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Review Article

The Genetic Etiology of Autoimmune Thyroid Diseases: The Neverending Story

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Abstract

Hashimoto's thyroiditis (HT) is the most prevalent autoimmune thyroid disorder. Autoimmune thyroid diseases (AITD), including Graves' disease (GD) and Hashimoto's thyroiditis, arise due to complex interactions between environmental and genetic factors. Each is presenting with distinct clinical features. Significant progress has been made in our understanding of the mechanisms leading to AITD. Because of the complex nature of AITD, caused by their polygenic nature and a complex mode of inheritance, there are still more questions to be answered than answers that can be given, especially about the nature of Hashimoto's thyroiditis. Unlocking the genetic contribution to AITD will hold one of the keys to understanding disease pathogenesis and developing improved treatments. Common HT and GD genes have been identified, as well as genes that are characteristic for only one of those diseases. In this review, we summarize the findings on the genetic susceptibility to AITD focusing on emerging mechanisms of susceptibility.

Abbreviations

HT - Hashimoto's thyroiditis.
AITD - Autoimmune thyroid disease
TPO - Thyroid peroxidase
Tg - Thyroglobulin
AITD - Autoimmune thyroid diseases
GD - Graves' disease
MHC - Histocompatibility complex
ATPO - Antithyroid peroxidase
TGAB - Antibodies and antithyroglobulin
AITDs - Graves' disease and Hashimoto's thyroiditis
nsSNPs - Nonsynonymous coding single-nucleotide polymorphisms protein tyrosine PTPN22 - phosphatase non-receptor type 22
PCR-RFLP - Polymerase chain reaction-restriction fragment length polymorphism
CTLA4 - Cytotoxic T lymphocyte-associated- 4
HLA - Human leukocyte antigens
Th - Helper T lymphocytes
Tg - Thyroglobulin
TPO - The thyroid peroxidase
TSHR - TSH Receptor
APCs - Antigen-presenting cells
IFN-α - Interferon-alpha
ICAM-1 - Intracellular adhesion molecule-1
XCI - X-chromosome inactivation

Introduction

Hashimoto's thyroiditis (HT), the most prevalent autoimmune thyroid disease (AITD), is becoming more and more commonplace in the recent years [1,2]. Since the entity of Hashimoto's thyroiditis (HT)described by Hashimoto in 1912, it has evolved as the most common aetiology of acquired hypothyroidism inadolescents and children [3,4]. Also, It's known as chronic lymphocytic thyroiditis or autoimmune thyroiditis.HT is primarily a disease of adult and senior women between 45-65 years. When compared to men it has 10 to 20 times more female sex predilection [5,6]. HT in children rarely occurs before four years of age with a peak age of incidence around adolescence (10-12 years) [7].

Goitre associated with hypothyroidism characterises HT, loss of thyroid follicular cells, circulating autoantibodies to thyroid-specific antigens: thyroid peroxidase (TPO) and thyroglobulin (Tg); infiltration of the thyroid by T and B cells reactive with these thyroid antigens [8]. The presence of autoantibodies against Tg and TPO is a hallmark of early stages of HT [9], and the lymphocytic infiltration of the thyroid gland leads to apoptosis of thyrocytes and hypothyroidism [10]. Hashimoto's thyroiditis cases have been increasing noticeably during the last few years [2]. Intrathyroidal lymphocytic infiltration is Fig. (1) followed by the gradual destruction of the thyroid gland which may lead to subclinical or overt hypothyroidism [1].

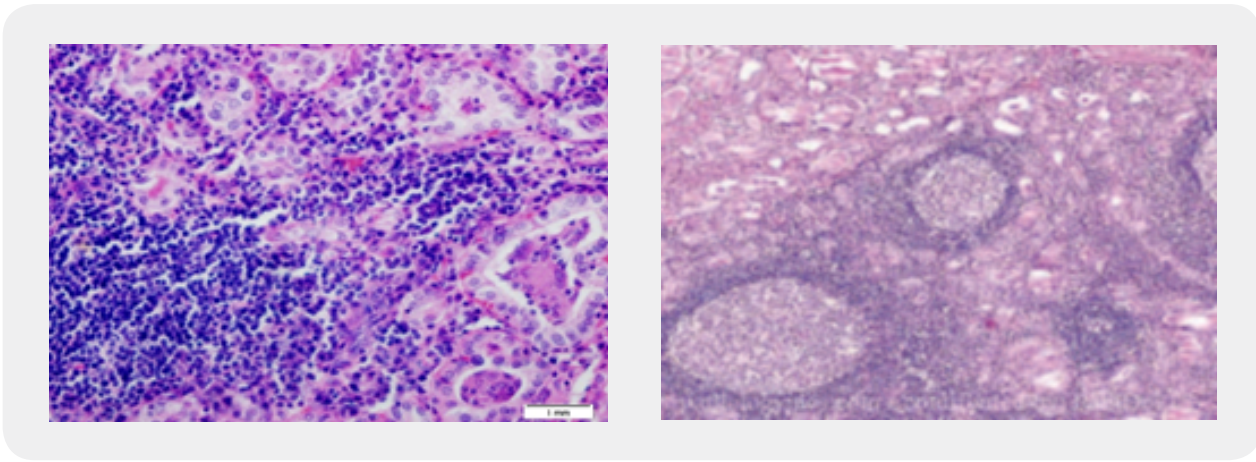

Fig. 1: *Histological section thyroid gland affected with Hashimoto's thyroiditis*

The classical clinical presentation of HT consists of three phases - initial hyperthyroid (subclinical or overt); euthyroid and final irreversible hypothyroid with/without goitre. The duration of these phases is neither discrete nor sequential in a given individual varying from weeks to months Years later after the diagnosis long euthyroid period ending up in hypothyroidism. The diagnosis of HT based on seropositivity of elevated anti-TPO antibody titer along with thyroid dysfunction with or without goitre. Radiologically, ultrasound criteria of diffuse hyperechogenicity [1,11] and pathologically, FNAC features of lymphocytic infiltration, fibrosis, Hurthle cells, plasma cells and thyrocyte destruction are additional confirmatory criteria [6,12].

