Genetic and epigenetic basis of Graves’ disease

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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

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).
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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**Table 1**

<table>
<thead>
<tr>
<th>Brand</th>
<th>Year</th>
<th>TSHR Gene</th>
<th>SNPs</th>
<th>Functional Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anvari</td>
<td>2010</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zhao</td>
<td>2019</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zimmermann</td>
<td>2015</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**References**

- Khalilzadeh et al. 2009
- Anvari et al. 2010
- Zimmermann et al. 2015
- Zhao et al. 2019
- Brand et al. 2009
- Tonacchera & Pinchera 2000
- Tomer 2014
- Tomer et al. 2013
- Rapoport & McLachlan 2007
- Colobran et al. 2011
- Mathis & Benoist 2009

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**Note:**

The content is a summary of the genetic and epigenetic basis of Graves' disease, focusing on TSHR and its polymorphisms, as discussed in the referenced articles. The table provides a brief overview of the SNPs identified in the TSHR gene and their potential functional effects.
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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 (fTSHR or TSH holoreceptor) and 2 main splicing isoforms 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.

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 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

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. 
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.

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

<table>
<thead>
<tr>
<th>Genetic variation</th>
<th>Function</th>
<th>Year</th>
<th>Population</th>
<th>Increased risk</th>
<th>Associated region</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2234919</td>
<td>Ameliorates G(s)alpha protein activation of TSHR</td>
<td>1995</td>
<td>Caucasian</td>
<td>Yes</td>
<td>Exon 1</td>
<td>(Ban et al. 2002)</td>
</tr>
<tr>
<td>DS14581, TSHR-AT</td>
<td>NR</td>
<td>1997</td>
<td>Caucasian</td>
<td>Yes</td>
<td>Chr. 14q31</td>
<td>(Tomer et al. 1999)</td>
</tr>
<tr>
<td>rs1991517</td>
<td>rs1991517 alters the binding affinity to cAMP, thus changes signaling pathways mediated by TSHR</td>
<td>2002</td>
<td>Russian</td>
<td>Yes</td>
<td>Exon 10</td>
<td>(Yin et al. 2008)</td>
</tr>
<tr>
<td>D14S258, rs2239610</td>
<td>NR</td>
<td>2003</td>
<td>Caucasian</td>
<td>Yes</td>
<td>Chr. 14q</td>
<td>(Tomer et al. 2007)</td>
</tr>
<tr>
<td>rs2268458, rs2268475, rs3783938</td>
<td>NR</td>
<td>2005</td>
<td>Caucasian</td>
<td>Yes</td>
<td>Intron 1, Intron 7, Intron 8</td>
<td>(Tomer et al. 2009)</td>
</tr>
<tr>
<td>rs3783941</td>
<td>NR</td>
<td>2007</td>
<td>Caucasian</td>
<td>Yes</td>
<td>Intron 8</td>
<td>(Płoski et al. 2010)</td>
</tr>
<tr>
<td>rs2268458</td>
<td>NR</td>
<td>2008</td>
<td>Caucasian</td>
<td>Yes</td>
<td>Intron 1</td>
<td>(Qu et al. 2010)</td>
</tr>
<tr>
<td>rs179247, rs12101255</td>
<td>Can increase the production of STS and change the TSHR expression</td>
<td>2009</td>
<td>Caucasian</td>
<td>Yes</td>
<td>Intron 1</td>
<td>(Colobran et al. 2011)</td>
</tr>
<tr>
<td>rs12101261</td>
<td>Decreases the intrathymic TSHR expression through signaling pathways mediated by promyelocytic leukemia zinc finger (PLZF) protein</td>
<td>2011</td>
<td>Chinese</td>
<td>Yes</td>
<td>Intron 1</td>
<td>(Chu et al. 2011)</td>
</tr>
<tr>
<td>rs12101255</td>
<td>By binding to PLZF protein decreases the intrathymic TSHR expression</td>
<td>2012</td>
<td>Chinese</td>
<td>Yes</td>
<td>Intron 1</td>
<td>(Yin et al. 2012)</td>
</tr>
<tr>
<td>rs2284720, rs179243</td>
<td>NR</td>
<td>2013</td>
<td>Caucasian</td>
<td>Yes</td>
<td>Intron 1</td>
<td>(Tomer et al. 2013)</td>
</tr>
<tr>
<td>rs12885526, rs179247, rs3783948</td>
<td>NR</td>
<td>2015</td>
<td>Brazilian</td>
<td>Yes</td>
<td>Intron 1</td>
<td>(Bufalo et al. 2015)</td>
</tr>
<tr>
<td>rs12101261, rs4903964, rs179247, rs2284722, rs17111394</td>
<td>rs179247 can increase the production of STS and change the TSHR expression, while rs12101261 changes TSHR gene expression through binding to PLZF protein.</td>
<td>2016</td>
<td>Chinese</td>
<td>Yes</td>
<td>Intron 1</td>
<td>(Jing et al. 2016)</td>
</tr>
<tr>
<td>rs4411444, rs4903961</td>
<td>NR</td>
<td>2017</td>
<td>Japanese</td>
<td>Yes</td>
<td>Intron 1</td>
<td>(Fujii et al. 2017)</td>
</tr>
</tbody>
</table>

