Graves' disease (Brand et al. 2007). *FOXP3* encodes a forkhead/winged helix transcription factor expressed in naturally arising T_{reg}, committing naïve T cells to become T_{reg}. Mutations in *FOXP3* result in the fatal IPEX syndrome (immunodysregulation, polyendocrinopathy, enteropathy, X-linked). Polymorphisms in *FOXP3* have been associated with AITD in Caucasians (especially with Graves' disease developing below the age of 30 years), but not in Japanese (Owen et al. 2006; Ban et al. 2007). The location of *FOXP3* on the X-chromosome might contribute to the female preponderance of AITD.

Mutations in *AIRE* (autoimmune regulator gene), expressed in thymic medullary epithelial cells, result in failure to present self-antigens correctly in the thymus leading to a loss of self-tolerance and thereby to autoimmune polyglandular syndrome type 1. However, *AIRE* mutations are rarely present in adult AITD patients (in about 0.3–0.6% of patients with Graves' disease and autoimmune hypothyroidism). Therefore *AIRE* is not considered as a susceptibility gene contributing to the more common autoimmune endocrinopathies (Nithiyananthan et al. 2000; Meyer et al. 2001).

4. *Genome-wide association studies (GWAS).* GWAS offer a hypothesis-free approach to understanding disease susceptibility, which allows the discovery of novel pathways (Simmonds 2013). A genome-wide direct comparison between Hashimoto's thyroiditis and Graves' disease revealed an SNP at the VAV3 locus, associated with Hashimoto's thyroiditis (Oryoji et al. 2015). A large GWAS investigated 18,297 individuals for TPOAb positivity (1,769 TPOAb positives and 16,528 TPOAb negatives) and 12,353 individuals for TPOAb serum levels, with replications in 8,990 individuals (Medici et al. 2014). Significant associations ($p < 5 \times 10^{-8}$) were detected at TPO-rs11675434, ATXN2-rs653178, and BACH2-rs10944479 for TPOAb positivity and at TPO-rs11675434, MAGI3-rs1230666, and KALRN-rs20110099 for TPOAb levels. Individuals with a high genetic risk score (based on the effects of these variants) had an increased risk of TPOAb positivity and an increased level of TSH and a decreased risk of goiter; the MAGI3 variant was also associated with an increased risk of hypothyroidism. The results provide insight into why individuals with thyroid autoimmunity do or do not eventually develop thyroid disease, and these markers may therefore predict which TPOAb-positive subjects are particularly at risk of developing clinical thyroid dysfunction.
5. *X-chromosome inactivation (XCI).* In female mammalian cells, one of the two X-chromosomes is inactivated in early embryonic life. Female tissues are thus mosaics of two cell lines, one with the paternal X-chromosome and the other with the maternal X-chromosome as the active X. Usually, there is a random 50:50 ratio of the two cell lines. A skewed X-chromosome inactivation (XCI) is defined as inactivation of the same X-chromosome in $\geq$80% of cells. The consequence could be that self-antigens on one X-chromosome are not expressed at sufficiently high levels in the thymus or at peripheral sites, thereby failing to induce tolerance to these self-antigens (Effraimidis and Wiersinga 2014; Brix et al. 2005). Skewed XCI could be an explanation for the female preponderance in AITD. A meta-analysis of one UK and four non-UK Caucasian studies reports significant skewing of XCI with Graves' disease (OR 2.54, 95% CI 1.58–4.10] and Hashimoto's thyroiditis (OR 2.40, 95% CI 1.10–5.26) (Brix et al. 2005; Simmonds et al. 2014).

Polymorphisms in immunoregulatory genes may promote AITD, but they are not specific for AITD as they are associated with other autoimmune diseases as well. It explains why various autoimmune diseases may occur in the same patient. The mechanism of action of many susceptibility loci is incompletely understood. Why

are so many SNPs located in noncoding parts of the gene? Gene-gene or gene-environment interactions have hardly been studied. GWAS continue to detect additional genes and loci conferring risk for AITD. The odds ratio of each locus for AITD is rather low in the order of 1.5–2.0, with slightly higher odds for *HLA*. Taking together the effects of known susceptibility loci, it accounts for only a small proportion of the heritability of AITD. It follows that there must be many undetected susceptibility genes, each locus contributing just a little to the development of AITD, and that our current understanding of the etiology of AITD is gravely underestimated (Effraimidis and Wiersinga 2014; Brix and Hegedus 2011).