Pathogenesis

Significant progressis made in our understanding of the mechanisms leading to AITD. AITD, including Graves' disease and Hashimoto's thyroiditis, arise due to complex interactions between environmental and genetic factors [13,14]. Each is presenting with distinct clinical features [15]. Still,there are more questions to be answered than answers that can bestated as a consequence of the complex nature of AITD, caused by their polygenic nature and a complex mode of inheritance, especially about the nature of Hashimoto's thyroiditis [2]. The exact mechanisms responsible for the disease development are still not completely understood in spite of a very high HT prevalence. Significant advancesin the knowledge of the causes and pathogenesis of autoimmune thyroid disease which most frequently occurs in the form of Graves' disease (GD) or HT [1].

The pathogenesis of Hashimoto's thyroiditis is a complicated multistep process which involves various genetic, environmental and immunological factors (Fig. 2). When individuals of genetically predisposed are exposed to the above mentioned ecological factors,the initial inflammatory changes in the disease process are triggered. The major histocompatibility complex (MHC) class 2 antigen-presenting cells, which include dendritic cells and macrophages, invade the thyroid gland after the initial inflammatory process. These cells present the autoantigen components of the thyroid gland to the immune system for processing. Within the myriad of potential auto-antigens, thyroglobulin is believed to play a central role in the pathogenesis of this disease [16]. The thyroglobulin was reported to have nearly 40 different types of epitopes, which play

anessentialrole in the aetiopathogenesis of the disease [17]. However, in contrast to the epitope recognition pattern ofhealthy individuals, the epitope recognition pattern of the antibodies in autoimmune thyroiddisease is altered activatinginflammatory and immune processes [18]. Thyroid peroxidase, an enzyme that catalyses the oxidation of iodine, also plays a significant role as an autoantigen in the disease pathogenesis. Moreover, 180 different types of thyroid peroxidase antibodies are identified, thus far.

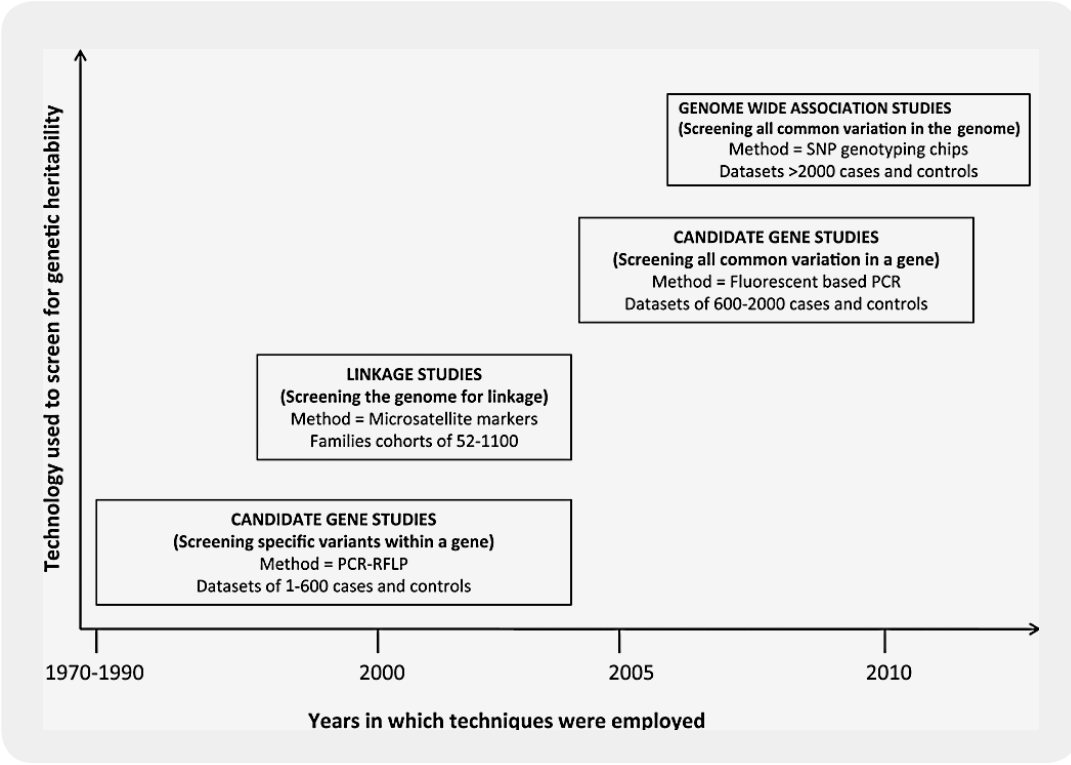

Fig. 2: *shows a timeline of the different techniques used to screen for the genetic heritability to AITD. Since the 1970s different techniques are used to screen for the genetic contribution to AITD. This figure shows the variousmethods that were used since the 1970s through to the present time, the size of datasets in which these techniques were applied and how much genetic variation was captured. PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism [28].*

Formation of autoreactive cells directed against the thyroid gland is considered asa significant step in the pathogenesis is the which could result from defects in central tolerance or errors in the peripheral tolerance. Loss of immune tolerance is associated with genetically determined immune abnormalities or with the lack of regulatory T-cells which force the suppressive function [19]. The previous processis followed by formation, clonal extension, and maturation of self-reactive T-lymphocytes and B-lymphocytes in the draining lymph nodes.

Then, this step is followed by a central phase of autoimmunity, characterised by the unconscious production ofautoantibodiesand self-reactive cells in response to the presented antigens. This process primarilytakes placein the lymph nodes but as the disease continuesthe production process shifts to the thyroid gland

where theevolution of lymphoid tissue follows. Stimulated B-lymphocytes produce antithyroid peroxidase (ATPO) antibodies and antithyroglobulin (TGAB)which are directedtoward thyroid cells. The autoreactive T-cellsinfiltrate the thyroid gland and mediate destruction through cytotoxicity with the help of CD+8 cells. The macrophages which are stimulated in this process produce numerous cytokines which, along with antibodies, initiate the process of tissue destruction through apoptosis [20].

Caspases, which are self-activated through proteolytic cleavage, induce enzymes which are directly involved in the destruction of the thyroid gland. In normal thyroid gland, the production of new cells and the removal of old cellsaredynamicallyadjustedso that a constant proportion of functioning cells is always present. During the disease, the control over the destruction of cells in the thyroid gland is lost. Etiopatho-genetically, HT has been held as an organ-specific immunological disease, later evidence and a surge of molecular researchers suggest that both genetic and environmental factors influence its genesis [21,22].

