Autoimmune thyroid disease: old and new players

Grigoris Effraimidis and Wilmar M Wiersinga

Department of Endocrinology and Metabolism, Academic Medical Center, Room FS-169, University of Amsterdam, Meibergdreef 9, Amsterdam 1105AZ, The Netherlands

Abstract

The last 10 years have seen some progress in understanding the etiology of autoimmune thyroid disease (AITD). The female preponderance can now be explained – at least in part – by fetal microchimerism and X-chromosome inactivation. The number of identified susceptibility genes for AITD is increasing (among others now including TSHR, TG, HLA, CTLA4, PTPN22, CD40, FCRL3, IL2RA, and FOXP3), but these genes together probably do not explain more than about 10% of the heritability of AITD. As twin studies indicate that genes contribute for 70% of AITD, it follows that there must be many more loci, each of them contributing a little. While the genetic studies have clarified why various autoimmune diseases so often cluster in the same patient, the molecular mechanism of action of these genetic polymorphisms (frequently located in introns) has hardly been explained. Polymorphisms in AITD susceptibility genes may become helpful in clinical practice, e.g. in assessing risk of recurrent Graves’ hyperthyroidism (GH) after a course of antithyroid drugs. Moderate alcohol intake decreases the risk on overt GH and overt Hashimoto’s hypothyroidism. Current smokers – as well known – are at increased risk for Graves’ disease, but – surprisingly – at diminished risk for Hashimoto’s thyroiditis. Low selenium and low vitamin D levels might increase the risk of developing AITD, but data are still inconclusive. Current options for preventive interventions in subjects at risk to develop AITD are very limited.

Introduction

Autoimmune thyroid disease (AITD) is a multifactorial or so-called ‘complex’ disease in which autoimmunity against thyroid antigens develops against a particular genetic background facilitated by exposure to environmental factors (Fig. 1). AITD encompasses a spectrum of conditions ranging from Hashimoto’s hypothyroidism (HH) at one end to Graves’ hyperthyroidism (GH) at the other end. Thyroid peroxidase (TPO) and thyroglobulin (Tg) are the major autoantigens in Hashimoto’s disease, but TPO-Ab and Tg-Ab occur also in ~70% of patients with Graves’ disease. The thyroid-stimulating hormone receptor (TSHR) is the major autoantigen in Graves’ disease, but TSHR antibodies occur also in some patients with Hashimoto’s disease. Graves’ and Hashimoto’s

Invited Author’s profile

W M Wiersinga MD, PhD is Professor Emeritus of Endocrinology at the University of Amsterdam in The Netherlands. He was Chair of the Division of Endocrinology and Metabolism in the Academic Medical Center, Amsterdam from 1995 to 2008. He is past president of the European Thyroid Association and served on the Executive Committee of the European Society of Endocrinology. His research interests are autoimmune thyroid diseases in particular Graves’ ophthalmopathy (he was one of the founders of EUGOGO), nonthyroidal illness syndrome, and amiodarone.
diseases share some but not all known AITD susceptibility genes. Similarly, for environmental exposures: one environmental factor may constitute a risk for both Graves’ and Hashimoto’s diseases or for just one of them, but another factor can be risky for Graves’ disease but protective for Hashimoto’s disease. In this review, we focus on what has been learnt in the last 10 years on the etiology of AITD: Is the predilection for female gender better understood? Have new susceptibility genes been detected? And has progress been made with respect to environmental insults? Have there been new players in this field, or have remaining issues with old players been clarified? Finally, if individuals are at risk to develop AITD in view of their family history of AITD, is there anything they can do in terms of AITD prevention?

**Existential factors**

There is a strong female preponderance among AITD patients, with a female:male ratio ranging from 5:1 to 10:1. The biologic explanation of the gender difference has puzzled physicians for a long time, and only recently there have been some clues.

