REVIEW

Graves' disease: introducing new genetic and epigenetic contributors

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Abstract

Autoimmune thyroid disease (AITD) accounts for 90% of all thyroid diseases and affects 2–5% of the population with remarkable familial clustering. Among AITDs, Graves' disease (GD) is a complex disease affecting thyroid function. Over the last two decades, case–control studies using cutting-edge gene sequencing techniques have detected various susceptible loci that may predispose individuals to GD. It has been presumed that all likely associated genes, variants, and polymorphisms might be responsible for 75–80% of the heritability of GD. As a result, there are implications concerning the potential contribution of environmental and epigenetic factors in the pathogenesis of GD, including its initiation, progression, and development. Numerous review studies have summarized the contribution of genetic factors in GD until now, but there are still some key questions and notions that have not been discussed concerning the interplay of genetic, epigenetic, and immunological factors. With this in mind, this review discusses some newly-identified loci and their potential roles in the pathogenicity of GD. This may lead to the identification of new, promising therapeutic targets. Here, we emphasized principles, listed all the reported disease-associated genes and polymorphisms, and also summarized the current understanding of the epigenetic basis of GD.

Key Words

- autoimmune hyperthyroidism
- autoimmune thyroid disease
- genetic and epigenetic factors
- Graves' disease

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Introduction

Graves' disease (GD) causes hyperthyroidism as a result of circulating IgG antibodies that activate the thyroid-stimulating hormone receptor (TSHR). This activation leads to follicular hypertrophy/hyperplasia, which in turn causes thyroid enlargement and augments thyroid hormone production, especially the ratio of triiodothyronine (T3) relative to thyroxine (T4) in thyroid secretions. Thyroid-function testing in GD shows typically low basal serum TSH levels that are followed by a high level of free T3 and T4 in serum (Brent 2008).

A combination of genetic, epigenetic, and environmental factors can account for autoimmune responses against the thyroid gland (Imani *et al.* 2017). These responses are limited to lymphocytic infiltration and autoantibodies targeting thyroid antigens, such as TSHR, thyroglobulin (TG), and thyroid peroxidase (TPO).

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T cells recognize various epitopes of the TSHR and induce B cells to secrete thyroid-stimulating antibodies. The uncontrolled thyroid hormone production and ensued hyperthyroidism are caused by mimicking the action of TSH through TSHR-stimulating autoantibodies.

Hereditary factors have been demonstrated to account for 75–80% of the risk of GD development (Khalilzadeh *et al.* 2009, Anvari *et al.* 2010). The incidence of GD is about 20 to 50 cases per 100,000 people and individuals can be affected at any age, but usually between 30 and 50 years (Zimmermann *et al.* 2015). Concordance among monozygotic twins is higher in comparison with dizygotic twins and the male-to-female ratio among patients with GD is between 1:5 and 1:10 (Zhao *et al.* 2019). Recent studies have shown the roles of interacting risk factors as in genetics, immunogenetics, epigenetics, and environmental factors. In the following, we discuss some essential genetic and epigenetic factors that play substantial roles in GD. We summarize and list the genes according to the functions in two distinct groups: Thyroid hormone synthesis and T cell response regulatory genes. We also enumerate variants/polymorphisms that are associated with heightened or decreased GD susceptibility. Ultimately, we focus on epigenetic factors and their possible roles in GD development.

Thyroid hormone synthesis

Besides its undeniable roles in the immune system, the main function of the thyroid gland is synthesizing T3 and T4 hormones that are essential for the regulation of metabolic processes. This process initiates with thyroglobulin synthesis and its secretion into the follicular lumen followed by iodine transportation and oxidation that lead to the iodination of thyroglobulin tyrosine residues. After endocytosis, lysosomes can hydrolyze the complex and prepare the secretion of T3 and T4. Each of these complex processes can be modulated by encoded proteins of *TSHR*, *TPO*, and *TG* (Fig. 1A). In the following, the roles of these genes in the immune system will be highlighted.

TSH receptor

The TSHR was a critical candidate for GD (Tonacchera & Pinchera 2000). To date, numerous SNPs associated with GD risk have been identified (Table 1). TSHR antibodies are present in GD patients and are directly related to disease severity (Tomer 2014). The most causative variants are located within intron 1 (Tomer *et al.* 2013) that probably change the splicing process. These variants downregulate TSHR in the thymus by developing autoreactive TSHR-targeting T cells that have escaped deletion. Regarding this, we can propose two possible mechanisms: peripheral and central tolerance.

According to peripheral tolerance, after *TSHR* expression, the protein undergoes different modifications such as glycosylation, dimerization, sulfation, disulfide-bond formation, and proteolytic cleavage (Rapoport & McLachlan 2007). The TSHR may undergo post-translational intramolecular cleavage of its A and B subunits which determines its fate: A subunit forms a large extracellular domain, while the B subunit sets up the seven-transmembrane domain. Several alternatively spliced variants in the *TSHR* gene have been detected that can change the balanced expression of these subunits (Table 1) (Brand *et al.* 2009). There is also evidence for up to 5 truncated TSHR transcripts, particularly ST4 (1.3Kb) and ST5 (1.7Kb), that encode a significant percentage of the entire ligand-binding extracellular region (Fig. 1B). The truncated mRNA transcripts ST4 and ST5 encode the majority of soluble A-subunit directly, hence increasing the chances of autoantibody production against the TSHR. Different polymorphisms, for example, rs179247 and rs12101255, have been reported in association with the production of the soluble A subunit (Colobran *et al.* 2011) (Table 1). In sum, the generation of this soluble form of TSHR can likely favor an autoimmune response, although the molecular mechanism is not clear.

The expression of self-antigens in the thymus is essential for 'Central Tolerance'. These antigens vividly play in a negative selection of autoreactive T cell clones. This process filters developing T and B cells and eliminates auto-reactive lymphocytes (Fig. 2). In medullary thymic epithelial cells, tissue-restricted autoantigens can induce the expression of promiscuous gene expression (*PGE*), providing various ligands that are vital for the negative selection of T cells. Genetic variations in the autoimmune-related genes, for example, *AIRE* gene, can also influence the expression of PGE and TSHR (Mathis & Benoist 2009). Hence, it seems fair to suggest that DNA alternations that affect central tolerance can change the TSHR signaling in GD.

Two mapped SNPs the intron 1 of the *TSHR*, rs12101255 and rs12101261, have an association with GD via epigenetic functions. Interferon-α (IFn-α) leads to a remarkable H3K4me1 enrichment only in the overlapping region of rs12101255 and rs1210126, proposing one of them is the causative SNP. Furthermore, a regulatory element has been identified that binds to

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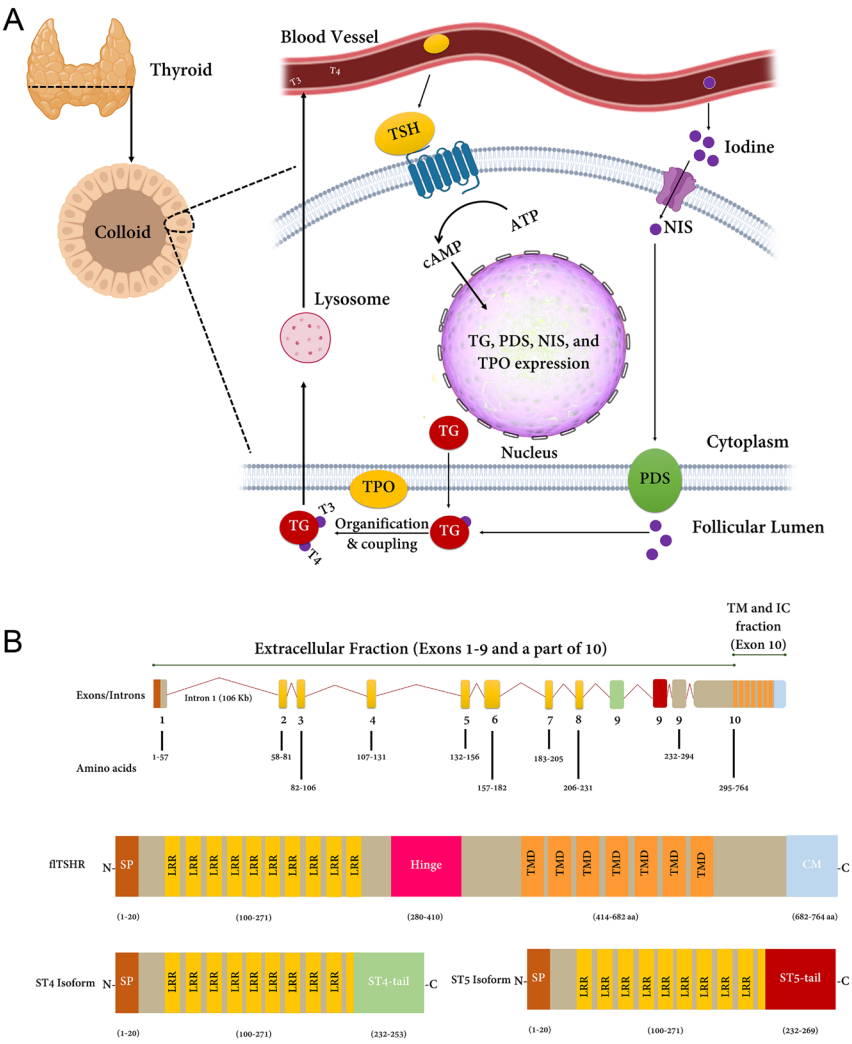