Genetic Susceptibility

One of the factors that play anessential role in the deregulation of the natural destructive mechanisms in the thyroid gland is genetic susceptibility. The genetic analysis of HT shows two types of susceptibility genes-immune regulatory and thyroid-specific genes [23,24]. Thyroid-specific genes act on inter- and intra-cellular milieu responsible for normal hormone synthesis. Immunomodulatory genes such as HLA-DR, CTLA-4, PTPN22 determine the thyroid auto-immunity [1]. Thyroid-specific genes such as Top, NIS, Duox2, thyroglobulin, TSH receptor have also implicated in pathogenesis and thyroid dysfunction [6]. Also, the exact genotype-phenotypic correlations and risk categorisation of hypothyroid phenotypes resulting from these known mutations are mainly speculative [24].

Genetic vulnerability in combination with external factors (e.g. dietary iodine) isthought to initiate the autoimmune response to thyroid antigens in AITD [25]. Although somereplicated, genetic associations are emerging, providing insights into the underlying disease mechanisms, a significant component of the genetic association to AITD remains unknown [26].

AITDs (GD and HT) are complex genetic diseases which most likely have more than 20 genes contributing to the clinical phenotypes [1,27]. Unlocking the genetic contribution to AITD will hold one of the keys to understanding disease pathogenesis and developing improved treatments [28]. Common HT and GD genes are identified, as well as genes that are characteristic for only one of those diseases [2]. Seven genes have been shown to contribute to the aetiology of AITD. The first AITD gene discovered inHLA-DR3is contributed with both GD and HT. The putative GD and HT susceptibility genes include both immune-modifying genes (e.g. HLA, CTLA-4) and thyroid-specific genes (e.g. TSHR, Tg) and it is likely that the final disease phenotype is a result of an interaction between these loci, as well as environmental influences [25].Candidate gene analysis, whole-genome linkage screening, genome-wide association studies (Figure - 2 timelines of genome-wide screening technologies),and the whole genome sequencing are the major technologies that have advanced this field that lead to the identification of at least seven genes whose variants are associated with AITD. One of the major ones is the HLA-DR gene locus [14].

A recent association scan using a genome-wide set of nonsynonymous coding single-nucleotide polymorphisms (nsSNPs) conducted in four diseases including GD, identified nine possible novel regions of association with GD [26]. The genes are known to be contributing fall into two categories: immune regulatory genes (including HLA, CTLA4, PTPN22, CD40, CD25, and FCRL3) and thyroid-specific genes (TG and TSHR) [14,27,28].

Candidate Genes

Several loci (genetic regions) that areassociated with AITD are mapped,and in some of these loci, putative AITD susceptibility genes areidentified (Fig. 3). Some of these loci predispose to a single phenotype (GD or HT), while other loci are common to both diseases, indicating that there is a shared genetic susceptibility to GD and HT [25,29]. Several other genes are also shown to confer susceptibility to AITD.It's currently believed that susceptibility to complex diseases including GD and HT involves mutation(s) in genes generally predisposing to autoimmune diseases, mutations in thyroid autoantigen-specific genes, and environmental factors [13,30]. Susceptibility to AITD varies between ethnic groups, which makes it more difficult to confirm linkage between the genes and disease rather than a simple association [31].

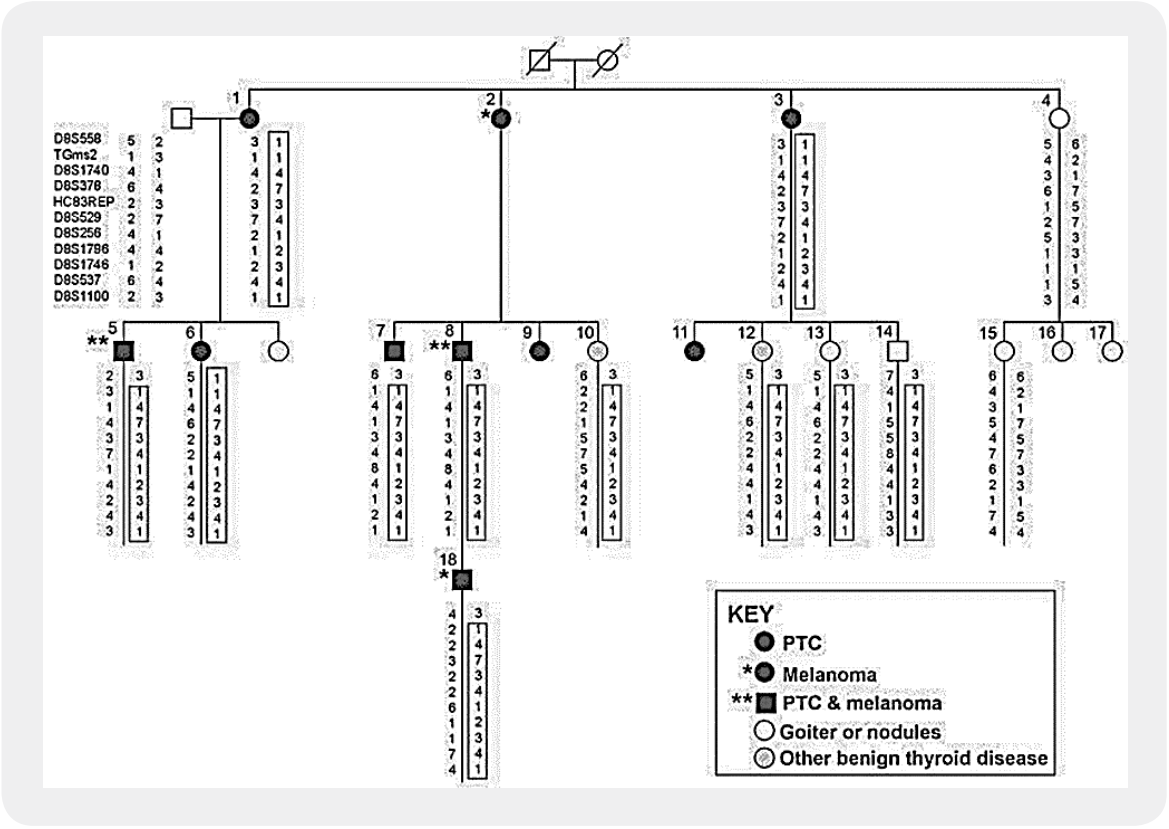

Fig. 3: *Chromosome loci found to be associated with Hashimoto's thyroiditis [2]. Haplotypes of microsatellite markers in members of family #1. A unique haplotype (boxed) co-segregates with PTC, melanoma, and some benign thyroid diseases.*