**Parity**

During pregnancy the serum concentrations of thyroid antibodies decrease, related to generation of maternal regulatory T-cells ($T_{reg}$) early in pregnancy that maintain a state of tolerance to fetal alloantigens in order to prevent rejection of the fetus (1). After delivery, there is a rebound with a transient rise in thyroid antibodies. The post partum period carries a risk for the onset of Graves’ disease although the risk might have been overestimated (2), and post partum thyroiditis is often the forerunner of permanent autoimmune hypothyroidism (3). In the prospective Amsterdam AITD cohort study, the prevalence of pregnancy and post partum was higher in those developing overt hyper-/hypothyroidism than in those remaining euthyroid (4). Parity as a risk factor for AITD has received much attention in relation to fetal microchimerism. There is transfer of fetal cells into the maternal circulation, already in the first trimester. Fetal microchimerism results when fetal cells persist in maternal tissues, and it is hypothesized that maternal immune responses against foreign fetal antigens might trigger autoimmune diseases. Fetal microchimerism has indeed been found in blood and thyroid tissue from women with Hashimoto’s or Graves’ disease (5). Using parity as a surrogate for increasing fetal cell exposure, one would expect the higher the number of pregnancies is, the higher the frequency and/or concentration of thyroid antibodies will be. However, the results have been conflicting. Several population-based studies have observed that there was no association between parity and TPO-Ab any longer after adjustment for maternal age and other important confounders (6, 7, 8). Other equally large studies do report associations that remain significant after adjustments, using higher cutoff values for TPO-Ab positivity. Odds ratio (OR) for AITD (defined as TPO-Ab $> 200$ kU/l) was increased in women with $\geq 1$ pregnancy compared with nulliparous women (OR, 1.8; 95% CI, 1.0–3.3) (9). In another study, the trend analysis for risk of TPO-Ab with increasing parity was significant only at TPO-Ab $> 500$ kU/l and not at lower levels (10). In a population-based Danish cohort, women with children, compared with childless women, were at a relative risk of 1.11 (1.08–1.14) for autoimmune diseases with female predominance; associations with parity were found for Hashimoto’s thyroiditis (1.11; 1.00–1.24) and Graves’ disease (1.19; 1.14–1.24) (11). Taken together, the data suggest that parity is somehow involved in the development of AITD but only to a modest extent, and parity explains only partially the female preponderance of AITD. Fetal microchimerism might contribute, but its effect is rather limited. The potential role of microchimerism in developing thyroid autoimmunity is further supported by twin studies: both female and male twins of opposite-sex pairs, as opposed to monozygotic pairs, have an increased frequency of thyroid antibodies (12).
X-chromosome inactivation

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 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 – arbitrarily – as inactivation of the same X chromosome in ≥80% of cells (13). 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 involved in tolerance induction. Loss of immunological tolerance to X-linked antigens might induce autoimmunity. Several studies have shown that skewed XCI is associated with an increased risk of developingAITD. A meta-analysis confirmed significant skewing of XCI in women with Graves’ disease (OR, 2.54; 95% CI, 1.58–4.10) and Hashimoto’s disease (OR, 2.40; 95% CI, 1.10–5.26) (14). The epigenetic phenomenon of skewed XCI may, in part, explain the strong female preponderance in AITD.