Figure 1
(A) Thyroid hormone synthesis (reviewed in Kopp & Solis-S 2009). Thyrotropin (TSH) as the main stimulator of TSHR can transduce the signal through cAMP production in the cytoplasm, which in turn can modulate thyroid hormone-responsive gene expression, for example, *TPO*, *TG*, sodium-iodide symporter (*NIS*), and pendrin (*PDS*). This figure introduces a pathway that includes TSHR, TPO, and TG. The figure is redrawn from Galligan *et al.* (2019). (B) Structure of the *TSHR* gene. This gene contains 10 exons and encodes a protein in a full-length version with 764 amino acids. The *TSHR* gene is transcribed to a full-length mRNA (flTSHR or TSH holoreceptor) and 2 main splicing isoform including ST4 and ST5. ST4 and ST5 are common in at the first 8 exons but differ in an additional unique 9th exon. These unique exons are highlighted in green and red. In the figure, C, C-terminal; N, N-terminal region; SP, signal peptide; LRR, leucine-rich repeat; TMD, transmembrane domain; CM, Cytoplasmic Motifs. In GD patients, LRRs are a subject of pathogenic stimulating antibodies. The figure is redrawn from Marín-Sánchez *et al.* (2019). A full color version of this figure is available at https://doi.org/10.1530/JME-20-0078.

the transcriptional repressor region of the promyelocytic leukemia zinc finger (PLZF) in the rs12101261 location. This polymorphism reduces the expression of PLZF in GD patients (Chen *et al.* 2018). *TSHR* expression was also reduced intrathymically in the homozygote individuals carrying this SNP (Kursawe & Paschke 2007). These findings established an understanding that non-coding SNPs of intron 1 within the *TSHR* have a genetic-epigenetic interaction that adjusts the *TSHR* expression in thymus and boosts evasion of *TSHR*-reactive T cells from central tolerance. Additionally, hypermethylation in intron 1 has been identified where various GD-associated polymorphisms are reported (Table 1). The results show the contribution of dysregulated DNA methylation and histone modifications of T cell signaling genes in patients with GD that affect 'Peripheral/Central Tolerance' (Limbach *et al.* 2016); however, the key mechanism of TSHR involvement in GD development is elusive.

TPO

TPO, a thyrocyte apical plasma glycosylated membrane-bound enzyme, involves in producing the thyroid hormones T3 and T4 by iodine oxidation/iodination of tyrosyl residues of the Tg molecules (Kopp *et al.* 2017) (Fig. 1A). As a marker of AITD, over 90% of GD patients show an increased amount of anti-TPO autoantibodies (Silva *et al.* 2003). The *TPO* gene is merely expressed in thyroid glands, while is imperative for proper functions of at least three thyroid-specific transcription factors, including NKX2-1, FOXE1, and PAX-8 (Grasberger *et al.* 2005). Some genetic variations in *TPO* are associated with GD; for example, rs11675434 is correlated with the development of Graves' ophthalmopathy (GO), especially in male patients with a late-onset GD (Kuś *et al.* 2017), while c.2268insT is the most frequently identified mutation in the *TPO* gene within the Taiwanese

Table 1 Most significant polymorphisms of *TSHR* that are associated with GD.

Genetic variation	Function	Year	Population	Increased risk	Associated region	Reference
rs2234919	Ameliorates G(s)alpha protein activation of TSHR	1995	Caucasian	Yes	Exon 1	(Ban *et al.* 2002)
DS14S81	NR	1997	Caucasian	Yes	Chr. 14q31	(Tomer *et al.* 1999)
TSHR-AT	NR	2000	Japanese	Yes	Intron 2	(Yin *et al.* 2008)
rs1991517	rs1991517 alters the binding affinity to cAMP, thus changes signaling pathways mediated by TSHR	2002	Russian	Yes	Exon 10	(Cuddihy *et al.* 1995)
D14S258	NR	2003	Caucasian	Yes	Chr. 14q	(Tomer *et al.* 2007)
rs2239610	This polymorphism is associated with higher serum concentrations of free thyroxin and TRAb with unknown mechanisms	2003	Chinese	Yes	Intron 1	(Hiratani *et al.* 2005)
rs2268458	NR	2005	Caucasian	Yes	Intron 1	(Brand *et al.* 2009)
rs2268475, rs3783938	NR	2005	Japanese	Yes	Intron 7, Intron 8	(Tomer *et al.* 1997)
rs3783941	NR	2007	Caucasian	Yes	Intron 8	(Płoski *et al.* 2010)
rs2268458	NR	2008	Caucasian	Yes	Intron 1	(Qu *et al.* 2010)
rs179247, rs12101255	Can increase the production of ST5 and change the TSHR expression	2009	Caucasian	Yes	Intron 1	(Colobran *et al.* 2011)
rs12101261	Decreases the intrathymic TSHR expression through signaling pathways mediated by promyelocytic leukemia zinc finger (PLZF) protein	2011	Chinese	Yes	Intron 1	(Chu *et al.* 2011)
rs12101255	By binding to PLZF protein decreases the intrathymic TSHR expression	2012	Chinese	Yes	Intron 1	(Yin *et al.* 2012)
rs2284720	NR	2013	Caucasian	Yes	Intron 1	(Tomer *et al.* 2013)
rs179243	NR	2014	Chinese	Yes	Intron 1	(Stefan & Faustino 2017)
rs12885526	NR	2015	Brazilian	Yes	Intron 1	(Bufalo *et al.* 2015)
rs179247, rs3783948	NR	2016	Italian	Yes	Intron 1	(Lombardi *et al.* 2016)
rs12101261, rs4903964, rs179247, rs2284722, rs17111394	rs179247 can increase the production of ST5 and change the TSHR expression, while rs12101261 changes *TSHR* gene expression through binding to PLZF protein.	2016	Chinese	Yes	Intron 1	(Jing *et al.* 2016)
rs4411444, rs4903961	NR	2017	Japanese	Yes	Intron 1	(Fujii *et al.* 2017)

NR, not reported.

population (Huang & Jap 2015). It seems that these kinds of mutations can change TPO protein activity, its expression in serum, or even the serum levels of TPOAb, confirmed by a study introducing nonsynonymous substitutions in *TPO* (including p.Ala373Ser, p.Ser398Thr, and p.Thr725Pro) in Bangladeshi patients (Begum *et al.* 2019). However, the molecular mechanisms behind the association between these variants and GD are not clearly understood.

TG gene

The thyroid gland produces TG playing a pivotal role in both the immune system and thyroid hormone synthesis; the *TG* gene is also a crucial candidate for GD (Yamashita *et al.* 1989). *TG* variants are common and likely contribute to the pathogenesis of autoimmune thyroid diseases (Tomer 2014). Anti-TG antibodies are found in 1 in 10 healthy individuals that cause falsely

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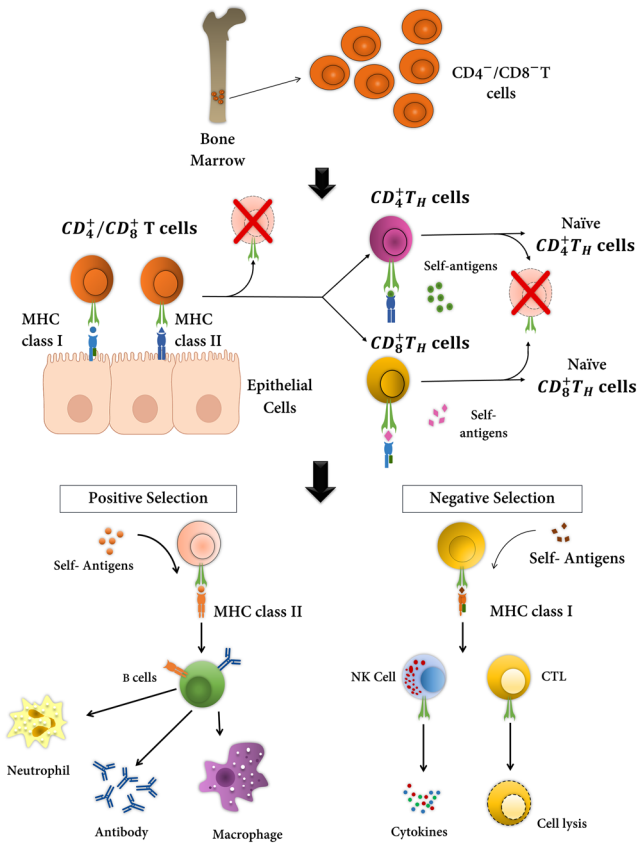