In Fig.3 grey bars on the left side of the chromosomes present regions pointed by whole-genome scans, based on references [29,32,33]. Black bars at the right, together with the descriptions, present regions accommodating the appropriate candidate genes.Crucial for antigen presentation seems to have the enormous impact. It is followed by protein tyrosine phosphatase non-receptor type 22 (PTPN22), the product of which – the protein Lyp – acts downstream of the T cell receptor, and cytotoxic T lymphocyte-associated- 4 (CTLA4), responsible for limiting immune responses. They serve as a background to the development of autoimmunity in general. Common allelic variations of genes encoding regulators of the immune system may underpin the molecular basis for gene-environment interactions [34]. Nevertheless, none of the immune-regulatory genes wasrevealed to be a dominant susceptibility gene for HT, as odds ratios for these chromosome loci in AITD were instead modest – 1.9 for HLA in HT [35], 1.45 for CTLA4 in HT [34] , and 1.43 for PTPN22 in GD [36], which suggested that the HLA region, as well as CTLA4 and PTPN22 genes,are contributed only to a lesser extent to the vulnerability to AITD. Therefore, other loci remain to be identified.

Thyroglobulin, encoded by the gene TG, is to date the thyroid-specific protein, which is believed to contribute to HT development. It is the main protein of the thyroid and plays a crucial role in the synthesis and storage of thyroid hormones. Anti-Tg is, together with anti-TPO, among the essential autoantigens in autoimmune thyroiditis [10].

Human Leukocyte Antigen (HLA) Genes

The first gene locus identified in association with the autoimmune thyroid disease was MHC region on the chromosome 6p21 which encodes HLA [37]. The compound of genes encoding HLA glycoproteins is located on chromosome 6p21, which is the region that has been stated to be of importance for AITD development [8]. This region contains many immune response genes. The HLA region is a greatly polymorphic region that composed of many immune response genes and is linked with the predisposition or protection to multiple autoimmune disorders [38,39].

The Controversy Over Major Genes in AITD

After the clarification that multiple genes are at work in AITD, it is likely that more than 20 potential genes contribute to the AITD phenotypes. But significant genes, those essential to disease development, have not been found [40]. A considerable gene should be involved in most of the patients with the disease, and the risk ratios, even for HLA, do not reveal such a gene. This most likely means that different genes combinations might produce similar clinical phenotypes or that epigenetic phenomenon are dominant.In the whole-genome screening of families, siblings, and populations with AITD, some sites areconfirmed for GD and HT susceptibility, but none of them has very high statistical values (LOD scores) [8,41].

The Role of T Cells

T lymphocytes play a serious role in the development of autoimmune diseases. In Hashimoto's thyroiditis, helper T lymphocytes (Th) become sensitised to thyroidal antigens by interacting with self-antigen presenting cells. This process causes an increase in the amount of autoreactive T and B lymphocytes, which infiltrate the

thyroid. B lymphocytes secrete thyroid autoantibodies, predominantly against Tg and TPO. The infiltration of lymphocytes destroys the thyroidal architecture, which clinically displays as hypothyroidism [42].

Cytotoxic T-Lymphocyte-Associatedprotein 4 (CTLA4) Gene

CTLA-4 gene, which is the second major immune-regulatory gene associated withautoimmune thyroid disease, located on chromosome 2q33, is one of the major known contributors to susceptibility to AITD [34,43]. CTLA4 is a T cell response suppressor that is initially contributed with susceptibility to autoimmune disorders, because evidence of linkage with chromosome 2q33, on which this gene is encoded, has been reported. The expression of CTLA-4 on the surface of T cells, induced by the activation of the T-cell receptor, results in suppression of T-cell activation [1]. Moreover, ctla4 knockout mice develop a lymphoproliferative disorder resulting in death from autoimmunity within 3–4 weeks postpartum [43].

CTLA-4 gene polymorphisms may reduce expression or function of the CTLA-4 antigen and may, therefore, contribute to the reduced inhibition of T-cell proliferation and subsequently increase susceptibility to an autoimmune response. Quite a few polymorphisms of the CTLA-4 gene in HT patients were studied. Among them, the initially reported (AT)n microsatellite CTLA-4 polymorphism in the 3' untranslated region (UTR) was found to be contributedwith HT in Caucasian [44] and Japanese patients [45], but not in Italian population [46].

Aside from being contributed with HT, CTLA-4 seems to be the significant TAbvulnerability gene. Linkage of the CTLA-4 region to the presence of TAbs was revealed by a whole genome linkage analysis [47], and subsequently, CTLA-4 was confirmed as thecentral locus for TAb status also in a broader information set [48]. There have, however also been studies that did not confirm the association of mutations in this gene with increased susceptibility to HT [13,49,50].

Thyroid Specific Genes: Thyroglobulin Gene

Thyroglobulin functions in the thyroid as the substrate for thyroid hormone synthesis. The thyroglobulin (Tg) protein is the major thyroidal protein antigen and is a precursor to thyroid hormones, also present in the circulation, which makes it an easy target of the autoimmune response. Tg is also a key antigen in AITD as evidenced by the fact that HT is characterised by anti-thyroglobulin antibodies which are recognised, in 75% of patients [51]. Two studies have found evidence for linkage between a locus on chromosome 8q24 and AITD. Tomer *et al.* group showedcompelling evidence for linkage at the Tg gene locus with an MLS of 3.5 between D8S514 and D8S284 [52]. This locus contained the Tg gene, and linkage of this locate with HT, and autoimmune thyroid disease was first recognised by a Japanese and an American whole genome studies [8,29]. Sequencing of the Tg gene identified several non-synonymous SNPs that were associated with AITD [53]. Following fine mapping of this locate exposed Tg gene as one of the significant thyroid specific vulnerability genes, linked and associated with the autoimmune thyroid disease [52,53]. Later, different alleles of various microsatellite markers and different SNPs of Tg gene were related to HT, possibly affecting its expression, antigenicity, iodination, or binding to HLA.