Environmental Factors

1. *Iodine intake*. As outlined in the above section on epidemiology, an increase in the ambient iodine intake in a population is followed by an increase in the prevalence and incidence of Hashimoto's thyroiditis: the frequencies of TPOAb, TgAb, and autoimmune hypothyroidism all rise. The mechanisms linking thyroid autoimmunity and iodine use in humans are incompletely understood. Using monoclonal TgAb-Fab directed to various epitopes on Tg, it has been suggested that the unmasking of a cryptic epitope on Tg contributes to iodine-induced thyroid autoimmunity in humans (Latrofa et al. 2013; Fiore et al. 2015). Excess iodine contributes further to autophagy suppression and apoptosis of thyroid follicular epithelial cells, which could be predisposing to increased risk of developing Hashimoto's thyroiditis (Xu et al. 2016).
2. *Smoking.* Whereas smoking is a clear risk factor for Graves' hyperthyroidism and even more so for Graves' ophthalmopathy, it has been recognized only in the last few years that smoking to a certain extent has a protective effect against the development of Hashimoto's thyroiditis (Wiersinga 2013). The prevalence of TPOAb is lower in smokers than in non-smokers, both in the Amsterdam AITD cohort (odds ratio 0.69, 95% CI 0.48–0.99) and in the third NHANES survey (odds ratio 0.57, 95% CI 0.48–0.67) (Strieder et al. 2003a; Belin et al. 2004). In the population-based HUNT study in Norway, the prevalence of subclinical hypothyroidism (OR 0.54, 95% CI 0.45–0.66) and overt hypothyroidism (OR 0.60, 95% CI 0.38–0.95) was lower in smokers compared to never smokers (Asvold et al. 2007). In a prospective study among healthy female relatives of AITD patients, discontinuation of smoking increased the risk of developing de novo TPOAb and/or TgAb (Effraimidis et al. 2009). In the prospective DanThyr study, patients diagnosed with autoimmune hypothyroidism had more often stopped smoking in the last 2 years before diagnosis than matched controls (16.4% vs 3.4%) (Carle et al. 2012a). The increased risk of autoimmune hypothyroidism after quitting smoking was transient: odds ratios <1 year, 1–2 years, and 3–10 years after cessation of smoking were 7.36 (95% CI 2.27–23.90), 6.34 (95% CI 2.59–15.3), and 0.75 (95% CI 0.30–1.87), respectively.
   Danish nationwide registration of maternal smoking during pregnancy adds further evidence that smoking reduces the risk of hypothyroidism (adjusted

hazard ratio 0.75, 95% CI 0.70–0.81) and increases the risk of hyperthyroidism (adjusted hazard ratio 1.38, 95% CI 1.27–1.49) (Andersen et al. 2014). The contrasting effects of smoking on the risks for Graves' hyperthyroidism and Hashimoto's hypothyroidism remain unexplained. One may hypothesize involvement of nicotine, which reduces experimental autoimmune encephalomyelitis. Anatabine – a tobacco alkaloid with a structure similar to nicotine – reduces the incidence and severity of experimental autoimmune thyroiditis (Caturegli et al. 2012).

3. *Alcohol.* Early studies suggested a direct toxic effect of alcohol on the thyroid gland, since thyroid volume was smaller in patients with alcoholic liver cirrhosis than in matched controls (Hegedus 1984). A nested case-control study in the Amsterdam AITD cohort did not find a relationship between alcohol consumption and de novo development of TPOAb, but participants who developed overt autoimmune hypothyroidism consumed less alcohol than those who remained euthyroid (Effraimidis et al. 2012a). A population-based case-control study in Denmark likewise observed that moderate alcohol consumption reduced the risk of overt autoimmune hypothyroidism: odds ratios were 1.98 (95% CI 1.21–3.33) for 0 units of alcohol per week, 1.00 for 1–10 units/week (reference), 0.41 (95% CI 0.20–0.83) for 11–20 units/week, and 0.90 (95% CI 0.41–2.00) for ≥21 units/week (Carle et al. 2012b). The observed associations were independent of gender, smoking, type of alcohol (beer or wine), and iodine intake. Interestingly, alcohol consumption also protects against Graves' hyperthyroidism (Carle et al. 2013). How alcohol exerts this protective effect is unknown; suffice it to say that the same protective effect of alcohol has been recorded in other autoimmune diseases like rheumatoid arthritis and type 1 diabetes mellitus.
4. *Selenium.* Recent epidemiological studies from China provide strong circumstantial evidence that low selenium intake is associated with Hashimoto's thyroiditis (Wu et al. 2015). Comparing prevalences between counties with low Se intake (serum Se 57 μg/L, IQR 39–82) and counties with adequate Se intake (serum Se 104 μg/L, IQR 80–136), prevalences were higher in counties with low Se intake for hypothyroidism (4.2% vs 2.0%, $p < 0.001$), subclinical hypothyroidism (21.4 vs. 11.7%, $p < 0.001$), and autoimmune thyroiditis (3.4% vs. 2.2%, $p = 0.007$). Upon dividing all participants in quintiles according to their serum Se concentration, those with serum Se in quintile 1 (<47 μg/L) and quintile 2 (47–69 μg/L) had higher prevalences of autoimmune thyroiditis, subclinical hypothyroidism, and hypothyroidism than those with serum Se in quintiles 3, 4, and 5 (69 −≥120 μg/L) (in whom prevalences were similar). Glutathione peroxidases are selenoproteins, protecting thyrocytes from oxidative stress generated by the action of H_2O_2. Low selenium levels have been associated with poor immune function. Thus, mild nutritional selenium deficiency may promote thyroid autoimmunity, and selenium supplementation might have a beneficial effect on thyroid autoimmunity. This has been investigated in eight randomized clinical trials, comparing the effect of selenium supplementation with placebo on the concentration of serum TPOAb in patients with Hashimoto's thyroiditis and TPOAb. Baseline TSH was either normal or slightly elevated, and exogenous