NR, not reported.

**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...
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 allore cognition (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

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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 IL28A. 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 withAITDs, 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).
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. 2020a).

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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R39
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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, rs1311445, 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. 2019a).

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-Kurylew et al. 2019).
and occasionally affected the GD onset in the Chinese Han population (Li-qun et al. 2010). Autoimmune PTNP22 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 (Stefanic 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 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 −3283, −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. 2020b).

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.
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 rs4006607 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 uroteroglobin-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 Coppè 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 (Coppè 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 (TNA 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
Table 2  Summary of the most relevant genes associated with AITDs and GD.

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

(Continued)
### Table 2 (Continued)

<table>
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<tr>
<th>Group</th>
<th>Gene</th>
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<th>Associated diseases</th>
<th>Used method(s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune system responses</td>
<td>IL2RA</td>
<td>10p15.1</td>
<td>Encodes CD25 which downregulates T-cell activity (\text{(Lowe et al. 2007)})</td>
<td>GD, MS, RA</td>
<td>SNP screening &amp; traditional case-control studies</td>
<td>(Chistiakov et al. 2011)</td>
</tr>
<tr>
<td></td>
<td>BAFF</td>
<td>13q33.3</td>
<td>As a cytokine is expressed in B cell lineage cells and functions as a potent B cell activator</td>
<td>AITD, GD</td>
<td>GWAS, SNP screening &amp; traditional case-control studies</td>
<td>(Lane et al. 2019)</td>
</tr>
<tr>
<td></td>
<td>HCP5</td>
<td>6p21.33</td>
<td>It is affiliated with the non-coding RNA class</td>
<td>AITD, GD, SLE, TC, Acquired Immunodeficiency Syndrome</td>
<td>GWAS &amp; SNP analysis</td>
<td>(Kuś et al. 2015)</td>
</tr>
<tr>
<td></td>
<td>SCGB3A2</td>
<td>5q32</td>
<td>is a downstream target of the thyroid transcription factor</td>
<td>Asthma, AITD, GD</td>
<td>GWAS</td>
<td>(Xue et al. 2014)</td>
</tr>
<tr>
<td></td>
<td>CD40</td>
<td>20q13.12</td>
<td>Activates B-cells and APCs</td>
<td>AITD, GD</td>
<td>Meta-analysis &amp; GWAS</td>
<td>(Wang et al. 2019)</td>
</tr>
<tr>
<td></td>
<td>GDCG4p14</td>
<td>4p14</td>
<td>Expressed in CD4+ T helper and CD8+ T cells (\text{(Antonelli et al. 2015)})</td>
<td>AITD</td>
<td>GWAS and Immunochip</td>
<td>(Antonelli et al. 2015)</td>
</tr>
<tr>
<td></td>
<td>RAC2</td>
<td>22q12.3</td>
<td>RAC2 (Ras-related C3 botulinum toxin substrate 2) is a signaling G-protein and induces peripheral immune tolerance</td>
<td>AITD</td>
<td>GWAS &amp; Immunochip</td>
<td>(Zhang et al. 2017)</td>
</tr>
<tr>
<td></td>
<td>SLAMF6</td>
<td>1q23.2</td>
<td>Is a costimulatory molecule in T cell-stimulation; it can also mediate inhibitory signals in NK cells from X-linked lymphoproliferative patients</td>
<td>AITD</td>
<td>GWAS &amp; Immunochip</td>
<td>(Zhao et al. 2013)</td>
</tr>
<tr>
<td></td>
<td>BACH2</td>
<td>6q15</td>
<td>Involves in NF-κB Signaling and controls B-cell development and antibody production (\text{(Simmonds 2011)})</td>
<td>AITD, T1D, CRD, CD, MS, etc.</td>
<td>GWAS and Immunochip</td>
<td>(Liu et al. 2014)</td>
</tr>
<tr>
<td></td>
<td>ITGAM</td>
<td>16p11.2</td>
<td>Has a role in immune response of Integrin in NK cells cytotoxicity (\text{(Hom et al. 2008)})</td>
<td>AITD, SLE</td>
<td>GWAS &amp; Immunochip</td>
<td>(Pujol-Borrell et al. 2015)</td>
</tr>
<tr>
<td></td>
<td>RNASET2-FGFR10P-CCR6</td>
<td>6q27</td>
<td>Are expressed in CD4+ T-helper and CD8+ T cells</td>
<td>AITD</td>
<td>GWAS and Immunochip</td>
<td>Reviewed in (Orygi et al. 2015)</td>
</tr>
<tr>
<td></td>
<td>FOXP3</td>
<td>Xp11.23</td>
<td>Contributes to immune system responses</td>
<td>GD, AITD</td>
<td>GWAS</td>
<td>(Zheng et al. 2015)</td>
</tr>
<tr>
<td></td>
<td>MMEL1</td>
<td>1p36.32</td>
<td>Involves in pain perception, phosphate metabolism, homeostasis, and immune responses (\text{(Danoy et al. 2011)})</td>
<td>AITD, RA, MS, etc.</td>
<td>GWAS &amp; Immunochip</td>
<td>(Cooper et al. 2012)</td>
</tr>
</tbody>
</table>
GWAS &
Genetic and epigenetic basis of Graves’ disease