Autoimmune thyroid disease susceptibility genes

The thyroid peroxidase (TPO) gene

The TPO gene is most likely not a dominant susceptibility gene for AITD. Studies in the Japanese population have shown associations of GD with HLA-B35 [54]. However, other class I and II HLA alleles have also been reported to be increased in Japanese GD patients [55]. In Chinese, an increased frequency of HLA-Bw46 was reported [56]. It is interesting that in Asians the HLA associations are with class I genes while in Caucasians they are with class II genes. Thesemight imply that other non-HLA genes in the region in linkage disequilibrium with class I genes are the susceptibility genes in Asians. In contrast, DR3 is believed to be the causative gene in Caucasians. In African–Americans an increased frequency of HLA DRB3*0202 has been reported [57]. Interestingly, one study of a mixed population in Brazil showed association with HLADR3 implying that this allele may confer susceptibility in other ethnic groups and not just Caucasians [58]. Also, HLA association studies in HT have not been consistent in non-Caucasian ethnic groups, e.g. HLADRw53 in Japanese [59], and HLADR9 in Chinese [60]. The association between GD and the CTLA-4 30 UTR microsatellite and A/G49 SNP are consistent across populations of different racial backgrounds such as Japanese [45], and Koreans [61], and in Caucasians [62]. The frequency of the G allele and the GG genotype of the CTLA-4 A/G49 SNP was significant to be reported higher in GD patients who did not go into remission after five years on anti-thyroid medications in Japanese [63]. Similarly, CTLA-4 has been reported to be associated with HT in non-Caucasians including Japanese [45].

Other immune-related genes

The IgH gene was found to be contributedby GD in the Japanese [64]. However, these results are not reproduced in Caucasians [65]. This might imply that the IgH gene may contribute to the susceptibility to GD only in the Japanese if a founder effect exists.

Antibodies against Tg are a common marker and one of the primary diagnostic criteria for AITD and appear at the very early stages of their development [10]. At first, the 8q24 locus containing the TG gene has been reported to be linked with AITD in general [8] or HT alone [29]. Afterwards, different mutations in the gene TG are identified, spread through its 48 exons, as well as the introns [13,51].

Because of the importance of Tg in thyroid functioning and the presence of anti-Tg antibodies in affected subjects, as well as a lot of evidence of linkage or association of the encoding region and/or mutations with AITD, TG is still stated as a highly probable susceptibility gene for AITD development. It is currently the only thyroid-specific gene that is being contributed with HT development. It has however been stressed that Tg mutations are not exclusively responsible for autoimmune thyroiditis and that the identification of additional HT susceptibility genes would help to understand the molecular mechanism underlying the induction of thyroid autoimmunity [66].

TSH Receptor (TSHR) Gene

Associations between AITD and TSHR microsatellite markers have been reported in the Japanese [45]. These results suggest that TSHR gene may contribute to the susceptibility to GD only in Japanese especially

if there is a founder effect [67]. The TSHR gene is located on chromosome 14q. It's a prime candidate gene for GD since GD is caused by autoantibodies that bind to and stimulate the TSH receptor. Several TSHR SNPs were tested for association with GD, including non-synonymous SNPs in the extracellular TSH receptor domain and the intracellular domain of the TSHR; all of these gave conflicting results. However, linkage studies demonstrated significant evidence for linkage of GD with a locus on chromosome 14q harbouring the TSHR gene [8]. Later, it wasreported that non-coding SNPs in intron 1 of the TSHR confer the association with GD [51].

Immune Regulatory Genes: CD40, CTLA-4, PTPN22

The CD40 Gene and Graves' Disease

The CD40 molecule, located on chromosome 20q, is crucial to both the innate and adaptive immune responses. It is present on the surface of antigen-presenting cells (APCs) including B cells. The T cell-APC interaction results in activation of CD40 as a co-stimulatory molecule. CD40 also plays a serious role in activating B lymphocytes permit them to finally differentiate and secrete antibodies [51]. It is no censorious that the CD40 gene is linked to numerous autoimmune disorders. Whole genome linkage scanning has identified strong linkage of CD40 to GD. The causative variant predisposing to GD is a C/T polymorphism in the Kozak sequence, a nucleotide sequence that isimportant for the initiation of translation of the CD40 molecule. The CC genotype was definitivelyrecognizedin Japanese, Koreans, and Caucasians to be associated with GD [52]. Functional studies demonstrated that the C allele of this SNP increased CD40 mRNA translation by ~20–30% when compared to the protective T allele [14]. The improved translation of CD40, driven by the C-allele increased the levels of CD40 expressed on B cells, consequently increasing the probability of their activation and antibody production [68]. Also, this SNP might increase translation of CD40 in the target tissue, i.e. the thyroid thereby resulting in cytokine production and activation of resident T-cells by bystander mechanisms [14,51].

The CTLA-4 Gene

The cytotoxic T lymphocyte-associated protein 4, CTLA-4, is a highly polymorphic gene that was first recognised to be corresponding with risk for AITD by the candidate gene approach. Located on chromosome 2q, under normal situation, the CTLA-4 protein acts to suppress T cell activation, and later immune response, to prevent T-cell overactivity [14] CD4+CD25- T cells only express CTLA-4 on their surface after the T cell receptor is activated, and its participation with its ligand suppresses the ongoing immune response. Decreased or absent CTLA-4 activity permits uninhibited T-cell movement and a lengthy, unregulated immune response [69], making CTLA-4 an attractive candidate gene for autoimmunity. Indeed, the CTLA-4 gene was found to be contributed with many other autoimmune diseases.

A microsatellite in 3′UTR of CTLA-4 is linked to AITD, the longer the AT repeat at this site, the less inhibitory activity CTLA-4 has. Other variants of the CTLA-4 gene are linked to AITD; a G allele replacementat an A/G SNP at position 49 was also found to be associated with AITD conferring a relative risk of ~2 for disease [51]. Additionally, and most recently discovered, an A/G SNP downstream from the 3′UTR, designated CT60, was also found to be associated with GD and has been suggested as the causative

variant, albeit this is not conclusively demonstrated [34]. Unlike other candidate genes, the CTLA-4 gene's association with AITD is not specific to specific ethnic groups or geographic locations [14].