Figure 2
The role of cells and molecules encoded by GD associated genes in T cell and other immune cells during central tolerance and immune response-related pathways. Progenitor T cells are generated in the bone marrow. The positive and negative selections in the thymus are required to deplete the nonfunctional and autoreactive T cells. During positive selection, antigen-presenting HLA molecules bind to immature T cells and provide a survival signal to T cells. During negative selection, autoreactive T cells are recognized by binding to self-antigens. These T cells are distinguished as autoreactive T cells and undergo apoptosis. Externally derived proteins are obtained by the antigen-presenting cells (APCs), converted to antigens, bound to MHC class II molecules, and presented on the APC surface to be recognized by $CD4^+$ T cells. If antigens are recognized as foreign antigens, B cells will be activated followed by antibodies secretion, and macrophages and neutrophils recruiting by cytokines' $CD4^+$ T cells. Internally derived antigens bound to HLA class I molecules and presented on the cell surface for recognition by CD8+ T cells. If the antigen is detected as a foreign antigen, the cell destruction is carried out by cytotoxic T cells and NK cells. The GD associated variants to this pathway have been reported in *CTLA4*, *PTPN22,* and *IL2RA*. The figure is depicted according to the data from Kyewski & Klein (2006) and Nemazee (2017). A full color version of this figure is available at https://doi.org/10.1530/JME-20-0078.

low or rarely high levels of reported TG. These antibodies are often detected in patients with AITDs, especially GD (Antonelli *et al.* 2014), and also in conditions such as Hashimoto's encephalopathy, papillary or follicular thyroid carcinoma, systemic lupus erythematosus (SLE), and type 1 diabetes (T1D) (Wallace & Stone 2003). Further reports demonstrated amino acid substitutions in TG (SNP cluster of exon 10–12 and an exon 33) raise the susceptibility to AITDs (Ban *et al.* 2003). Indeed, the exon 33 SNPs demonstrate adequate evidence of interaction between TG and *HLA-DR3* that can lead to elevating GD susceptibility (Ban *et al.* 2003).

As an SNP in the promoter region of the *TG* gene, rs180195 has been identified to increase susceptibility to AITD by an interferon α-modulated mechanism (Stefan *et al.* 2011). This SNP has an epigenetically-important interaction with interferon regulatory factor 1 (IRF-1) to develop GD (Tomer 2014). Stefan *et al.* detected that −1623A/G SNP modifies the binding site for IRF-1, in fact, the disease-associated allele (G) limited the increase of *TG* promoter activity through IRF-1 binding (Stefan *et al.* 2011). Therefore, a novel mechanism incorporating both epigenetically-important interaction (IFn-α) and genetic factors (*TG*) can be implicated in GD development.

T cell response regulatory genes

Various proteins have been described to play important roles in T cell differentiation, maturation, and activation. In the following, we list some of the well-established genes and summarize their possible roles in GD development.

Major histocompatibility complex

Major histocompatibility complex (MHC), also known as Human Leukocyte Antigen (HLA) in humans, are encoded proteins on the cell surface that are essential for the acquired immune system to recognize antigens. There are three major subgroups of HLAs playing roles in antigen presentation, autoimmune reactions, and tissue allorecognition (Simpson 1988). A strong correlation between the HLA class I and class II regions with GD has been identified (Wellcome Trust Case Control Consortium, Australo-Anglo-American Spondylitis Consortium (TASC) *et al.* 2007, Zeitlin *et al.* 2008), that is, HLA class I allele HLA-B8 and HLA class II alleles are strongly associated with GD risk (Chen *et al.* 2000).

An interaction of *TG* SNPs in exon 33 has been identified that can synergistically facilitate the interaction of HLA-DRβ1-Arg74 with TG genotype as a disease-associated genotype of Trp1980Arg SNP (Simmonds *et al.* 2005). An arginine at β74 is encoded by *HLA-DRB1*03*, while *HLA-DRB1*07*, as a member of a protective DR7 haplotype, encodes glutamine at the same location (Simmonds *et al.* 2005). Moreover, a statistical interaction has been observed between such amino acid variants in

TG and HLA-DRβ1-Arg74 leading to higher susceptibility to GD (Hodge *et al.* 2006) and other autoimmune diseases (Menconi *et al.* 2010, Bernecker *et al.* 2012). Li *et al.* showed that TSHR peptides that bind to the HLA-DRβ1-Arg74 with high affinity represent key pathogenic TSHR peptides triggering GD and that blocking their presentation to CD4+ T-cells can be used as a novel therapeutic approach in GD (Li *et al.* 2020*a*).

DQB1* alleles and the amino acid residues have been shown to contribute to AITD in South Indian populations. In fact, DQB1*02:02, *06:03, *06:09, *03:02, and *03:03 alleles show a higher risk, while *02:01, *05:02, and *06:02 alleles can be deemed as a protecting factor toward AITD (Ramgopal *et al.* 2018). Similarly, investigations on populations of African descent showed a high association of DRB3*01:01 in Jamaicans (Smikle *et al.* 2001) and an association of DRB3*02:02 and DQA1*05:01 in African-Americans (Chen *et al.* 2000). In these studies, only DRB1*05:31 and DRB1*14:03 could raise the GD risk. Although various studies show HLA interactions and their associations with GD, the distinct mechanisms have remained unclear. However, it seems that HLA haplotypes exert their functions through an epistatic mechanism affecting the regulation of the intensity of GD by T-cells. Such T-cells recognize a protective HLA motif on antigen-presenting cell (APCs) surfaces, for example, DRB1*13:02, or interfere with anti-TSHR production (interfere with thyroid hormone synthesis) (Sasazuki *et al.* 2016).

CD40

As a member of the tumor necrosis factor (TNF) superfamily, *CD40* is expressed on a wide range of immune cells, such as B-cells, macrophages, and dendritic cells. Furthermore, CD40 ligand (CD40L), also known as CD154 that binds to the CD40 receptor is predominantly expressed by activated CD4+ T cells (Fig. 3A). The interaction of CD40-CD154 is vital for more activation of humoral immunity through triggering B-cells (Ferrari *et al.* 2001) that is supposed to trigger hyperthyroidism. Several groups have aimed to show the role of CD40 in GD, for example, Iscalimab is an antibody that has been assessed in various autoimmune conditions (e.g. RA and GD) because of its ability to prevent the CD40-CD154 interaction (Genere & Stan 2019), increasing hopes to treat GD patients.

Several studies have established *CD40* expression in the thyroid follicular cells in GD patients in which CD40 was associated with uncontrolled HLA class II expression and *ICAM1* overexpression (Bottazzo *et al.* 1983, Tolosa *et al.* 1992). Thus, it is hypothesized that thyroid follicular cells could act as APCs under special circumstances (Jacobson *et al.* 2007), so affecting T cells production/regulation.

CD40 rs1883832 (−1T>C) in the Kozak sequence is associated with GD (Hiromatsu *et al.* 2005), confirmed by a meta-analysis in other populations (Houston *et al.* 2004, Kurylowicz *et al.* 2005, Wang *et al.* 2019). It appears that the C allele of rs1883832 provokes a pro-inflammatory endothelial cell phenotype, compensated by enhanced CD40 shedding to neutralize excess CD40 ligand (Sultan *et al.* 2020). Besides, high concentrations of soluble CD40L has been identified in children with newly diagnosed GD and a correlation between soluble CD40L and both TSHR antibodies and thyroid volume, which may indicate a biologically active role for soluble CD40L in the pathogenesis of GD (Metwalley *et al.* 2020). However, there is little information showing how CD40 contributes to GD pathogenesis.

Interleukins

Interleukins (ILs) can significantly participate in inflammation, cell differentiation, and immune responses, and thus play essential roles in various immunological diseases (Sabzevary-Ghahfarokhi *et al.* 2018). Previously, we confirmed that different polymorphisms in proinflammatory cytokines can contribute to GD susceptibility in Iranian patients. We also demonstrated a remarkable correlation between GD and IL-2-330G, IL-12-1188C, and *IFNG* UTR 5644T alleles (Anvari *et al.* 2010). Other studies showed the correlation between ILs and GD; for example, a considerable positive association between polymorphisms of *IL1A* and *IL-1RA* genes and predisposition to GD have been demonstrated (Khalilzadeh *et al.* 2009); although, it was reported earlier by Cuddihy *et al.* that none of the A2 alleles of the *IL-1* receptor antagonist gene and the *IL1A* exon 5 polymorphism allowed for increased susceptibility to GD (Cuddihy & Bahn 1996). This significant difference can be justified by the founder effect, sample size, and technical issues in immunogenetic tests.