Genetic factors

Susceptibility genes for AITD can be divided into thyroid-specific genes and immunoregulatory genes. Identified thyroid-specific genes are TSHR and TG, but not TPO. One explanation for this discrepancy could be that TSHR and TG (but not TPO) are polymorphic. Single-nucleotide polymorphisms (SNPs) in TSHR have been specifically associated with Graves’ disease and not with autoimmune hypothyroidism in a Caucasian population (15, 16), although more recently associations with Hashimoto’s thyroiditis as well are described in a Chinese Han population (17). The functional consequences of these intronic SNPs are not entirely clear. They could give rise to RNA splice variants, increasing the level of potential autoantigenic TSHR-A subunits (16). Alternatively, SNP carriers may have fewer thymic TSHR mRNA transcripts, which may decrease central tolerance to TSHR (18). Multiple SNPs in TG have been associated with both Graves’ and Hashimoto’s diseases, but SNPs are located in exons in Caucasians and in introns in Japanese (19, 20). The immunoregulatory genes HLA class II, CTLA4, and PTPN22 were already recognized as risk factors in the 20th century by case–control studies. They are all involved in the immunological synapse, in which antigenic peptides complexed in HLA molecules are presented by antigen-presenting cells (not only macrophages and dendritic cells, but also B-cells) to T-cell receptors on T-cells. Polymorphisms in these genes are not specific for AITD, as they also confer susceptibility for other autoimmune diseases. A single amino acid variation in the peptide-binding cleft of HLA-DR, resulting in an arginine residue at position 74 of the β-chain, is strongly associated with Graves’ disease. An interaction with the SNP in exon 33 of TG has been described, suggesting that HLA-DRβ-Arg74 presents the disease-associated TG SNP alleles more efficiently to T-cells: an example of gene-gene interaction resulting in synergism (19, 21). Many more susceptibility loci have been detected since 2005 by genome-wide association studies (22). Among these are HLA class I molecules, which present endogenous antigens to the immune system (including those derived from viruses which are possible environmental triggers for AITD). HLA-C demonstrated a much stronger association with Graves’ disease in Caucasians than HLA-DRB1, DQA1, and DQB1, with HLA-C*07 predisposing and HLA-C*03 and HLA-C*16 being protective; HLA-B was found to be associated independent of HLA-C (23). In Han Chinese, HLA-B was a risk factor for Hashimoto’s disease (24). CD40, a co-stimulator of antigen-presenting cells, is also expressed on nonimmune cells such as thyrocytes. An SNP in the Kozak sequence of CD40 is associated with Graves’ disease in Caucasians and Koreans, but not in Taiwanese (19, 25). It increases the expression of CD40 mRNA and protein in the thyroid. A polymorphism in the Fc receptor-like 3 gene (FCRL3) is predisposing for several autoimmune diseases including Graves’ disease, in both Caucasians and Chinese Han population (26, 27). The association with FCRL5 is secondary to the effect of FCRL3 (26). The gene encodes for a member of the immunoglobulin receptor superfamily, expressed on B-cells. FCRL3 polymorphisms may be involved in the pathogenesis of Graves’ disease by excessive inhibition of B-cell receptor signaling and the impairment of suppressing function of T_{reg} (27). Two other genes, IL2RA and FOXP3, encode markers for T_{reg}-cells and are involved in immunological tolerance. CD25 is a marker for the interleukin 2 (IL2) receptor α chain present predominantly on CD25+T-cells, and it is a susceptibility locus for Graves’ disease (28). 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 immunodysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome. 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 (29, 30). The location of
FOXP3 on the X chromosome might contribute to the female preponderance of AITD. Mutations in autoimmune regulator (AIRE) 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); thus AIRE is not considered as a susceptibility gene contributing to the more common autoimmune endocrinopathies (31,32). Genome-wide association studies continue to detect additional genes and loci conferring risk for AITD (21).

What can be learnt from this multitude of genetic studies? First, it has provided a deeper insight into the molecular mechanisms involved in the immunopathogenesis of AITD. But at the same time it must be acknowledged that the mechanism of action of many susceptibility loci is incompletely understood (why are so many SNPs located in noncoding parts of the gene?), that the mechanisms are much more complex than previously thought, and that we have hardly started studying gene–gene and gene–environment interactions. Secondly, we have a better understanding as to why autoimmune diseases are associated with each other, and so often cluster in the same patient. Thirdly, the OR of each locus for AITD is rather low in the order of 1.5–2.0, with slightly higher OR of 2.0–4.0 for HLA. Combining the effects of four HLA loci and five non-HLA loci, they accounted for only 9.3% of heritability of Graves' disease in a Chinese study (33). Twin studies have convincingly demonstrated that genetic factors contribute about 70% to the development of AITD, leaving only 30% for environmental factors (34). The data strongly suggest that there must be many more still undetected susceptibility genes, each variant contributing just a little to the development of AITD. Fourthly, application of knowledge about susceptibility genes is slowly entering clinical practice. Some polymorphisms have predictive value for whether or not GH will recur after a course of antithyroid drugs (35, 36). One may expect that susceptibility genes are going to play a substantial role in personalized medicine.