Protein tyrosine phosphatase-22 (PTPN22) Gene

The protein tyrosine phosphatase-22 (PTPN22) gene is the most recently recognised immune-regulatory gene associated with the autoimmune thyroid disease, which is located on chromosome 1p13. PTPN22, which is principally expressed in lymphocytes, acts as a negative regulator of T-cell activation. This gene encodes for the lymphoid tyrosine phosphatase (LYP), a molecule that, similar to CTLA-4, functions to inhibit T-cell activation [70]. Weaker T-cell signalling may lead to diminishingthymic deletion of autoreactive T cells, or an increased PTPN22 function may result in inhibition of regulatory T cells (Tregs), which protect against autoimmunity [71]. A non-synonymous SNP in the PTPN22 gene, R620W, was found to be associated with GD, as well as other autoimmune diseases. This replacement results in a functional change in the LYP protein resulting in activation of T-cells, but the mechanism is unclear.

Protein Tyrosine Phosphatase Nonreceptor-Type 22 (PTPN22) Gene

An early study in HT patients demonstrated a significant association with 1858C/T SNP (OR 1.77; 95% CI, 1.56-3.97) [72]. In a small group of patients with both HT and autoimmune diabetes, T allele was determined in 50% compared with only 14% inhealthy controls[73]. Five other PTPN22 SNPs were tested in Japanese patients, showing no relation with HT, but a novel protective haplotype is containing those SNPs weredetected [74].

Copy Number Variants

Copy number variants (CNVs) are large duplications or deletions of a DNA sequence that are inherited. Several CNVs are reported to be associated with autoimmune disorders [75]. Therefore, we have analysed the three immune-regulatory genes known to be contributed with GD, CD40, CTLA-4, and PTPN22, for the presence of CNVs that are associated with the disease. Surprisingly, no CNVs were identified in the CD40 and CTLA-4 genes, while only two subjects out of 190 had a rare PTPN22 CNV. Asignificant difference in the CNV analysis was foundwhen using DNA obtained from fresh blood compared to DNA acquired for Epstein Barr virus-immortalized B-cells. Consequently, these results have potential implications for studies of CNV in complex diseases. It is now clear that immortalising cell lines with EBV create artificial CNVs and therefore, CNV analysis should only be done on DNA acquired from blood [76].

Epigenetics: The Interface Between Genes And Environment In Disease Etiology

While it is clear that genetic and environmental factors interact toproduce thyroid autoimmunity, the nature of this interaction is still uncertain. One potential mechanism for gene-environment interaction in complex diseases, which has emerged in recent years, is through epigenetic effects. Epigenetic effects are explained as inheritable effects on gene expression that are not coded in the DNA sequence. The term epigenetics is broadened to include any non-DNA sequence encoded impacts on gene expression whether inherited or not. The classical epigenetic factors include DNA methylation, histone modifications (usually acetylation, deacetylation, and methylation) and micro-RNAs [77,78].

In several autoimmune diseases including type-I diabetes [79], systemic and lupus erythematosus [80] epigenetic changes wereshown to play a role in the etiology of disease, and epigenetic factors are likely to be associated with other autoimmune disorders including AITD [81].Epigenetic changes are likely to besignificant in AITD. As mentioned before infections were shown to play a critical role in activating AITD[82].A likely mechanism by which infections in susceptible individuals can trigger AITD is through epigenetic effects. Indeed, epigenetic modifications were recognized following viral diseases such as HIV [83].

The Impact of Environmental Factors (Environmental Triggers)

Environmental factors that are supposed to be involved in the development of Hashimoto's thyroiditis include high dietary iodine intake, medications (especially interferon (IFN-α and amiodarone), infections (bacterial – Yersinia enterocolitis or viral – hepatitis C virus), smoking, stress, pregnancy, irradiation, and pollutants. They have been reviewed in more detail elsewhere [13,84]. Postulated possible mechanisms include molecular mimicry, bystander activation, and Toll-like receptor activation by viruses [14,85]. Only recently, the search for a possible explanation of how genetics may render sensitivity to environmental are addressed by an elegant study carried out by Stefan *et al.* [30]. The authors found that carriers of a susceptibility allele in the TG promoter are prone to produce high amounts of Tg when encountering IFN-α. Surprisingly, the disease-associated polymorphism was conserved among vertebrates, and a disease-protective mutation in this region seems to be unique to human. This phenomenon would explain why AITD is a common consequence of IFN-α treatment (e.g. in chronic hepatitis C patients).

Iodine Intake

Excessive iodine intake is a well-established environmental factor for triggering thyroid autoimmunity. Several putative mechanisms by which iodine may promote thyroid autoimmunity weresuggested. Firstly, iodine exposure leads to higher iodination of Tg and thus increases its immunogenicity by creating novel iodine-containing epitopes or exposing cryptic epitopes. Thesefeatures may facilitate a presentation by APC and enhance the binding affinity of the T-cell receptor which may lead to particular T-cell activation [86]. Secondly, iodine exposure is shown to increase the level of reactive oxygen type in the follicular cells of the thyroid which is produced during TPO oxidation of excessive amounts of iodine. They enhance the expression of the intracellular adhesion molecule-1 (ICAM-1) on the follicular cells of the thyroid gland which could attract the immunocompetent cells into the thyroid gland [84]. Thirdly, iodine toxicity to the follicular cells of the thyroid gland was reported, since highly reactive oxygen species may bind to membrane lipids and proteins, causing thyrocyte damage and release of autoantigens [87]. Fourthly, iodine excess is shown to promote follicular cell apoptosis by inducing an abnormal expression of tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) and its death receptor (DR)-5 in the thyroid [88]. Fifthly, in vitro evidence also suggests an enhancing influence of iodine on the cells of the immune system, including augmented maturation of dendritic cells, increased number of T cells and stimulated B-cell immunoglobulin production [87]. Studies of several large population-based demonstrated a higher prevalence of TAbs in the areas with higher iodine supply. Moreover, up to four-fold increase in the incidence of TAbs was shown after the exposure to excess iodine intake due to the improvement of iodine prophylaxis in previously

iodine-deficient areas [89]. Precious evidence was also donated by using experimental animal models of autoimmune thyroiditis, where the prevalence and severity of thyroid autoimmunity significantly increased when the dietary iodine was added [86].