It seems that IL-6 plays a substantial role in GD, for example, a considerable association of *IL6*-174 G/C polymorphism and also the increased risk of GD in dominant, recessive, and homozygote contrast models have been reported and confirmed by some meta-analysis data (Imani *et al.* 2017). Moreover, it has been demonstrated that rs1800795 of IL-6 can increase susceptibility for GD (Tu *et al.* 2017). These data have been verified on the protein level as well, for example, augmentation of IL-6

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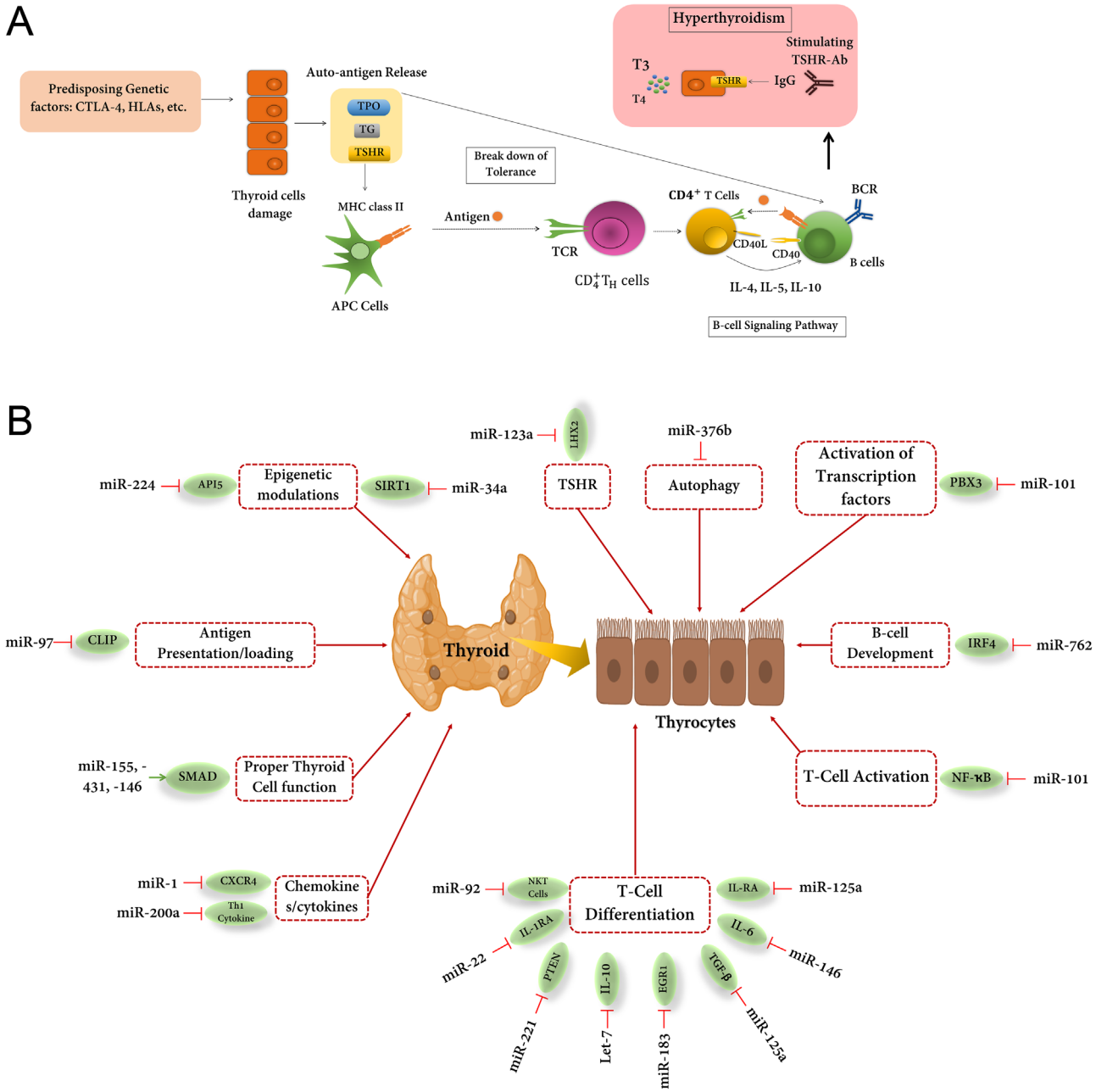

Figure 3

(A) A possible mechanism that involves TPO, CD40, HLA Class II, TG, and TSHR in GD. GD can be characterized by the presence of thyroid autoantibodies against TPO and TG in serum and thus, various degrees of thyroid dysfunction are expected. During GD, environmental factors along with genetic susceptible loci make a situation in which thyroid cells will be damaged and TSHR will be recognized as the most critical autoantigen. After breaking down the tolerance, aberrant production of stimulating TSHR antibodies exacerbate the condition and pave the way to hyperthyroidism. Antibodies mimic the effects of the hormone on thyroid cells, TSH, stimulating autonomous production of thyroxine (T3), and triiodothyronine (T4), so causing hyperthyroidism. Figure A is redrawn from Ramos-Leví & Marazuela (2016). (B) Most important miRNAs contributing to GD. These miRNAs can be used as a diagnosis/prognosis biomarker of GD patients. A full color version of this figure is available at https://doi.org/10.1530/JME-20-0078.

and IL-6R expression in sera of 49 GD patients (Salvi *et al.* 1996) have been reported.

IL-17 expression is significantly correlated with thyroid-associated ophthalmopathy pathogenesis and development (Chen 2019). IL-17 can play dual functions in GD: a predisposing or protecting factor; for example, the portion of *IL-17F*/rs763780 genotypes in GD patients varied considerably from the control groups; the frequency of A allele of rs3819025 was lower in GD patients. These data show that *IL-17F*/rs763780 polymorphism can increase predisposition to GD with unknown molecular mechanisms. On the other hand, *IL-17A*/

rs3819025 SNP has been identified as a likely protective allele of GD in Chinese populations (Yan *et al.* 2012).

The genetic association of *IL-16* and *IL-23R* has been also identified. The interactions of IL-16 recruit T helper cells in GD. Gu *et al.* showed an association of rs4778889, rs1131445, and rs4778641 of *IL-16* with an increased risk of GD in the Chinese population (Gu *et al.* 2008). Variants in the *IL-23R* gene, namely A, C, and T alleles of rs2201841, increase the susceptibility of GD by changing the expression and/or function of IL-23R, thereby triggering a pro-inflammatory signaling cascade (Huber *et al.* 2008).

Some studies suggested that interleukins might be used as a diagnostic marker for GD. For instance, *IL1B* gene promoter (−511 C/T) polymorphism may be used to predict GD susceptibility (Chen *et al.* 2005). Similarly, Yao *et al.* suggested that IL-32 and IL-32α^{+} cells may be associated with the pathogenesis of GD and also introduced IL-32 as a promising target and a marker for, respectively, treatment and diagnosis of GD (Yao *et al.* 2019*a*).

In some cases, the conclusion about the involvement of *IL* polymorphism in GD is controversial; for instance, there is an association between a promoter polymorphism of the *IL-4* gene and GD although Heward *et al.* showed that this polymorphism does not confer protection against the GD development in Caucasians in the United Kingdom (Heward *et al.* 2001). Furthermore, polymorphisms of the *IL-13* gene could confer susceptibility of Japanese populations to GD, that is, a decrease of allele frequency of 2044A in exon 4 and −1112T in GD patients was shown (Hiromatsu *et al.* 2005); however, another study suggested that these polymorphisms do not show any genetic susceptibility to GD at all (Bednarczuk *et al.* 2003). To some extent, this can be justified by genetic diversity and population structures that are unique for each population. These are the most important limitations in such studies. In sum, this is conclusive that ILs can predispose to GD through aberrant inflammatory signaling cascades.

CTLA4

Cytotoxic T-lymphocyte-associated protein 4 (CTLA4), also known as CD152, is a protein receptor involved in immune checkpoint and immune repression responses. Transmembrane protein CD152 quenches T cell responses and therefore helps to make self-antigen tolerance (Rahman *et al.* 2019). Several variants in *CTLA4* have been reported with increased risk of GD, T1D, RA, and SLE susceptibility (Wang *et al.* 2014); for example, rs231775 was correlated with a higher risk of GD susceptibility (Liu & Zhang 2013). The distinct relationships of *CTLA4* polymorphisms with GD and AITDs are still debatable (Ueda *et al.* 2003); however, it has been proposed that the decreased expression of the soluble form of CD152 (e.g. influenced by rs231775) contributes to GD (Waterhouse *et al.* 1995, Oaks & Hallett 2000).

