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Review article

Immunogenetics of autoimmune thyroid diseases: A comprehensive review

Hanna J. Lee [a], Cheuk Wun Li [a], Sara Salehi Hammerstad [a, b], Mihaela Stefan [a], Yaron Tomer [a, c, *]

[a] Division of Endocrinology, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA
[b] Department of Pediatrics, Oslo University Hospital, Ullevål, Oslo, Norway
[c] Bronx VA Medical Center, Bronx, NY, USA

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ABSTRACT

Both environmental and genetic triggers factor into the etiology of autoimmune thyroid disease (AITD), including Graves' disease (GD) and Hashimoto's thyroiditis (HT). Although the exact pathogenesis and causative interaction between environment and genes are unknown, GD and HT share similar immune-mediated mechanisms of disease. They both are characterized by the production of thyroid autoantibodies and by thyroidal lymphocytic infiltration, despite being clinically distinct entities with thyrotoxicosis in GD and hypothyroidism in HT. Family and population studies confirm the strong genetic influence and inheritability in the development of AITD. AITD susceptibility genes can be categorized as either thyroid specific (Tg, TSHR) or immune-modulating (FOXP3, CD25, CD40, CTLA-4, HLA), with HLA-DR3 carrying the highest risk. Of the AITD susceptibility genes, FOXP3 and CD25 play critical roles in the establishment of peripheral tolerance while CD40, CTLA-4, and the HLA genes are pivotal for T lymphocyte activation and antigen presentation. Polymorphisms in these immune-modulating genes, in particular, significantly contribute to the predisposition for GD, HT and, unsurprisingly, other autoimmune diseases. Emerging evidence suggests that single nucleotide polymorphisms (SNPs) in the immunoregulatory genes may functionally hinder the proper development of central and peripheral tolerance and alter T cell interactions with antigen presenting cells (APCs) in the immunological synapse. Thus, susceptibility genes for AITD contribute directly to the key mechanism underlying the development of organ-specific autoimmunity, namely the breakdown in self-tolerance. Here we review the major immune-modulating genes that are associated with AITD and their potential functional effects on thyroidal immune dysregulation.

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1. Introduction

Autoimmune thyroid disease (AITD), which includes Graves' disease (GD) and Hashimoto's thyroiditis (HT), affects an estimated 5% of the general population, making it one of the most prevalent autoimmune diseases [1,2]. Autoimmunity to the thyroid, defined by the presence of antibodies to thyroid antigens, is even more common and reported to be as high as 10–20% of all women. In addition to the generation of thyroid autoantibodies and abnormal thyroid hormone production, AITD histologically involves the infiltration of self-targeting T and B lymphocytes in the thyroid gland [3]. The etiology of AITD is currently understood to be multifactorial and is due to a complex interplay of specific susceptibility genes and environmental exposures. In fact, the influential role of susceptibility genes in the development of AITD is highlighted by epidemiological studies showing that approximately 50% of the siblings of GD patients test positive for thyroid antibodies and that up to 33% of those with AITD share the diagnosis with their siblings (resulting in a sibling risk ratio or λs as high

Abbreviations: TSHR, thyrotropin receptor; FOXP3, forkhead box P3; CTLA-4, cytotoxic T-lymphocyte-associated molecule-4; PTPN22M, protein tyrosine phosphatase nonreceptor type-22; LYP, lymphoid tyrosine phosphatase; FCRL3, Fc receptor-like 3; GD, Graves' disease; HT, Hashimoto's thyroiditis; IPEX, immune dysregulation polyendocrinopathy enteropathy X-linked syndrome; T1D, type I diabetes mellitus; RA, rheumatoid arthritis; PBC, primary biliary cirrhosis; SLE, systemic lupus erythematosus; MS, multiple sclerosis; IBD, inflammatory bowel disease; NMO, neuromyelitis optica.

* Corresponding author. Division of Endocrinology, Box 1055, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, New York, NY 10029, USA.
E-mail address: yaron.tomer@mssm.edu (Y. Tomer).

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as 16.9) [4–6]. Twin studies are even more convincing with higher concordance for AITD between monozygotic twins than dizygotic twins [7,8] and have suggested that the overall heritable, genetic contribution to the development of GD is about 75% [7,9]. Racial variations in AITD prevalence, as was demonstrated in a recent NHANES (the National Health and Nutritional Examination Surveys) study, further accentuate potential genetic differences and the role of genetic susceptibility in the etiology of GD and HT [10].

Of the susceptibility genes, the majority are general immune-regulatory genes involved in the complex process of ensuring robust immune responses against appropriate, foreign antigens while maintaining tolerance to self-antigens. These include genes involved in the proper progression from central tolerance, peripheral tolerance to antigen presentation and lymphocyte activation in the immunologic synapse. Aberrant activity of these immune-regulatory genes due to polymorphisms would logically and potentially lead to a breakdown in immune tolerance and ultimately autoimmunity. Through linkage and association analyses, genome screening, and genome wide association studies (GWAS), several single nucleotide polymorphisms (SNPs) in genes including FOXP3, CD25, CTLA-4, CD40, the HLA family, and others have been discovered to be associated with AITD. Interestingly, certain polymorphisms uniquely predispose to GD, HT or both while others are not thyroid-specific and increase the likelihood of autoimmunity in general (Table 1).