Solute Carrier Family 26 Member 4 (SLC26A4)

Admittedly, there are some other suspected thyroid-specific genes apart from TG, but to this day little work has been done to prove their influence on the development of HT. One of them, SLC26A4/PDS (solute carrier family 26 members 4; Pendred syndrome), located on chromosome locus 7q31, has been recently connected with AITD. There is growing evidence that mutations in this gene are associated with HT and GD, but only a few works are linking it with AITD were reported [90,91].

Gene Interactions

Recently it has been suggested that two weakly associated genes need to interact to become significantly associated with AITD. Alternatively, the low ORs found for the presence of patient subsets might cause most AITD genes. Different genes would be associated considerably only with some subsets, and these results could therefore not be generalised to the whole population [14]. Supporting the first hypothesis, evidence for gene-gene interactions bearing susceptibility to AITD was recorded. An interaction between an SNP in exon 33 of the TG gene and the Arg-74 encoding variant of HLA-DRB1 has been identified for GD [92], and a similar interaction has been suggested for HT [93]. The authors hypothesise that the reported HLA-DR pocket variants would accommodate mutated peptides like thyroglobulin with the exon 33 SNP, the subsequent presentation of which may initiate the disease [13]. Another research revealed evidence of interaction between the CTLA4 gene and the locus containing the TG gene triggering susceptibility to high levels of thyroid autoantibodies and clinical AITD [94]. It has been shown in Japanese HT patients that the interaction between the HLA-DRB4 and CTLA4 genes might determine the thyroid function of TPO-positive goitrous HT patients [95]. Genetic interactions between PTPN22 and HLA-DR in autoimmune disorders could not be identified [96]. Baki *et al.* [97]discovered that the simultaneous presence of IL-10 c.-1082G>A and TNFα c.-308G>A, as well as IL-10 c.-1082G, >A and IL-6 c.-174G>C polymorphisms significantly raised the risk for HT, while none of these changes alone did have any significant impact on the disease.

Vitamin D Receptor Gene

Vitamin D, which acts via vitamin D receptor (VDR), possesses immunomodulatory properties and its deficiency isinvolved in the development of autoimmune diseases. Many immune cells express VDR, dendritic cells in particular, where VDR stimulation is shown to enhance their tolerogenicity. Tolerogenic dendritic cells promote the development of Tregs with suppressive activity and therefore peripheral tolerance [98]. VDR gene is located on the chromosome 12q12, and its polymorphismsare related to different autoimmune disorders such as type I diabetes or Addison's disease. A decade ago, the association between VDR-FokI SNP in exon 2 and HT had been identified [99] which was later confirmed in the observation of Taiwanese Chinese patients [100]. In the Croatian population VDR gene 3' region polymorphisms were related to HT, possibly affecting VDR mRNA expression [101]. A significant relation has also been discovered between

HT and both promoter and intron six gene polymorphisms of CYP27B1 hydroxylase, which is located on chromosome 12q13, catalyzing the conversion of 25 hydroxyvitamin D3 to its active form [102].

Cytokine Genes and other Immune-Related Genes

Lately, several genes encoding different inflammatory cytokines were studied in HT, some of them also influence the severity of the disease. Interferon (IFN)-γ, produced by T-helper type 1 (Th1) cells, promotes cell-mediated cytotoxicity which underlies thyroid destruction in HT. T allele of the +874A/T IFN-γ SNP, causing the increased production of IFN-γ, was associated with severity of hypothyroidism in HT patients [103]. Higher frequency of severe hypothyroidism was also detected in patients carrying CC genotype of -590C/T interleukin 4 (IL-4) SNP, leading to lower production of IL-4, one of the critical Th2 cytokines which suppress cell-mediated autoimmunity [104]. Gene polymorphism of transforming growth factor (TGF)-β, an inhibitor of cytokine production, was also associated with HT. T allele of +369T/C SNP, leading to lower secretion of TGF-β, was more frequent in severe hypothyroidism than in mild hypothyroidism [105]. Similarly, a more severe form of HT was associated with -2383C/T SNP of the gene for forkhead box P3 (FoxP3), an essential regulatory factor for the Tregs development [106]. Unlike the severity of hypothyroidism, the development of HT itself was integrated with C allele of tumour necrosis factor (TNF)- α -1031T/C SNP. Namely, C-allele carriers present with a higher concentration of TNF-α which acts as the stimulator of the IFN-γ production [107].

The Role of Female Sex and Reproduction

Female Sex

As indicated by numerous epidemiological studies, females present with positive TAbs up to three times more often than males [108-110]. The largest NHANES III study has shown that females were positive for TPOAbs and TgAbs in 17% and 15.2%, respectively, while males only in 8.7% and 7.6%, respectively [111]. As stated inthe estimation provided by the study of Danish twins, the genetic contribution to TPOAb and TgAb susceptibility in females was 72% and 75%, respectively, while in males it was only 61% and 39%, respectively [112]. The possible explanation for high female predominance in thyroid autoimmunity might be associated with the X chromosome containing some sex and immune-related genes which are of crucial importance in the preservation of immune tolerance [113]. Increased immunoreactivity might, therefore,be associatedwith the genetic fault of the X chromosome, such as structural abnormalities or monosomy. Appropriately, a higher incidence of thyroid autoimmunity was recordedin patients with a higher ratio of X chromosome monosomyin peripheral white blood cells[114] or patients with Turner's syndrome [115]. Another potential mechanism of impaired immunotolerance in females is skewed X-chromosome inactivation (XCI) results inthe escape of X-linked self-antigens from an exhibition in thymus with subsequent loss of T-cell tolerance. Skewed XCI was detected with a higher risk of developing autoimmune thyroid diseases[116].

Pregnancy and Postpartum Period

The tolerance of the fetal semi-allograft during pregnancy is permitted by the state of immunosuppression which is a result of hormonal interchange and trophoblast expression of key immunomodulatory molecules.