Regulation of $CD4^{+}$ T cell-related memory responses by *CTLA4* may also play a role in developing autoimmune diseases (Devarajan 2014) (Fig. 3A). Indeed, activating heterozygote mutations in *CTLA4* increased the rate of autoimmunity, while treating with anti-CTLA4 monoclonal antibodies suppressed T cell activation and reduced the incidence of AITDs (Torino *et al.* 2013). It seems that the polymorphisms/genetic variations in the *CTLA4* can affect gene expression. Hence, low concentrations of intracellular CTLA4 may be associated with low cell surface expression of CTLA4 and therefore with reduced negative control of T cell proliferation, ultimately leading to T cell hyperresponsiveness and predisposition to GD.

PTPN22

PTPN22 encodes human lymphoid tyrosine phosphatase and shows a significant association with autoimmune diseases including GD, RA, SLE, and T1D (Stanford & Bottini 2014, Zhang *et al.* 2018). The interaction of lymphoid tyrosine phosphatase with the Csk and Fyn kinases functions as negative regulators of T cell receptor signaling, such as pattern recognition receptors (PRR), type 1 IFN pathway signaling, and IFn-γ-dependent activation (reviewed in Bottini & Peterson 2014).

There are some genetic variations in *PTPN22* showing a great association with GD; for example, rs2476601 that is associated with T1D, RA, SLE, and GD (Vang *et al.* 2005) is in the C-terminal of the protein presumably affects the interaction of this domain with adaptor TRAF3 and Csk kinase and results in PRR signaling reduction despite TCR signaling enhancement (Bottini & Peterson 2014). PRRs are categorized based on the recognition of ligands from two distinct groups: Pathogen-Associated Molecular Patterns and Damage-Associated Molecular Patterns. The contribution of these groups in the etiology of GD has been discussed (reviewed in Kawashima *et al.* 2013). Although many studies confirmed the association of rs2476601 with GD, one study showed that this polymorphism was not associated with GD in Kashmiri populations (Shehjar *et al.* 2018). The SNP might be linked with a higher risk of GD within the adult north-eastern Polish population (Wawrusiewicz-Kurylonek *et al.* 2019)

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and occasionally affected the GD onset in the Chinese Han population (Li-qun *et al.* 2010). Autoimmune *PTPN22* rs2476601 risk allele A controls the frequency of regulatory T cells in human peripheral blood that is decreased in GD (Valta *et al.* 2020). Other genetic variations in this gene also show the association with GD although there is not enough information about underlying molecular mechanisms.

FCRL3

Fc receptor-like protein 3 (FCRL3) protein involves immunoreceptor tyrosine-based activation motifs (ITAMs) and may act as an activator of the immune system. Different studies confirmed the association of *FCRL3* promoter SNPs with RA, AITDs, and SLE (Kochi *et al.* 2005), for example, three polymorphisms as in *FCRL3_3C*, *FCRL3_5C*, and *FCRL3_6A* were associated with multiple sclerosis (MS) and also were remarkably tied with a higher risk of GD in the Chinese Han population (Yuan *et al.* 2016). Additionally, several meta-analyses showed that the impressions of these novel variants on GD predisposition are different between Asian and Caucasian populations (Fang *et al.* 2016).

A/G SNP at position −169 in the promoter region of the *FCRL3* is strongly correlated with the predisposition of GD among the Chinese population. This allele is tightly pertinent to positive TSHR autoantibodies that in turn result in thyroid diseases (Jin *et al.* 2015). It seems that the genetic variations can exert their effects through changing the gene expression; for example, Stefanic *et al.* confirmed increased mRNA levels of *FCRL3* in peripheral blood T cells from end-stage, long-standing, and/or more aggressive autoimmune thyroid diseases were related to disease severity (Štefanić *et al.* 2019). This study acknowledges that co-inhibitory receptors, for example, FCRL3 and T cell immunoglobulin and ITIM domain, play an essential role in AITDs though their primary roles are uncertain.

Other important genes in the immune system

Several gene abnormalities may promote GD susceptibility. For example, it has been acknowledged that the *BACH2* is critical for class switch recombination and somatic hypermutation (Muto *et al.* 2004) and is an essential regulator of $CD4^+$ T-cell differentiation and hinders inflammatory disease by keeping a balance between tolerance and immunity (Roychoudhuri *et al.* 2013). A significant association of *BACH2* rs9344996 with GD was reported, which can be clarified by its linkage to *BACH2* rs2474619 in diverse populations (Liu *et al.* 2014). The genetic variants in the *BACH2* are associated with different autoimmune diseases such as asthma, coeliac disease, vitiligo, MS, and T1D (Cooper *et al.* 2008, Dubois *et al.* 2010, Sawcer *et al.* 2011, Jin *et al.* 2012). It was also shown that rs3757247 can increase the risk of autoimmune Addison's disease in humans (Pazderska *et al.* 2016). Despite these studies, the exact molecular mechanism by which BACH2 polymorphisms increase the risk of AITD needs further studies.

A genome-wide association study (GWAS) with >500,000 SNPs detected a new susceptible region located in 6q27 loci (*Ribonuclease T2 (RNASET2)-FGFR1* oncogene partner *FGFR1OP-CCR6*) and also an intergenic region at 4p14 (*GDCG4p14*) (Ban *et al.* 2013). *RNASET2* rs9355610 was associated with the susceptibility to GD in the Chinese Han population (Chen *et al.* 2015) and shown in other populations (Ban *et al.* 2013). Moreover, the G allele of rs9355610 may be a protective factor for liver damage in patients with GD, suggesting that RNase T2 has a potential intervention effect on GD and liver damage. This can *per se* provide a new target for the diagnosis and targeted therapy of GD combined with liver damage (Zhang *et al.* 2018).

Forkhead box P3 (FOXP3), also is known as Scurfin, is involved in immune system responses and may have a role in the etiopathogenesis of AITDs. FOXP3 is a master regulator in proper T cell development and also functions of Tregs. In the Chinese Han population, four SNPs including −2383, −3279, −3499 in the promoter region and IVS9+459 in the intron were genotyped and it was shown that these polymorphisms were highly tied with GD susceptibility (Zheng *et al.* 2015). Li *et al.* found that rs3761548 and rs3761549 polymorphisms in *Foxp3* were associated with a higher risk of GD among Asians, possibly because of the suppressed function of regulatory T cells and extended autoimmune responses (Li *et al.* 2020*b*).

PRICKLE1 protein can negatively regulate the Wnt/beta-catenin signaling pathway. Wnt signaling is vital for dendritic cells to appropriately regulate immunity and tolerance (Swafford & Manicassamy 2015). An association between *PRICKLE1* rs4768412 and GD was reported using an immunochip study (Consortium *et al.* 2012) that led to this notion that rs4768412 was nominally more frequent in pediatric-onset GD than adult-onset GD patients, which might be linked to the age of GD onset (Kus *et al.* 2019).

The elevated concentration of B lymphocyte activating factor (BAFF) that is vital for B cell-survival, -activation, and -differentiation has been also found in GD patients. In fact, various genetic variants within the *BAFF* gene can change the *BAFF* expression in GD

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patients (Kuo *et al.* 2008), confirmed by a study showed that the expression of *BAFF* and its particular receptor (BAFF-R) was elevated in infiltrating lymphocytes in GD-derived thyroid tissue (Campi *et al.* 2015). Similarly, the association of rs9514828 and rs4000607 in UK patients with GD have been reported that can change the gene expression (Lane *et al.* 2019). As an underlying molecular mechanism, Wang *et al.* showed that the skewed expression profile of BAFF receptors on B lymphocytes may mediate autoimmunity in GD, suggesting that restoring the normal expression profile can be a new strategy for GD treatment (Wang *et al.* 2020). In other words, blocking the interaction of BAFF with its receptor negatively affects B-cell proliferation, indirectly decreasing B-cell survival and reducing the production of autoantibodies in GD (Lane *et al.* 2020).

Lastly, the *SCGB3A2* gene, which encodes uteroglobin-related protein 1, plays important role in inflammation and immunologic responses (Yoneda *et al.* 2016). *SCGB3A2* −112G>A promoter polymorphism has been reported in association with GD in the Chinese population (Xue *et al.* 2014). This polymorphism was investigated in Caucasian cohorts, proposing this polymorphism can be noticed as a potential marker linking susceptibility to allergy/asthma and GD (Chistiakov *et al.* 2011). The main function of *SCGB3A2* in GD remains elusive. The most important genetic factors contributing to GD are summarized in Table 2.

How epigenetic factors contribute to GD

Epigenetic modulations have been suggested to influence susceptibility to AITD. Environmental factors such as stress, iodine diet, infections, and smoking can regulate and alter DNA methylation and histone modifications (Tomer & Huber 2009). These alternations along with gene silencing triggered by non-coding RNAs are the main epigenetic mechanisms that contribute to T cell differentiation and activity (Cai *et al.* 2015). The epigenetic mechanisms, indeed, regulate the chromatin structure and switch genes from 'on' to 'off', reversibly and temporarily. In the following, we summarized the important epigenetic mechanisms identified in GD.