Environmental factors: new players

Smoking

Smoking is a well-established risk factor for Graves' disease. The OR for GH is 3.30 (95% CI, 2.09–5.22) in current smokers when compared with never smokers. The OR for Graves' ophthalmopathy is even higher: OR 4.40 (95% CI, 2.88–6.73) in ever smokers vs never smokers. The risk disappears a few years after cessation of smoking: the OR for Graves' disease in ex-smokers vs never smokers is 1.41 (95% CI, 0.77–2.58), not significant any longer. These figures were already published in a 2002 meta-analysis, which at that time also concluded that smoking was not associated with hypothyroidism (37). In the last few years, however, convincing evidence has been obtained that current smoking protects against (autoimmune) hypothyroidism. Although smoking is an old player in the relation between environmental exposures and autoimmunity, the discovery of its protective effect allows the label 'new player' (Fig. 2). The evidence is derived from large cross-sectional population-based surveys and from prospective observational studies. In NHANES III, the prevalence of TSH >4.5 mU/l is lower in smokers than in nonsmokers (2.6 vs 5.5%, relative risk (RR) 0.5 with 95% CI, 0.4–0.6), in a dose-dependent manner (38). In the Norwegian HUNT study, the ORs (current smokers vs never smokers) for subclinical and overt hypothyroidism are 0.54 (95% CI, 0.45–0.66) and 0.60 (95% CI, 0.38–0.95) respectively in women; the corresponding figures in men are 0.37 (95% CI, 0.26–0.52) and 0.51 (95% CI, 0.15–1.73). ORs for former smokers were not significant (39). In this study, the cause of hypothyroidism was not specified but most probably it was Hashimoto's thyroiditis because subjects with previously known and treated thyroid disease had been excluded. Subsequent studies indeed demonstrate a protective effect...
against Hashimoto’s disease. In NHANES III, TPO-Abs were present in 11% of smokers vs 18% in nonsmokers (OR 0.57; 95% CI, 0.48–0.67), with a dose–response relationship (38). In the Amsterdam AITD cohort study at baseline, 25% of subjects, with TPO-Ab at baseline, smoked vs 38% of subjects without TPO-Ab (OR 0.69; 95% CI, 0.48–0.99) (40). In Denmark, smoking was also negatively associated with Tg-Ab and to a lesser degree with TPO-Ab (41). Follow-up of subjects in the Amsterdam AITD cohort, who had no thyroid antibodies at baseline, revealed that cessation of smoking increased the risk of de novo development of TPO-Ab and Tg-Ab (42); there was a significant trend toward more quitters in subjects who progressed to autoimmune hypothyroidism than in subjects who remained euthyroid (4). In the prospective DanThyr study, patients with newly diagnosed autoimmune hypothyroidism had more often quit smoking in the last 2 years than controls (16.4 vs 3.4%) (43). The increased risk after smoking cessation, however, was transient: ORs < 1, 1–2, and 3–10 years after quitting are 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. Finally, using Danish nationwide registers, mothers who smoke during pregnancy have a subsequent decreased risk of developing hypothyroidism (hazard ratio (HR), 0.75; 95% CI, 0.70–0.81) and an increased risk of hyperthyroidism (HR, 1.38; 95% CI, 1.27–1.49) (44). Thus, current smoking diminishes the risk of developing TPO-Ab/Tg-Ab and autoimmune hypothyroidism in a dose-dependent manner, but the protective effect disappears a few years after quitting smoking. The contrast with the increased risk of smoking for GH is striking, but poorly understood. One may hypothesize involvement of nicotine, which reduces the severity of experimental autoimmune encephalomyelitis by a shift from pathogenic Th1 and Th17 responses to protective Th2 responses. Anatabine – a tobacco alkaloid with a structure similar to nicotine – reduces the incidence and severity of experimental autoimmune thyroiditis (45).