2. Genetic disruption of central tolerance – TSHR gene

An attractive hypothesis is that GD is triggered by a defect in negative selection of autoreactive T cells to the TSHR (thyrotropin receptor), either in the thymus or the peripheral immune system. Indeed, a number of genetic variants associated with GD were shown to impact central tolerance (TSHR) or peripheral tolerance (FOXP3 and CD25, see section 3.1). The hallmark of GD is the presence of the stimulating TSHR antibodies. Consequently, TSHR has been considered an important candidate gene predisposing to GD even before the era of GWAS studies. Subsequent GWAS and other association studies have confirmed TSHR as a disease specific locus [11–13]. Consecutive comprehensive sequence analyses of the TSHR gene/locus localized the causative variant(s) to a 40-kb region within intron 1 where at least five GD-associated SNPs were identified (rs179247; rs2284720; rs12101255; rs12101261; and rs2268458) [11,14,15]. Further functional analyses of TSHR intron 1 polymorphisms provided direct evidence of a link between central tolerance and TSHR intron 1 SNPs. Recently, our group showed that the disease-predisposing genotype (TT) of SNP rs12101261 was associated with decreased thymic expression levels of TSHR mRNA [16].

By mapping epigenetic modifications induced by interferon alpha (IFNα), a key cytokine secreted during viral infections that was previously shown to trigger autoimmunity, we showed that the disease-associated variant of rs12101261 (TT) interacts through chromatin remodeling with the transcriptional repressor, promyelocytic leukemia zinc finger protein (PLZF), to reduce TSHR gene expression [16]. We proposed that loss of adequate epigenetic interactions in the thymus due to micro-environmental influences (e.g., cytokines, viral infections) would affect TSHR gene expression through genetic variants. Decreased intra-thymic expression of TSHR would facilitate pathogenic T cell escape from central tolerance and increase the risk of autoimmunity to TSHR. In agreement with these findings, Colobran et al. showed a correlation between TSHR thymic mRNA levels and rs179247, a SNP in tight linkage disequilibium (LD) with rs12101261. They showed that individuals homozygous or heterozygous for the GD-associated allele at the rs179247 SNP (AA or AG) have significantly lower TSHR mRNA expression levels in the thymus than individuals homozygous for the protective allele (GG) [17]. This unbalanced allelic expression likely represents defective central tolerance, contributing to GD through the escape of T cell clones targeting the TSHR.

3. Genetic disruption of peripheral tolerance – FOXP3 and CD25 genes

3.1. FOXP3

FOXP3 (forkhead box P3), an X-linked gene [18], belongs to the forkhead/winged-helix family of DNA-binding transcription factors [19,20]. It can act as both a transcriptional repressor [21] and activator [22–24] for primarily immunological genes. For example, FOXP3 can bind to Runt-related transcription factors to inhibit IL-2 and IFNγ expression [25] or, by binding to other transcription factors, it can also activate CD25 [26]. To maintain immunological self-tolerance, regulatory T cells (Tregs) suppress peripheral self-reactive lymphocytes that have escaped central tolerance in the thymus [27,28] and FOXP3 is a known crucial regulator of Treg differentiation and function. The role of FOXP3 in autoimmunity was first revealed by the mouse mutant *Scurfy*, a line defective in FOXP3. As expected, the *scurfy* mutant phenotype is characterized by massive hyperproliferation and multi-organ infiltration of $CD4^+$ T cells and is lethal in hemizygous males [20]. In humans, mutations in FOXP3 lead to an X-linked syndrome characterized by immune dysregulation, polyendocrinopathy and enteropathy (IPEX) [29–33].

Various FOXP3 polymorphisms have been reported to be associated with autoimmune thyroiditis (AITD). For example, a DXS573 microsatellite that is in LD with FOXP3 was found to be associated with AITD in Caucasian female AITD patients [34]. An A/C polymorphism in position -3279 has been associated with the development of treatment-resistant GD [35] while the CC genotype at position -2383 has been associated with severe HT [35]. Our group found an association between the $(TC)_n$ microsatellite in intron 5 of the FOXP3 gene and AITD in Caucasian males (p-0.011) [24]. We also identified that this microsatellite is associated with a variant of autoimmune polyglandular syndrome type 3 (designated APS3v) [36], characterized by the co-occurrence of AITD and type 1 diabetes (T1D) [37].

Mechanistically, we hypothesized that the $(TC)_n$ microsatellite in intron 5 may affect splicing because of its location and size, as intronic microsatellites have been shown to be regulators of gene splicing [38,39]. Although no significant difference in splicing efficiency was observed when human embryonic kidney cells (HEK 293) were transfected with the long or short repeats of the FOXP3 intron 5 $(TC)_n$ microsatellite, our study identified a new splice variant designated FOXP3Δ6 (Fig. 1). FOXP3Δ6 was expressed in the thymus and lymph nodes, as well as in Tregs [40]. The role of this splice variant in thyroid autoimmunity warrants further investigation. Despite the fact that we did not find a difference in the splice variant levels associated with the long or short microsatellite repeats, epigenetic interactions and changes, which are known to regulate gene expression, can potentially influence splicing [16]. It is possible that different FOXP3 splice variants, including the novel splice variant FOXP3Δ6 that we identified to be expressed in Tregs, may modulate immune responses, although further evidence is needed.

3.2. CD25

CD25 (also known as IL-2Rα receptor or the α-subunit of the IL-2 receptor) is involved in the regulation of T cell function. More specifically, it is encoded by the CD25 region on chromosome

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