DNA methylation

DNA methylation is a process in which methyl groups are added to target DNA, mediated by DNA methyltransferases (DNMTs). DNA methylation can silence gene expression by the addition of a methyl group to cytosine in CpGs, which recruits methyl-CpG-binding domain proteins that, in turn, are a starting signal for other modulators altering chromatin remodeling and transcriptional repression (reviewed in Coppedè 2017). Similar to many autoimmune diseases, GD is more common in females than men, a process that can be justified by skewed X chromosome inactivation (XCI) in women, that is, inactivation of either the maternal or paternal X chromosome. Various important immune-related genes are located in the X chromosome (e.g. *CD40L*, *FOXP3*, and *toll-like receptor 7*) that can be silenced in the XCI process. The fact is that the skewed XCI is associated with clinically overt AITD, particularly GD (Simmonds *et al.* 2014), and it has been also suggested that XCI is related to the AITD prognosis, not to its development (Coppedè 2017).

Different polymorphisms have been investigated in DNA methylation genes that can affect GD susceptibility. For example, rs2228612 in *DNMT1* was reported in association with DNA hypomethylation and with the intractability of GD (Arakawa *et al.* 2012). On the other hand, rs1801133 in methylenetetrahydrofolate reductase (imperative for a chemical reaction involving the vitamin folate as the early substrate of methylation) was associated with reduced GD risk in women (Mao *et al.* 2010).

Genome-wide DNA methylation studies in GD have exhibited DNA methylation profiles in new CpG sites, among them many genes and pathways are related to IFN signaling, immune responses, lymphocyte activation, and HLA loci. The results indicate that GD patients have many hypomethylated CpGs sites in their $CD8^+$ T cells. For instance, hypomethylation of the *NOTCH1* gene that regulates T cell differentiation has been found in AITD (Yui & Rothenberg 2014, Limbach *et al.* 2016). Limbach *et al.* identified a preferable differential methylation cluster at the MHC region on the 6p22.1 to 6p21.3 and methylation distinguished peaks at the HLA class I locus (*HLA-A*, *HLA-B*, *HLA-E*, and *TRIM39*). They identified alternations in methylation marks at HLA class II (*HLA-DRB1, HLA-DMB, PSMB8*, and *TAP1*) and class III (*TNFA* and *LTA*) genes. Approximately 40% of the CpGs undergone hypo/hypermethylation are located within intragenic regions, and less than 30% are in 5' regions. Gene expression analysis detected 46 and 980 differentially expressed genes in $CD4^+$ and $CD8^+$ T cells, respectively; for example, hypomethylation was observed at the *CD3E* gene in $CD4^+$ and $CD8^+$ T cells. Moreover, several genes were detected in $CD8^+$ T cells of GD patients that had different methylation profiles

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Table 2 Summary of the most relevant genes associated with AITDs and GD.

Group	Gene	Chr. location	Protein function(s)	Associated diseases	Used method(s)	Reference
Thyroid hormone synthesis	*TSHR*	14q31.1	Encodes the receptor for TSH as a primary auto-antigenic target of GD (Brand *et al.* 2009)	GD	GWAS and case–control studies	(Dechairo *et al.* 2005)
	TPO	2p25.3	Plays a central role in thyroid gland function	AITD, GD	GWAS, SNP screening and traditional case–control studies	(Begum *et al.* 2019)
	Thyroglobulin	8q24.22	Plays vividly in thyroid gland	AITD, GD	GWAS, SNP screening and traditional case-control studies	(Sakai *et al.* 2001)
	TRIB2	2p25.1	TG increases the canine *TRIB2* expression, which also plays a critical role in stimulating TSH to release (Wilkin *et al.* 1997)	AITD	GWAS and Immunochip	(Pujol-Borrell *et al.* 2015)
	FOXE1	9q22.33	Plays in thyroid gland morphogenesis and binds to response elements in the thyroglobulin (Tg) and thyroid peroxidase promoters (Castanet and Polak 2010)	AITD, TC, etc.	GWAS and Immunochip	(Campbell *et al.* 2016)
T-cell Response Regulatory	*HLA class I*	6p21	Presents endogenous antigens to $CD8^+$ T cells (Simmonds *et al.* 2005)	AITD, PS, RA, SLE, AS, etc.	GWAS & case–control studies	(Pujol-Borrell *et al.* 2015)
	HLA class II	6p21	presents exogenous antigens for recognition by $CD4^+$ T-helper cells (Simmonds *et al.* 2005)	AITD, T1D, CD, SLE, MS, etc.	SNP screening & traditional case–control studies	(Zamani *et al.* 2000)
	CTLA4	2q33.2	Inhibits T-cell signaling (Ueda *et al.* 2003)	AITD, T1D, CD, SLE, etc.	SNP screening & traditional case–control studies	(Zhao *et al.* 2010)
	PTPN22	1p13	Interacts with molecules essential for T-cell receptor signaling and involved in T-cell signal transduction (Smyth *et al.* 2004)	AITD, T1D, RA, SLE, etc.	SNP screening & traditional case–control studies	(Skórka *et al.* 2005)
	FCRL3	1q23.1	Has either positively and negatively role in regulating B-cell signaling (Kochi *et al.* 2005)	AITD, RA, MS, SLE, etc.	GWAS & case-control studies	(Simmonds *et al.* 2006)

(*Continued*)

Table 2 (Continued).

Group	Gene	Chr. location	Protein function(s)	Associated diseases	Used method(s)	Reference
Immune system responses	*IL2RA*	10p15.1	Encodes CD25 which downregulates T-cell activity (Lowe *et al.* 2007)	GD, MS, RA	SNP screening & traditional case-control studies	(Chistiakov *et al.* 2011)
	BAFF	13q33.3	As a cytokine is expressed in B cell lineage cells and functions as a potent B cell activator	AITD, GD	GWAS, SNP screening & traditional case-control studies	(Lane *et al.* 2019)
	HCP5	6p21.33	It is affiliated with the non-coding RNA class	AITD, GD, SLE, TC, Acquired Immunodeficiency Syndrome	GWAS & SNP analysis	(Kuś *et al.* 2015)
	SCGB3A2	5q32	is a downstream target of the thyroid transcription factor	Asthma, AITD, GD	GWAS	(Xue *et al.* 2014)
	CD40	20q13.12	Activates B-cells and APCs	AITD, GD	Meta-analysis & GWAS	(Wang *et al.* 2019)
	GDCG4p14	4p14	Expressed in CD4+ T helper and CD8+ T cells (Antonelli *et al.* 2015)	AITD	GWAS and Immunochip	(Antonelli *et al.* 2015)
	RAC2	22q12.3	RAC2 (Ras-related C3 botulinum toxin substrate 2) is a signaling G-protein and induces peripheral immune tolerance	AITD	GWAS & Immunochip	(Zhang *et al.* 2017)
	SLAMF6	1q23.2	Is a costimulatory molecule in T cell-stimulation; it can also mediate inhibitory signals in NK cells from X-linked lymphoproliferative patients	AITD	GWAS & Immunochip	(Zhao *et al.* 2013)
	BACH2	6q15	Involves in NF-ĸB Signaling and controls B-cell development and antibody production (Simmonds 2011)	AITD, T1D, CRD, CD, MS, etc.	GWAS and Immunochip	(Liu *et al.* 2014)
	ITGAM	16p11.2	Has a role in Immune response of Integrin in NK cells cytotoxicity (Hom *et al.* 2008)	AITD, SLE	GWAS & Immunochip	(Pujol-Borrell *et al.* 2015)
	RNASET2-FGFR10P-CCR6	6q27	Are expressed in $CD4^+$ T-helper and $CD8^+$ T cells	AITD	GWAS and Immunochip	Reviewed in (Oryoji *et al.* 2015)
	FOXP3	Xp11.23	Contributes to immune system responses	GD, AITD	GWAS	(Zheng *et al.* 2015)
	MMEL1	1p36.32	Involves in pain perception, phosphate metabolism, homeostasis, and immune responses (Danoy *et al.* 2011)	AITD, RA, MS, etc.	GWAS & Immunochip	(Cooper *et al.* 2012)

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Genes with Unknown function in AITD	*LPP*	3q27.3/3q28	LPP (LIM Domain Containing Preferred Translocation Partner in Lipoma) is a Protein-Coding gene. Diseases associated with LPP include Lipoma and Leukemia, Acute Myeloid (Schoenmakers *et al.* 1995)	AITD, CD	GWAS & Immunochip	(Pujol-Borrell *et al.* 2015)
	Gene desert	11q21	This association was reported in a gene desert and recently is little known about this region's potential functions	AITD	GWAS & Immunochip	(Pujol-Borrell *et al.* 2015)
	PRICKLE1	12q12	Is expressed in the brain and associated with Epilepsy-Ataxia Syndrome (Pujol-Borrell *et al.* 2015)	AITD	GWAS & Immunochip	(Hamilton *et al.* 2001)
	GPR174-ITM2A	Xq21.1	GPR174 is associated with Autoimmune Addison's Disease (Napier *et al.* 2015). ITM2A is induced during Thyrocyte selection and T cell activation and plays a role in Osteo- and Chondrogenic differentiation (Tuckermann *et al.* 2000)	AITD	GWAS & Immunochip	(Zhao *et al.* 2013)

AITD, autoimmune thyroid disease; AS, ankylosing spondylitis; CD, Crohn's disease; GD, Graves' disease; GPCR, G protein-coupled receptor; GWAS, genome-wide association study; MME, membrane metallo-endopeptidase; NEP, neutral endopeptidase; PS, psoriasis; RA, rheumatoid arthritis; SLE, systemic lupus erythematous; TC, thyroid cancer.

including *BCL11B*, *CXCR4*, *HLA class I*, *FYB*, *TNFRSF1B*, *IFNG* genes (Deng *et al.* 2019).