**Alcohol**

Case–control studies nested in the Amsterdam AITD cohort did not observe an association between alcohol consumption (defined as >10 units/week) and de novo occurrence of TPO-Ab, but the prevalence of alcohol consumption was lower in subjects developing autoimmune hypothyroidism during the 5-year follow-up than in those remaining euthyroid (6.7 vs 23.7%) (46). A population-based case–control study in Denmark also provided evidence for a protective role of alcohol consumption for the development of autoimmune hypothyroidism. ORs were as follows: 1.98 (95% CI, 1.21–3.33) for 0 alcoholic units/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 (47). Abstainers are 2.17 times more likely to develop autoimmune hypothyroidism than nonabstainers (≥ 1 unit/week). Remarkably, moderate alcohol consumption is likewise associated with a considerable reduction in the risk of GH. ORs were 1.73 (95% CI, 1.17–2.56) for 0 units/week, 1.00 for 1–2 units/week (reference), 0.56 (95% CI, 0.39–0.79) for 3–10 units/week, 0.37 (95% CI, 0.21–0.65) for 11–20 units/week, and 0.22 (95% CI, 0.08–0.60) for ≥ 21 units/week (48). No interaction was found with the type of alcohol (wine vs beer), smoking habits, gender, or iodine intake. Two previous case–control studies reported similar protective effects of alcohol for GH with ORs of 0.3 (95% CI, 0.2–0.7) and 0.4 (95% CI, 0.2–0.8) (49, 50), although not confirmed in another study (51). The observed associations likely indicate a cause-and-effect relationship in view of the strength and the consistency of the associations and the presence of a dose–response effect. Moreover, alcohol is known to protect against other autoimmune diseases such as rheumatoid arthritis and type 1 diabetes. The most likely explanation for the protective effect of alcohol on AITD is that moderate alcohol consumption has a beneficial impact on the immune system compared with alcohol abuse or abstinence (52). However, the effect of alcohol on the immune system is complex, and how alcohol suppresses autoimmunity remains incompletely understood. Alcohol has also a direct toxic effect on the thyroid gland, which, however, is difficult to reconcile with its protective effect against hypothyroidism.

**Selenium**

Glutathione peroxidases and thioredoxin reductases are selenoproteins involved in regulation of the redox state and protection from oxidative damage. The thyroid gland contains more selenium per gram of tissue than any other organ. The enzyme GPx3 protects thyrocytes from oxidative stress generated by the action of H2O2. Low selenium levels have been associated with poor immune function (53, 54). It has thus been hypothesized that even mild nutritional selenium deficiency may promote the initiation or progression of thyroid autoimmunity. To test the hypothesis that selenium supplementation decreases the serum concentration of TPO-Ab, six randomized clinical trials have been performed (55, 56, 57, 58, 59, 60).
The results are equivocal: selenium compared with placebo decreased TPO-Ab in three studies but not in the other three studies. The discrepant results could not be explained from the use of sodium selenite or seleno-methionine, sample size, baseline serum concentrations of Se (ranging from 69 to 75 µg/l), and TPO-Ab (ranging from 172 to 1875 kU/l), nor be explained from the concomitant use of levothyroxine (l-T₄) (60). At present, it cannot be excluded that selenium supplementation will decrease TPO-Ab specifically in regions with iodine deficiency (which increases the amount of oxidative stress to the thyroid gland), or when given for a period longer than 6 months. Selenium supplementation has proven to prevent deterioration of mild Graves’ ophthalmopathy (61) and the post partum surge of TPO-Ab and thyroid dysfunction (62). These randomized clinical trials were performed in countries with marginally low selenium blood levels, and it is not known if equally favorable results are seen in selenium-replete areas. The selenium dose used so far is 200 µg daily. In the Dutch trial, it increased plasma Se concentrations from 73 µg/l at baseline to 96 µg/l at 3 months and 95 µg/l at 6 months; obviously, a plateau had been reached within 3 months, which was also true for Selenoprotein P (60). Supplementation of selenium to people who already have adequate Se intake with their food might increase their risk of developing type 2 diabetes, particularly when baseline plasma Se concentrations are ≥122 µg/l (63). There may be health benefits and no extra risk for people with baseline (Se) <122 µg/l by raising their selenium status, perhaps to 130–150 µg/l, which is associated with minimal mortality (63). Finally, it might be possible that individual requirements for selenium differ because of polymorphisms in selenoprotein genes. A Cochrane systematic review concludes that data at present do not allow confident decision making about the use of selenium supplementation for Hashimoto’s thyroiditis (64).