The pivotalparticipants inthe regulation of the immune response are Tregs, which quicklyincrease during pregnancy. Accordingly, both cell-mediated and humoral immune responses are attenuated with a shift towards humoral immune response, resulting in immune tolerance of the conceptus tissues and suppression of autoimmunity [117]. Accordingly, the decrease in both TPOAb and TgAb concentrations during pregnancy has been reported, reaching the lowest values in the third trimester. The rapid postpartumreduction of Tregs and re-establishment of the immune response to the pre-pregnancy state may result in the occurrence or aggravation of the autoimmune thyroid disease [118]. The increase of TPOAb concentrations occurred as soon as six weeks after delivery [119], reaching the baseline level at approximately 12 weeks and the maximum level at about 20 weeks after birth [120].

In up to 50% of women with positive TPOAbs in the early pregnancy, thyroid autoimmunity in the postpartum periodexacerbatein the form of postpartum thyroiditis. It may occur in the first year after delivery, usually clinically presented with transient thyrotoxicosis and transient hypothyroidism, while in about a third of females persistent hypothyroidism may evenevolve [121]. Significant higher secretion of IFN-γ and IL-4 together with a lower median plasma cortisol concentration in 36th week of gestation was reported in females with postpartum thyroiditis than in euthyroidwomen, indicating that weaker immunosuppression in the former group of women in late pregnancy could contribute to the postpartum thyroid dysfunction [122].

Protection Against Thyroid Autoimmunity

Two mechanisms enable the maintenance of self-tolerance. The central tolerance refers to thymic deletion of autoreactive T cells during fetal life. Those cells that escape central tolerance are prevented from triggering autoimmunity by mechanisms of peripheral tolerance where Tregs play the pivotal role [118,123]. They have a suppressive effect on the effector T cells, APCs and B cells, thereforecontinuing the immunological unresponsiveness to self-antigens and suppressing the excessive immune response [124]. They may directly containtarget cells or act through secreted suppressor cytokines. Tregs derive from thymus as a subpopulation of T cells or from naive T cells in the periphery and express CD25 (α chain of the IL-2 receptor) and FoxP3. Consequently, the critical role in the immune system is played by CD4+CD25+Foxp3+ Tregs [123]. Cells with the highest CD25 expression (CD4+CD25high) are responsible for suppressive regulatory effects [125]. In humans, CD25 expression was higher in patients with HT than in healthy subjects [126]. When patients with autoimmune thyroid disease were studied, the proportion of Tregs was lower intrathyroidal than in peripheral blood [127]. The expression of Foxp3 and generation of Treg cells are both induced by TGF-β produced in Tregs, fibroblasts, macrophages, endothelial cells in inflammatory thyroid tissue as well as in thyrocytes. TGF-β is a key regulator of immune tolerance which stimulates suppressive Tregs and inhibits T cell differentiation [128]. Accordingly, in patients with HT, serum levels of TGF-β were lower than in controls which did not change after the treatment with levothyroxine. Therefore, levels of TGF-β seem to be associated with the HT and not thyroid dysfunction [129].

Summary

Despite tremendous progress made in the understanding of Hashimoto's thyroiditis during the past decade, the exact mechanisms of its progression are yet to be clarified. Hopefully, shortly, unravelling the genetic contribution to complex diseases such asHT, new evidencewill enable better insight into the disease

pathogenesis which may help us identify subjects at risk and may even enable us to prevent the development of clinical disease.

A lot of work has been undertaken to aim for understanding the aetiology of AITD, especially disease-specific risk factors. Hashimoto's thyroiditis is a common disorder, and many studies were undertaken to detect its genetic background; yet have no unambiguous results been obtained in genome-wide studies. HT is one of the most prevalent autoimmune diseases provoked in genetically susceptible individuals by several triggers, including female sex, immune changes after delivery, fetal microchimerism, iodine intake, and other environmental factors. Multiple susceptibility genes may be involved in the disease development, some of which are also common for other autoimmune diseases, while others are specific for thyroid autoimmunity. It is now clear that immune-regulatory genes such as HLA, CTLA-4, and PTPN22 play a major role in the aetiology of HT, GD, and several other autoimmune diseases. The only thyroid-specific gene currently showing the association with HT is a gene for Tg which is also GD susceptibility gene. VDR gene is another HT predisposing gene, common for other organ-specific autoimmune diseases such as type I diabetes or Addison's disease. Moreover, recent studies of cytokine genes such as IFN-γ, IL-4, or TGF-β indicate the association with the development and severity of HT.

So, we could expect that there are multiple different genetic risk factors that conduce to the genetic susceptibility of Hashimoto's thyroiditis, each bearing a modest influence. The predisposition of HT patients to other autoimmune disorders additionally confirms the complexity of the genetic background, suggesting the involvement of not only specific mutations in the predisposition to this disorder but most importantly variants that influence the immune system. While genetic effects are generally contributing to AITD are quite well investigated, the genetic factors predisposing to the HT phenotype remain to be accounted for. The thyroid-specific gene the role of which in HT development has been confirmed encodes Tg, a protein that functions as the matrix for thyroid hormone synthesis. However, it is impossible to identify a single etiological polymorphism or even gene, which would be responsible for the etiopathology of Hashimoto's thyroiditis. It seems that there are at least some genes responsible for this disease and that different mutations could be responsible for the different phenotypes of this disorder like the age of symptom onset, postpartum thyroiditis, the presence of goitre, or coexisting thyroid carcinoma. The comparison of studies searching for genes that would have an impact on the pathogenesis of Hashimoto's thyroiditis is difficult because of different criteria of disease diagnosis, and difficulties in the creation of a uniform group of tested patients. The clinical outcome may differ among individuals, even in one single family. The diagnosis of HT is based on thyroid ultrasound and the presence of antibodies against TPO, with a coexisting thyroid insufficiency.

Conclusion

In conclusion, it is an interesting observation that despite the many surveys performed so far, researchers are still not able to unambiguously indicate the genes that would contribute to the development of HT. Many approaches address gene-gene and gene-environment interactions rather than searching for further potential candidate genes for AITD development, as it has become clear that the genetics of these diseases is rather sophisticated.

Conflicts of interest

The authors declare that they have no conflict of interest related to this article.

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