Reference
Limbach
Cai
Yan
LPP (LIM Domain Containing Preferred Protein) is expressed in the brain and is associated with neurological and psychiatric diseases. This protein is involved in the LIM domain-containing protein network, which plays a crucial role in axon guidance and neurite outgrowth.

Protein function
GPR174 is associated with autoimmune thyroid disease (AITD) and other immune-mediated disorders. 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).

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 the genome, and are involved in the regulation of various biological processes in GD patients (Deng et al. 2019).
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. 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. 2017a). A full color version of this figure is available at https://doi.org/10.1530/JME-20-0078.

**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, TH, follicular helper T cells; Th, CD4+ T helper (Th) cells; and Treg, regulatory T cells. The figure is redrawn from Wang et al. (2017a). (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. 2017a). A full color version of this figure is available at https://doi.org/10.1530/JME-20-0078.

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(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. 2017a). A full color version of this figure is available at https://doi.org/10.1530/JME-20-0078.

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 inAITDs; however, little information is provided about the miRNAs' contribution to GD. In this review, we summarized some important miRNAs that show aberrant expression inAITD, 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.

<table>
<thead>
<tr>
<th>Non-coding RNAs</th>
<th>Abnormal expression (↑ or ↓)</th>
<th>Sample type</th>
<th>Function</th>
<th>AITD</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-200, miR-34a, miR-143, miR-1238</td>
<td>ND</td>
<td>PBMC of GD patients and healthy individual</td>
<td>NR</td>
<td>AITD, GD</td>
<td>(Glinsky 2008)</td>
</tr>
<tr>
<td>miR-154-5p, miR-376b, and miR-431-5p</td>
<td>↓</td>
<td>PBMC of GD patients and healthy individual</td>
<td>NR</td>
<td>GD</td>
<td>(Liu et al. 2012)</td>
</tr>
<tr>
<td>miR-200a1</td>
<td>↑</td>
<td>Thyroid tissue of HT and GD patients</td>
<td>NR</td>
<td>GD, HT</td>
<td>(Bernecker et al. 2012)</td>
</tr>
<tr>
<td>miR-146a1</td>
<td>↓</td>
<td>Thyroid tissue of GD patients</td>
<td>NR</td>
<td>GD</td>
<td>(Bernecker et al. 2012)</td>
</tr>
<tr>
<td>miR-155</td>
<td>↑</td>
<td>PBMC, Fibroblasts</td>
<td>Increased miR-155 promotes ocular inflammation.</td>
<td>GD, GO</td>
<td>(Li et al. 2014)</td>
</tr>
<tr>
<td>miR-146a</td>
<td>↓</td>
<td>PBMC, Fibroblasts</td>
<td>Decreased miR-146a may promote ocular inflammation and proliferation in GO patients.</td>
<td>GD, GO</td>
<td>(Li et al. 2014)</td>
</tr>
<tr>
<td>miR-200a1, miR-200a2-5p, miR-155</td>
<td>↓</td>
<td>CD4+ T cells</td>
<td>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.</td>
<td>GA, HT</td>
<td>(Bernecker et al. 2014)</td>
</tr>
<tr>
<td>miR-125a</td>
<td>↓</td>
<td>PBMCs</td>