**Vitamin D**

Many immunocompetent cells (monocytes, macrophages, dendritic cells, T-lymphocytes, and B-lymphocytes) express the vitamin D-activating enzyme CYP27B1 and the vitamin D receptor (VDR). The active hormone 1,25(OH)₂D (derived from the systemic circulation or from local conversion of 25(OH)D) binds to VDR and modulates both the innate and adaptive immune systems (65, 66). Low vitamin D levels have been identified as a risk factor for various autoimmune diseases such as type 1 diabetes, rheumatoid arthritis, multiple sclerosis, and Crohn’s disease. The situation with respect toAITD is less clear. The prospective Amsterdam AITD cohort was used for a nested case–control study: cases and controls had baseline normal TSH and no thyroid antibodies, but during the 5-year follow-up, cases did develop de novo TPO-Ab whereas controls remained without antibodies. Controls were carefully matched to cases not only for the duration of follow-up, but also for conditions known to affect vitamin D levels such as age, BMI, smoking status, estrogen use, and month of blood sampling (not for sex because the Amsterdam AITD cohort consists of women only). Serum 25(OH)D and 1,25(OH)₂D concentrations were not different between cases and controls, neither at baseline nor at the time of seroconversion (67). The remaining studies are all cross-sectional in nature. Some of them do find lower vitamin D levels in subjects with TPO-Ab (68, 69, 70), while others do not (71, 72). Interpretation of their study results is difficult because controls were not always matched to cases for the many other conditions affecting vitamin D levels. For example, in one study, the inverse relationship between vitamin D status and thyroid antibodies was lost after correcting for sex and age (72). However, a recent study from Korea in 6685 subjects undergoing routine health checkups tried to avoid confounding factors and observed lower 25(OH)D levels in women with TPO-Ab than in control women without TPO-Ab (22.0 vs 23.5 ng/ml; P=0.03); there was no difference in men (73). The prevalence of TPO-Ab in women with vitamin D deficiency, insufficiency, and sufficiency was 21.2, 15.5, and 12.6% respectively (P=0.027). ORs adjusted for age, BMI, serum calcium, smoking, menopause, and season were (relative to an OR of 1.00 for vitamin D sufficiency) 1.95 for vitamin D deficiency and 1.31 for vitamin D insufficiency. Whether vitamin D supplementation will prevent AITD has not been studied. Finally, polymorphisms in the VDR gene are associated with AITD: whereas Apal or Fokl carry no risk, the BsmI and TaqI polymorphisms decrease the risk for AITD (OR, 0.80; 95% CI, 0.71–0.91 and OR, 0.85; 95% CI, 0.76–0.96 respectively) (74).