Histone modifications

Various histone modifications have been postulated to either open or condense chromatin structure and can, in turn, change gene expression. These alterations include histone tail acetylation, methylation, phosphorylation, ubiquitination, and sumoylation. Among these, acetylation and methylation have been studied very well, but little in GD. A reduced global histone H4 acetylation (required for chromatin decompaction) levels with increased levels of histone deacetylase proteins have been reported in peripheral blood mononuclear cells in GD patients (Yan *et al.* 2015).

Methylation can occur in histone levels as well. For instance, it has been reported that histone methylation is aberrant in peripheral blood mononuclear cells of GD patients (Yan *et al.* 2019). This process can be attributed to the deregulation of epigenetic modifier genes, suggesting that abnormal histone methylation modification may be involved in the pathogenesis of GD, for example, the hypermethylation of *CD3* gene family members, the first intron of *TSHR*, *CTLA4,* and *B3GNT2* (regulates lymphocyte activation) has been found (Coppedè 2017). On the other hand, the hypomethylation of *intercellular adhesion molecule 1* has been reported in association with GD (Cai *et al.* 2015).

Studies also revealed reduced-trimethylated lysine 4 at histone H3 (H3K4me3) and acetylation of lysine 27 at histone (H3K27ac) marks at genes that are involved in T cell activation. To date, plenty of genes have been identified that play role in T cell signaling and activation, for example, *CD247*, *CD3D, CD3E*, *CD3G*, *CD8A, LCK*, *ZAP70,* and *CTLA4*; the common feature of these genes is that they have a low level of H3K4me3 marks in their promoter regions (leading to the decreased gene expression) in both $CD4^+$ and $CD8^+$ T cells of GD patients. Reduced expression of *CD3* gene family members (Limbach *et al.* 2016) has been also found.

Non-coding RNAs

A growing body of research shows that non-coding RNAs including microRNAs (miRNAs or miRs) and long non-coding RNAs (lncRNAs) have an impaired expression in AITD. miRNAs are small (~22 nt), single-stranded, and highly conserved molecules that regulate gene expression via base-pairing with complementary sequences within

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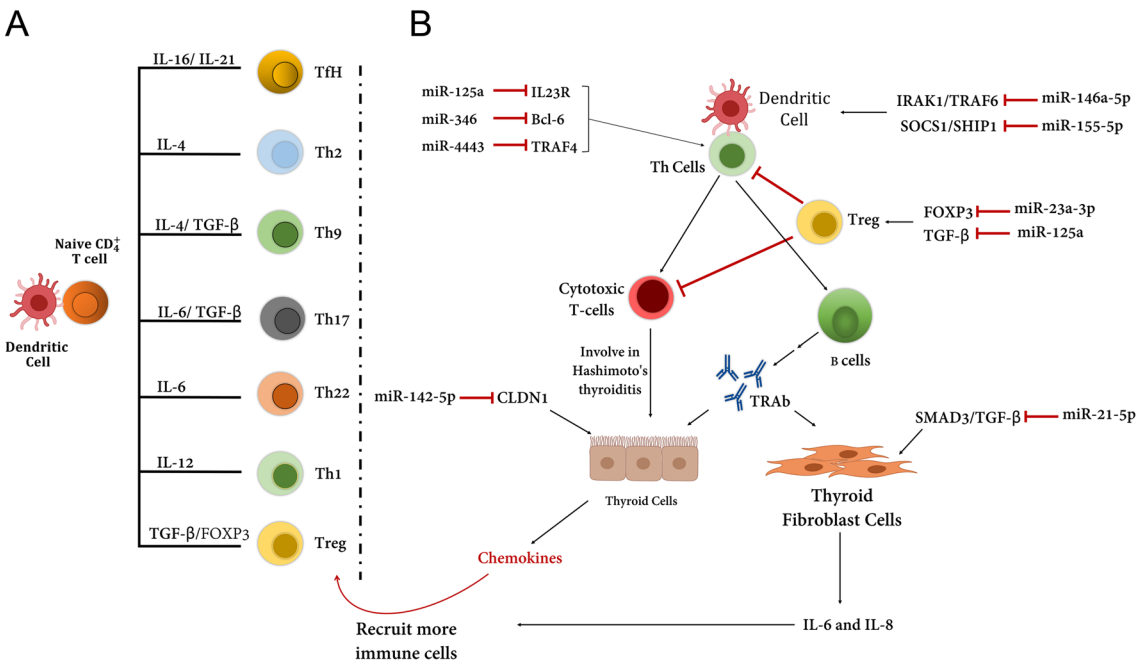

Figure 4

(A) The development of T cells depends on the stimulation/expression of various genes, for example, *ILs*. Naive CD4⁺ T cells activated by dendritic cells (DC) can be differentiated into various T cells. Under normal conditions, normal functions of T cells maintain immune tolerance (immune homeostasis). In this figure, TfH, follicular helper T cells; Th, CD4⁺ T helper (Th) cells; and Treg, regulatory T cells. The figure is redrawn from Wang *et al.* (2017*a*). (B) The aberrant expression of miRNAs can lead to breaking down of immune homeostasis that, in turn, causes immune attacks toward thyroid tissues during the GD development. For example, miR-146a-5p can inhibit the IL-1R-associated kinase 1 (IRAK1) and TNF-receptor-associated factor 6 (TRAF6) that are critical for dendritic cell maturation and development (Kobayashi *et al.* 2003). FOXP3, determining natural Treg development and function, can be repressed by miR-23a-3p. Although cytotoxic T cells do not play a role in GD, they malfunction in Hashimoto's disease. Thyroid fibroblast cells are often involved in graves ophthalmopathy and they can increase the expression of IL-6 and IL-8 that along with other chemokines contribute to recruiting other immune cells. MiR-142-5p targeting CLDN1 results in the reduced expression of claudin-1 and also increased permeability of thyrocytes monolayer. Overexpression of miR-142-5p in thyrocytes has been reported in GD patients. The figure is redrawn (Wang *et al.* 2017*a*). A full color version of this figure is available at https://doi.org/10.1530/JME-20-0078.

mRNA molecules. They often bind to 3'-UTR of target mRNAs and influence their translational efficiency. At least 60% of human genes contain target sites for miRNAs. Regarding GD, it has been identified that the differential expression of let-7b and miR-146a-5p in GD patients in comparison with controls is associated with GD development. miR-146a-5p is positively associated with TSHR-Abs, suggesting that let-7b and miR-146a-5p may serve as a biomarker for diagnosis and follow-up of GD patients (Al-Heety *et al.* 2020) (Fig. 3B). miRNAs can predict the predisposition of a worsening clinical outcome in patients with GD. For instance, miR-let7d-5p, miR-21-5p, miR-96-5p, miR-142-3p, and miR-301a-3p are significantly expressed in AITD and especially in GD patients, and can be implied as an indicator of higher severity of disease including active ophthalmopathy, goiter, higher antibody titers, and/or higher recurrence rates (Martínez-Hernández *et al.* 2018). Dendritic cells (DC), as an antigen-presenting cell, can activate naive CD4⁺ T cells which in turn differentiate into various T helper subsets that are characterized by different cytokine profiles and specific transcription factors. The balance of those immune cells is imperative for the maintenance of immune homeostasis (Fig. 4A). It seems that dysregulated miRNAs can change this homeostasis toward thyroid diseases (Fig. 4B). Aberrant miRNA expression is often detectable in AITDs; however, little information is provided about the miRNAs' contribution to GD. In this review, we summarized some important miRNAs that show aberrant expression in AITD, particularly GD (Table 3).

Aberrant lncRNAs expression or function has been also reported to contribute to GD development; lncRNAs are non-coding RNAs that length more than 200 nucleotides. For example, *HCP5* encodes a lncRNA and in terms of the sequence, this gene is pertinent to human endogenous retroviruses HERV-L and HERV-16. Interestingly, this gene is located within the MHC class I region. The encoded lncRNA is involved in adaptive and innate immune responses and is associated with the induction of some autoimmune diseases (Kulski 2019). Several variants in this gene have been linked to drug-related Stevens-Johnson syndrome, SLE, Kawasaki disease,

Table 3 Most important microRNAs (miRs) that have a great association with AITDs.