<td>miR-125a acts as a negative regulator of interleukin (IL)-6 and transforming growth factor (TGF)-β.</td>
<td>HD, AITD, GD</td>
<td>(Inoue et al. 2014, Peng et al. 2015)</td>
</tr>
<tr>
<td>miR-22, miR-183</td>
<td>↑</td>
<td>Specimens of thyroid tissue from GD patients</td>
<td>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.</td>
<td>GD</td>
<td>(Qin et al. 2015)</td>
</tr>
<tr>
<td>miR-101, miR-197, miR-660</td>
<td>↓</td>
<td>Specimens of thyroid tissue from GD patients</td>
<td>miR-101 targets JAK/STAT and nuclear factor-kappa B (NF-kb) 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.</td>
<td>GD</td>
<td>(Qin et al. 2015)</td>
</tr>
<tr>
<td>miR-346</td>
<td>↑</td>
<td>circulating CD4+ T cells and plasma</td>
<td>miR-346 inhibits Bcl-6 expression and regulates the activation of CD4+ T cells.</td>
<td>GD</td>
<td>(Chen et al. 2015)</td>
</tr>
<tr>
<td>miR-224-5p</td>
<td>↓</td>
<td>Serum of GD and GO patients</td>
<td>Overexpression of miR-224-5p can restore glucocorticoid sensitivity via targeting GSK-3β in GO cell models.</td>
<td>GD, GO</td>
<td>(Shen et al. 2015)</td>
</tr>
<tr>
<td>miR-23b-5p, miR-92a-39</td>
<td>↑</td>
<td>PBMC of GD patients after and before remission</td>
<td>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.</td>
<td>GD</td>
<td>(Hiratsuka et al. 2016)</td>
</tr>
<tr>
<td>let-7g-3p and miR-339-5p</td>
<td>↓</td>
<td>PBMC of GD patients after and before remission</td>
<td>They can upregulate cytokine production in GD patients.</td>
<td>GD</td>
<td>(Hiratsuka et al. 2016)</td>
</tr>
<tr>
<td>let-7e</td>
<td>↑</td>
<td>PBMC</td>
<td>let-7e regulates intracellular IL-10 expression in HD patients.</td>
<td>HD, GD</td>
<td>(Kagawa et al. 2016)</td>
</tr>
<tr>
<td>miR-4443, miR-10a, miR-125b</td>
<td>↓</td>
<td>CD4+ T cells from untreated GD (UGD) patients</td>
<td>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.</td>
<td>GD</td>
<td>(Qi et al. 2017)</td>
</tr>
<tr>
<td>miR-1a</td>
<td>↓</td>
<td>Serum of GD patients</td>
<td>NR</td>
<td>GD</td>
<td>(Wang et al. 2017b)</td>
</tr>
<tr>
<td>miR-16-1-3p, miR-122-5p, miR-221-3p, miR-762</td>
<td>↑</td>
<td>Plasma</td>
<td>NR</td>
<td>GD</td>
<td>(Yao et al. 2019b)</td>
</tr>
<tr>
<td>miR-23a-3p</td>
<td>↓</td>
<td>PBMC</td>
<td>miR-21-5p regulates lymphocyte differentiation and activation in GD patients.</td>
<td>GD, GO</td>
<td>(Zhang et al. 2019)</td>
</tr>
<tr>
<td>miR-21-5p</td>
<td>↑</td>
<td>Plasma</td>
<td>miR-21-5p regulates lymphocyte differentiation and activation in GD patients.</td>
<td>GD, GO</td>
<td>(Al-Heety et al. 2020)</td>
</tr>
</tbody>
</table>

GD, Graves’ disease; GO, Graves ophthalmopathy; HD, Hashimoto’s disease; HT, Hashimoto’s thyroiditis; NR, not reported; PBMC, peripheral blood mononuclear cell.
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 (Kuš 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 toAITDs 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 (Cai 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**

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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