**Environmental factors: old players**

**Iodine**

Thyroid antibodies and autoimmune hypothyroidism are more common in iodine-replete areas than in iodine-deficient areas. Further elegant proof of this has recently been obtained from population-based studies in Denmark. Prevalence figures before and after mandatory iodization
of salt were 14.3 and 23.8% for TPO-Ab, and 13.7 and 19.9% for Tg-Ab respectively; ORs were 1.80 (TPO-Ab) and 1.49 (Tg-Ab). The increase in frequency was most pronounced in young females and at low concentrations of antibodies (75). The incidence rate of hypothyroidism at baseline was 38.3/100 000 per year, increasing to 47.2/100 000 per year 5–7 years after iodine fortification of salt (RR, 1.23; 95% CI, 1.07–1.42) (76). Voluntary iodine prophylaxis in a small rural community in Italy also resulted in an increased frequency of thyroid antibodies (12.6 vs 19.5%) and hypothyroidism (2.8 vs 5.0%) 15 years later (77). Implementation of iodine prophylaxis in this study contributed to thyroid autoimmunity by unmasking a cryptic epitope on Tg (78).

**Stress**

It has been known for a long time that stress could be a provocative factor for GH, although the evidence is circumstantial (79). Annual assessment of stressful life events and daily hassles in the prospective Amsterdam AITD cohort demonstrated no association between stress exposure and *de novo* occurrence of TPO-Ab or autoimmune hypothyroidism (80). Thus, in contrast to...
Graves’ disease, stress is apparently not involved in Hashimoto’s thyroiditis.

Infections

Infection with *Yersinia enterocolitica* (YE) has long been implicated in the pathogenesis of AITD, because IgG from Graves’ patients inhibit binding of TSH to outer membranes of YE, and IgG from patients with YE infection inhibit binding of TSH to thyroid membranes. Biological plausibility of an association has recently been enhanced by cross-reactivity between YE outer membrane proteins (YOP) and epitopes of TSHR antibodies (81, 82, 83). Clinical evidence about the role of YE infection remains controversial. In twin pairs discordant for Graves’ disease, subjects with Graves’ disease had an increased OR of YE infection (IgA, 1.84; 95% CI, 0.99–3.45 and IgG, 1.90; 95% CI, 1.02–3.55) (84). The Amsterdam AITD cohort, the only prospective study in this area, however, did not find any evidence of an association between YOP IgA or IgG status and *de novo* occurrence of thyroid antibodies or development of overt autoimmune hyper/hypothyroidism (85). Hepatitis C virus (HCV) might be ‘the only infectious agent that is clearly associated with an increased risk for autoimmune thyroiditis’ (86). HCV can infect human thyrocytes resulting in production of proinflammatory cytokines, which may enhance the autoimmune response (87). Indeed, thyroid antibodies are more frequent in children with untreated HCV infection than in controls (88). Enteroviruses have been detected in thyroid tissue of subjects with Hashimoto’s thyroiditis (89). It is hypothesized that gut microbiota may trigger Hashimoto’s thyroiditis (90). One can even speculate that the profound changes in gut microbiota induced by smoking cessation (91) are related to the loss of the protective effect of current smoking for Hashimoto’s disease.

Drugs

Treatment with interferon α frequently results in Hashimoto’s thyroiditis (less often in Graves’ disease), especially in women with pre-existent TPO-Ab (92). It may induce autoimmune hypothyroidism by direct toxic effects on thyrocytes as well as provocation of destructive bystander immune responses (93). Treatment with alemtuzumab (anti-CD52 MAB) or highly active anti-retroviral therapy causes depletion of lymphocytes; in the recovery phase, when CD4+ T-cells rise, there is a risk of the emergence of autoreactive clones. Thus, both drugs could induce the immune reconstitution syndrome, expressed as GH and less frequently as hypothyroidism (94).