Non-coding RNAs	Abnormal expression (↑ or ↓)	Sample type	Function	AITD	Reference
miR-200, miR-34a, miR-143, miR-1238	ND	PBMC of GD patients and healthy individual	NR	AITD, GD	(Glinsky 2008)
miR-154-5p, miR-376b, and miR-431-5p	↓	PBMC of GD patients and healthy individual	NR	GD	(Liu *et al.* 2012)
miR-200a1	↑	Thyroid tissue of HT and GD patients	NR	GD, HT	(Bernecker *et al.* 2012)
miR-146a1	↓	Thyroid tissue of GD patients	NR	GD	(Bernecker *et al.* 2012)
miR-155	↑	PBMC, Fibroblasts	Increased miR-155 promotes ocular inflammation.	GD, GO	(Li *et al.* 2014)
miR-146a	↓	PBMC, Fibroblasts	Decreased miR-146a may promote ocular inflammation and proliferation in GO patients.	GD, GO	(Li *et al.* 2014)
miR-200a_1, miR-200a2-5p, miR-155	↓	CD4+ T cells	miR-155 can modulate the differentiation and function of cells of the innate and adaptive immunity and also can downregulate *SMAD4* in PBMCs of GD patients.	GD, HT	(Bernecker *et al.* 2014)
miR-125a	↓	PBMCs	miR-125a acts as a negative regulator of interleukin (IL)-6 and transforming growth factor (TGF)-β.	HD, AITD, GD	(Inoue *et al.* 2014, Peng *et al.* 2015)
miR-22, miR-183	↑	Specimens of thyroid tissue from GD patients	miR-22 targets estrogen receptor alpha mRNA, resulting in the repression of estrogen signaling, which is required for T cell differentiation. miR-183 is a key factor in TGF-β1-mediated immune suppression.	GD	(Qin *et al.* 2015)
miR-101, miR-197, miR-660	↓	Specimens of thyroid tissue from GD patients	miR-101 targets JAK/STAT and nuclear factor-kappa B (NF-κB) pathway inhibitors, so can change TNF production. miR-197 targets CILP and IL6R that are upregulated in GD. No conclusive roles of miR-660 in GD pathogenesis were detected.	GD	(Qin *et al.* 2015)
miR-346	↑	circulating $CD4^+$ T cells and plasma	miR-346 inhibits *Bcl-6* expression and regulates the activation of $CD4^+$ T cells.	GD	(Chen *et al.* 2015)
miR-224-5p	↓	Serum of GD and GO patients	overexpression of miR-224–5p can restore glucocorticoid sensitivity via targeting GSK-3beta in GO cell models	GD, GO	(Shen *et al.* 2015)
miR-23b-5p, miR-92a-39	↑	PBMC of GD patients after and before remission	miR-23b regulates NF-κB signaling pathway in GD, while miR-92a induces IL-6+ IL-10+ Natural Killer Cells, suppressing cytotoxic $CD8^+$ T cells.	GD	(Hiratsuka *et al.* 2016)
let-7g-3p and miR-339-5p	↓	PBMC of GD patients after and before remission	They can upregulate cytokine production in GD patients.	GD	(Hiratsuka *et al.* 2016)
let-7e	↑	PBMC	let-7e regulates intracellular IL-10 expression in HD patients.	HD, GD	(Kagawa *et al.* 2016)
miR-4443, miR-10a, miR-125b	↓	CD4+ T cells from untreated GD (UGD) patients	miR-4443 causes $CD4^+$ T cells dysfunction by targeting TNFR-associated factor 4 in GD. No molecular function of miR-10a and -125b was detected in GD.	GD	(Qi *et al.* 2017)
miR-1a	↓	Serum of GD patients	NR	GD	(Wang *et al.* 2017*b*)
miR-16-1-3p, miR-122-5p, miR-221-3p, miR-762	↑	Plasma	NR	GD	(Yao *et al.* 2019*b*)
miR-23a-3p	↓	PBMC	NR	GD	(Zhang *et al.* 2019)
miR-21-5p	↑	Plasma	miR-21-5p regulates lymphocyte differentiation and activation in GD patients.	GD, GO	(Al-Heety *et al.* 2020)

GD, Graves' disease; GO, Graves ophthalmopathy; HD, Hashimoto's disease; HT, Hashimoto's thyroiditis; NR, not reported; PBMC, peripheral blood mononuclear cell.

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and psoriasis (reviewed in Kus *et al.* 2019). Regarding AITD, *HCP5* rs3094228 polymorphism has been reported in association with TPO antibody levels and also GD susceptibility in Polish-Caucasian populations (Kuś *et al.* 2015). The number of *HCP5* risk alleles is inversely associated with the age of GD onset. This suggests *HCP5* as one of the GD risk loci. LncRNA Heg, as a GD-associated lncRNA, was demonstrated by Christensen *et al.* and was found to be related to the degree of mRNA as well as CD14 TRAb in mononuclear cells of GD patients (Christensen *et al.* 2008). Some lncRNAs are limited to AITDs and their roles in GD are still unclear. For example, SAS-ZFAT, an antisense transcript of the *ZFAT* gene, was reported to increase susceptibility to AITD (reviewed in Wu *et al.* 2015). How the lncRNAs regulating network affects GD mechanisms is still elusive and we believe that it is an important point for or discussion and further research.

Exosomes

Extracellular vesicles (EVs) can be in a range of 50–200 nm (Bæk *et al.* 2016). EVs are secreted by all cells and play roles in various physiological functions containing signaling, communication, and defense (Stahl & Raposo 2019). It has been shown that exosomes and their pertinent molecules, such as proteins and miRNAs, are tightly correlated with the pathogenesis in the majority of human malignancies. Exosomes have been also recently shown to play roles in the pathogenesis of GD. For example, Hiratsuka *et al.* showed that exosomes from intractable GD patients stimulated mRNA expression for IL-1β and TNF-α compared with GD patients in remission or healthy controls. Thus, it seems that serum exosomes of patients with intractable GD can activate immune cells, which in turn play an important role in GD pathogenesis (Hiratsuka *et al.* 2016). It has been also discussed that thyrocyte-derived exosome-targeted dendritic cells (harbored TPO, heat shock protein 60, MHC-II, and activated dendritic cells) can strongly stimulate $CD4^+$ T lymphocyte responses and play a role in the occurrence and development of AITD (Cui *et al.* 2020). This study increases the chance of establishing a proper therapeutic approach to treat AITD, therefore, future research should be conducted in more realistic settings to support this need.

Conclusions and future perspectives

Global efforts have been committed to elucidating the susceptibility loci that are responsible for GD risk ever since genetics were identified as a contributing factor to AITD susceptibility. Even though there are currently numerous associated genes, figuring out the disease etiopathogenesis will be improved with cutting-edge technologies and universal endeavors that are developed to a wide range of novel genes, variants, and various contributing factors. The synchronized genome-wide assay of gene expression, GWAS, and using next-generation sequencing techniques allow mapping of the genetic contributors that emphasize individual differences in quantitative levels of expression. In addition to genetic factors, the contribution of epigenetic modifications to GD pathogenesis should be addressed more than before, as data are lacking in this regard. The vital issue now is to specify how these novel discovered variants and epigenetic modifications influence GD pathogenesis. The functional analysis of these genes will provide more opportunities to convert these genetic findings into a better understanding of GD pathogenesis and apply them to devise new potential therapeutic options.

In this review, we observed that there are various possible genes and epigenetic modifications that are related to GD development and/or susceptibility. These observations raise very fundamental questions of how these genes, encoded proteins, or RNAs play role in a tortuous network of signaling pathways that contribute to GD initiation or development. We also realize that some points of GD etiology remain to be discovered; for example, how epigenetic modulations in combination with genetic and environmental interventions play roles in GD. Not much is known about why there is a great difference between susceptible loci in different populations; are there environmental factors (e.g. specific dietary habits) modulating susceptibility to GD? Most applied studies to GD have been performed by using small populations which is, in turn, a drawback of such studies; however, we believe that coming investigations will cast light on GD, which in turn provides valuable information about different biological aspects of 'GD etiology' and will pave the way to utilize them effectively in therapeutic purposes.

Declaration of interest

The authors declare that no conflict of interest could be perceived as prejudicing the impartiality of this review.

Author contribution statement

E R and M M involved in conceptualization. E R, A R B, H Y and M S contributed to methodology of the study. M S, M M, S A, A R B, J R C and H Y involved in the validation of the data. E R, A R B and MS involved in the

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writing – original draft preparation. A R B, H Y, M M, J R C and S A involved in the writing – review and editing. E R and A R B involved in visualization. M M involved in the supervision. All authors approved final version of the article.

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