levothyroxine was used in some of the trials. TPOAb concentrations decreased in four trials, and did not change in the other four trials (Wiersinga 2016). The contrasting outcomes could not be explained from baseline serum Se or TSH concentrations, type of selenium preparation (sodium selenite or selenomethionine), concomitant use of levothyroxine, sample size, or glutathione peroxidase genotypes. A systematic review and meta-analysis of the controlled trials investigating the effect of selenium supplementation concluded that selenium supplementation reduced serum TPOAb levels; whether this effect was clinically relevant remained doubtful (Wichman et al. 2016; Winther et al. 2016). Consequently, it is difficult to make confident decisions about the use of selenium supplementation for Hashimoto's thyroiditis. The outcome of a large randomized controlled trial is therefore eagerly awaited (Winther et al. 2014). Of interest is a polymorphism in the promotor region of the selenoprotein S gene (*SEPS1*), which contributes to genetic susceptibility for Hashimoto's thyroiditis (odds ratio 2.24) but requires replication (Santos et al. 2014).

Two more placebo-controlled studies on selenium supplementation have been done, both in pregnant women. Selenium in a dose of 200 μg daily given as of gestational week 12 up to 1 year after delivery lowered the postpartum surge of TPOAb and reduced the incidence of postpartum thyroid dysfunction (Negro et al. 2007). Selenium supplementation with 60 μg/day as of 12–14 gestational weeks did not change the prevalence of TPOAb, TgAb, or subclinical hypothyroidism in the second and third trimesters (Mao et al. 2016). Adequate nutritional supply of selenium that saturates expression of circulating selenoprotein P, together with optimal iodine and iron intake, is required for a healthy thyroid development (Kohrle 2015), but the utility of selenium supplementation to combat thyroid autoimmunity has not yet been established (Hegedus et al. 2016).

5. *Vitamin D.* Immunocompetent cells express the vitamin D-activating enzyme CYP27B1 and the vitamin D receptor (VDR). The hormone $1,25(OH)_2D$ (converted locally from 25(OH)D or derived from the blood) binds to VDR modulating innate and adaptive immunity (van Belle et al. 2011). Low vitamin D levels have been identified as risk factors for various autoimmune diseases like rheumatoid arthritis, type 1 diabetes mellitus, and multiple sclerosis. Whether this is true also for AITD is presently uncertain. AITD patients (either with Graves' disease or Hashimoto's thyroiditis) had lower 25(OH)D levels than controls in a meta-analysis of 20 case-control studies: standardized mean difference was −0.99 ng/ml with 95% CI −1.31 to −0.66 ng/ml (Wang et al. 2015). This meta-analysis, however, did not adjust for the many confounders of vitamin D measurements (such as age, sex, body mass index, smoking, estrogen use, and seasonal variation). Avoiding these confounders, a Korean study in subjects undergoing routine health checkups observed lower 25(OH)D levels in women with TPOAb than in women without TPOAb (22.0 ng/ml vs. 23.5 ng/ml, $p = 0.03$) (Choi et al. 2014). The difference was observed in premenopausal women, but not in postmenopausal women and men. The prevalence of TPOAb was 21.2%, 15.5%, and 12.6% in women with vitamin D deficiency (<10 ng/ml), vitamin D insufficiency (10–30 ng/ml), and vitamin D sufficiency (>30 ng/ml),