Natural history of AITD and prevention

Figure 3 schematically depicts the natural history of AITD. In stage I, all thyroid function tests are normal and thyroid antibodies are absent in serum. But at this stage in subjects at risk to develop AITD (like those with a family history of AITD), evidence of immune activation can already be found from a characteristic pattern of serum proteins related to growth/connective tissue abnormalities and migration/accumulation abnormalities of macrophages and dendritic cells (95). In stage II, thyroid antibodies become detectable in serum. The concentration of thyroid antibodies gradually increases, TSH but not FT₄ becomes abnormal and we are entering stage III: subclinical thyroid dysfunction. The duration of subclinical hyperthyroidism until overt GH occurs (stage IV) is much shorter than the duration of subclinical hypothyroidism until overt HH develops (stage IV) (4, 96). A number of prospective population-based studies have identified serum TSH and thyroid antibodies as predictors of future hypothyroidism and hyperthyroidism (97, 98, 99, 100). The quantitative risk of developing overt AITD can be estimated by the Thyroid Events Amsterdam (THEA) score based on TSH, TPO-Ab, and family history of AITD (97). The question arises whether a person at risk can take preventive action in order to diminish the risk. As shown in Table 1, the options are very limited. To stop smoking will steer the natural history away from Graves’ disease toward Hashimoto’s disease. Moderate alcohol intake would make sense, but there is insufficient evidence to justify preventive medication with selenium or vitamin D. Moreover, to refrain from pregnancy and to avoid stress are not very helpful recommendations either.

<p>| Table 1 Possible preventive interventions to decrease the risk of developing autoimmune thyroid disease. |
|--------------------------------------------------|-------------------------------------------------|------------------------------------------|--------------------------------------------------|</p>
<table>
<thead>
<tr>
<th>Preventive intervention</th>
<th>Risk of TPO-Ab</th>
<th>Risk of Hashimoto’s hypothyroidism</th>
<th>Risk of Graves’ hyperthyroidism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stop smoking</td>
<td>Increase</td>
<td>Increase</td>
<td>Decrease</td>
</tr>
<tr>
<td>Use alcohol</td>
<td>No change</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Use selenium</td>
<td>?</td>
<td>Decrease</td>
<td>Decrease</td>
</tr>
<tr>
<td>Use vitamin D</td>
<td>Decrease</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>Avoid pregnancy</td>
<td>No change</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>Avoid stress</td>
<td>No change</td>
<td>No change</td>
<td>No change</td>
</tr>
</tbody>
</table>

www.eje-online.org
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

Funding
This review did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

References
12 Brix TH, Hansen PS, Kyrivik KO & Hegedus L. Aggregation of thyroid antibodies in twins from opposite-sex pairs suggest that microchimerism may play a role in the early stages of thyroid autoimmunity. Journal of Clinical Endocrinology and Metabolism 2009 94 4439–4443. (doi:10.1210/jc.2009-0813)
23 Simmonds MJ, Howson JM, Heward JM, Carr-Smith J, Franklin JA, Todd JA & Gough SC. A novel and major association of HLA-C in Graves’ disease that eclipses the classical HLA-DRB1 effect. Human Molecular Genetics 2007 16 2149–2153. (doi:10.1093/hmg/ddm165)
27 Simmonds MJ, Howson JM, Heward JM, Carr-Smith J, Franklin JA, Todd JA & Gough SC. A novel and major association of HLA-C in Graves’ disease that eclipses the classical HLA-DRB1 effect. Human Molecular Genetics 2007 16 2149–2153. (doi:10.1093/hmg/ddm165)


41 Bulow Pedersen I, Laurberg P, Knudsen N, Jorgensen T, Perrild H, Ovesen L & Rasmussen LB. Smoking is negatively associated with the presence of thyroglobulin autoantibody and to a lesser degree with thyroid peroxidase autoantibody in serum: a population study.


93 Akeno N, Smith EP, Stefan M, Huber AK, Zhang W, Keddache M & Tomer Y. IFN-α mediates the development of autoimmunity by both direct tissue toxicity and through immune cell recruitment mechanisms. *Journal of Immunology* 2011 **186** 4693–4706. (doi:10.4049/jimmunol.1002631)


97 Strieder TG, Tijssen JG, Wenzel BE, Endert E & Wiersinga WM. Prediction of progression to overt hypothyroidism or hyperthyroidism in female relatives of patients with autoimmune thyroid disease using the Thyroid Events Amsterdam (THEA) score. *Archives of Internal Medicine* 2008 **168** 1657–1663. (doi:10.1001/archinte.168.15.1657)