respectively. Corresponding odds ratios adjusted for age, BMI, serum calcium, smoking, menopause, and season were 1.95, 1.31, and 1.00, respectively. A prospective study embedded in the Amsterdam AITD cohort, in contrast, did not find differences in serum 25(OH)D or $1,25(OH)_2D3$ between cases (women who developed de novo TPOAb) and controls (women who remained TPOAb negative), neither at baseline nor at the time of the occurrence of thyroid antibodies; in this study controls were matched to cases for age, BMI, smoking, estrogen use, season, and duration of follow-up (Effraimidis et al. 2012b). Matters become even more complicated by reported associations between polymorphisms in *VDR* and AITD (Feng et al. 2013; Inoue et al. 2014; Meng et al. 2015). Recent studies either confirm or refute an association between vitamin D deficiency and Hashimoto's thyroiditis (Mazokopakis et al. 2015; Yasmeh et al. 2016). Caucasian patients with Hashimoto's thyroiditis living on the island of Crete had low serum 25(OH)D, inversely correlated with serum TPOAb; serum TPOAb concentration decreased by 20% after 4 months of treatment with oral cholecalciferol in a daily dose of 1,200–4,000 IU (Mazokopakis et al. 2015).

6. *Infections.* MHC class II molecules are present on thyroid follicular cells in patients with Hashimoto's thyroiditis, but not in normal subjects. Expression of these molecules can be induced by IFN-γ and indirectly by viruses, enabling thyrocytes to present antigens (either foreign or self) to T cells, thereby activating T cells and initiating a thyroid autoimmune response. High endogenous IFN-α levels are seen in patients infected with certain viruses. Infections thus may provoke thyroid autoimmunity (Weetman 2016; Prummel and Laurberg 2003). No association was found between *Helicobacter pylori* infection and Hashimoto's thyroiditis in women (Shmuely et al. 2016). The relationship between *Yersinia enterocolitica* infections and AITD has been studied extensively. In the Amsterdam AITD cohort study, the prevalence of antibodies against YOP (*Y. enterocolitica* outer membrane protein) in healthy female relatives of AITD patients was higher than in controls (Strieder et al. 2003b). During follow-up, the proportion of subjects with YOP antibodies did not differ between cases (those who developed TPOAb) and controls (those who remained TPOAb negative), neither at baseline, at 1 year before seroconversion, nor at the time of seroconversion; the same negative results were obtained when analyzing hypothyroid cases and their respective controls (Effraimidis et al. 2011b). The data argue against a role of *Yersinia enterocolitica* in the pathogenesis of Hashimoto's thyroiditis. The higher prevalence of YOP antibodies in AITD relatives and in twins affected with Graves' disease (Brix et al. 2008) might be explained by assuming that susceptibility genes for AITD may also confer risk to *Y. enterocolitica* infection.
7. *Stress.* Whereas it is widely believed that stress exposure may provoke Graves' hyperthyroidism, there is a paucity of data about the role of stress in Hashimoto's thyroiditis (Wiersinga 2016). Stress exposure was assessed annually by questionnaires on recent life events (both pleasant and unpleasant) and daily hassles during the 5-year follow-up of the Amsterdam AITD cohort (Effraimidis et al. 2012c). No association was found between stress exposure and de novo occurrence of TPOAb or the development of overt autoimmune hypothyroidism.

Modulation of exposure to environmental factors in order to decrease the risk of developing Hashimoto's thyroiditis is maneuvering between Scylla and Charybdis (Laurberg et al. 2011). One should use iodized salt in order to prevent iodine deficiency disorders, but too high iodized salt intake has an unfavorable effect on blood pressure and increases the risk on Hashimoto's thyroiditis. To continue smoking may decrease the likelihood of developing Hashimoto's thyroiditis but increases the risk of Graves' disease, cardiovascular diseases, and cancer. To consume alcohol protects to a certain extent against Hashimoto's thyroiditis, but excessive amounts of alcohol are detrimental for health. There is no good evidence that supplementation with selenium or vitamin D will prevent Hashimoto's thyroiditis. To avoid pregnancy will indeed decrease the risk of developing Hashimoto's thyroiditis, but that kind of recommendation is not very realistic. Taken together, preventive interventions to diminish the risk of Hashimoto's thyroiditis are nowadays few, not always feasible, and probably of limited efficacy (Wiersinga 2016). Obtaining better data, and being able to predict the consequences of the interaction between susceptibility genes and the environmental triggers, at the level of the individual, is needed to implement better preventive measures.

Modified from Caturegli et al. (2014)

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