Genetic susceptibility to Grave's disease

Hong Li[1], Qiuying Chen[1]

[1]Department of Endocrinology, Long Hua Hospital Affiliated to Shanghai University of Traditional Chinese Medicine

TABLE OF CONTENTS

1. ABSTRACT

The variety of clinical presentations of eye changes in patients with Graves' disease (GD) suggests that complex interactions between genetic, environmental, endogenous and local factors influence the severity of Graves' ophthalmopathy (GO). It is thought that the development of GO might be influenced by genetic factors and environmental factors, such as cigarette smoking. At present, however, the role of genetic factors in the development of GO is not known. On the basis of studies with candidate genes and other genetic approaches, several susceptibility loci in GO have been proposed, including immunological genes, human leukocyte antigen (HLA), cytotoxic T-lymphocyte antigen-4 (CTLA-4), regulatory T-cell genes and thyroid-specific genes. This review gives a brief overview of the current range of major susceptibility genes found for GD.

2. INTRODUCTION

Graves' disease (GD) is a systemic autoimmune syndrome with manifestation in thyroid and orbital connective tissue. Graves' ophthalmopathy (GO) is clinically characterized by exophthalmos, periorbital edema, eyelid retraction, extraocular muscle dysfunction, pain and optic neuropathy (1,2). These symptoms are related to the pathologic processes within the orbit of the eye that increase the volume of retro-ocular tissue, in which the orbital fibroblasts are the principal target of autoimmune attack and are the key to the pathophysiology of GO (3–5).

Even weak to moderately severe ophthalmopathy impacts greatly on and reduces the quality of life in affected patients (6). The course of GD is unpredictable and a rapid worsening of GO can occur at any time (7,8). As the treatment of GO is often inadequate, there is a need to recognize

potential predisposing factors and to develop sensitive and specific diagnostic procedures with which to identify GD patients at high risk of developing ophthalmopathy (9,10).

The exact etiology of the immune response to the thyroid is not known but there is evidence for a strong genetic influence on the development of autoimmune thyroid diseases (AITDs), including GD. It is currently accepted that AITDs are complex diseases in which susceptibility genes, in combination with environmental triggers, initiate the autoimmune response to the thyroid (11).

Both endogenous (genetic factors, increasing age, male gender) and exogenous factors (cigarette smoking, thyroid dysfunction (hyperthyroidism and hypothyroidism) and treatment with radiolabeled iodine) could contribute to the development and/or severity of GO (12). On the basis of studies with candidate genes and other genetic approaches, several susceptibility loci in GO have been proposed; for example, immunological genes, human leukocyte antigen (HLA), cytotoxic T-lymphocyte antigen-4 (CTLA-4), regulatory T-cell genes and thyroid-specific genes (13). It is widely agreed that complex diseases are not controlled by an individual gene or DNA variation alone but by a combination of genes or DNA variations. We give a brief overview of the current range of the susceptibility genes found for Graves' disease.

3. IMMUNOLOGICAL GENES
The immunological synapse is the interface between antigen-presenting cells (APCs) and T-cells that is formed during T-cell activation. This immunological complex interface involves a peptide antigen bound to an HLA class II molecule and to the T-cell receptor, co-stimulatory molecules, receptors on the APC and T-cells, integrins and other molecules (14). Interestingly, several GD susceptibility genes participate in the immunological synapse, suggesting that abnormalities in antigen presentation are important mechanisms in the pathway leading to GD.

3.1. CD40/CD40L
CD40, which is expressed primarily on B-cells and other APCs (15), has a fundamental role in B-cell activation and antibody secretion (16,17). The whole-genome linkage study done by Tomer identified a locus on chromosome 20q that was linked with GD. Fine mapping identified the CD40 gene as the GD susceptibility gene at this locus, and further sequencing studies of the CD40 gene demonstrated that a C/T single nucleotide polymorphism (SNP) in the Kozak sequence of CD40 was the likely causative variant (18–23). The CC genotype of this SNP is associated with GD (20–23) but one study did not find this association, possibly because of ethnic differences among populations (24); however, a meta-analysis confirmed the association (22). Intriguingly, we showed recently that the association of the CC genotype was stronger in a subset of GD patients with persistently high levels of thyroid antibodies (25). Mechanistically, the CC genotype is located in the Kozak sequence of CD40 and can alter CD40 translation and expression.

Other studies have shown that the C-allele of the polymorphism increases the translation of CD40 mRNA transcripts by 20–30% compared to the T-allele. CD40 is expressed on B-cells (15) and on thyroid follicular cells (25,26), which are both involved in the development of GD, and it is possible that increased expression of CD40 on B-cells and or thyrocytes driven by the C-allele predisposes to disease. Thus, increased expression of CD40 on B-cells can result in enhanced production of anti-TSHR-stimulating antibodies, whereas increased expression of CD40 on thyrocytes can trigger an autoimmune response to the thyroid by resident T-cells. These mechanisms are not mutually exclusive and could both be operative.

Because CD40 is a major APC and B-cell co-stimulatory molecule, it is plausible that CD40 will have a role in the genetic susceptibility to other autoimmune diseases. Indeed, recent data show that CD40 is associated with and/or linked to high levels of IgE in asthma, rheumatoid arthritis, systemic lupus erythematosus and multiple sclerosis (27).

CD40L, which is expressed on activated T-cells, and its receptor CD40 have been shown to have a role in the onset and maintenance of autoimmune inflammation. Zhao et al. showed that CD40L can potently induce ICAM-1 expression in orbital fibroblast cells through multiple signal pathways, such as the p38 MAPKs and NF-κB pathways in orbital fibroblast cells.

3.2. The Fc receptor-like (FCRL) family
SNPs are the most common form of DNA variation and have great potential as a medical diagnostic tool. Wei et al. analyzed the association between combinations of SNPs and GD by investigating 108 SNPs in 384 cases and 652 controls. When this method was evaluated by differentiating between cases and controls in a five-fold cross-validation test, it achieved a prediction accuracy of 72.9% with a combination of 17 SNPs. The experimental results showed that SNPs, even those with a high *P*-value, have a greater effect on GD when acting in combination compared to their effect when acting individually (79).

The FCRL gene family, which encodes products that have a key role in controlling B-cell signaling (28,29) has been shown to be associated with several autoimmune diseases. A genome-wide screening of 15,000 non-synonymous SNPs in a UK Caucasian GD cohort confirmed the association of the previously detected GD susceptibility locus FCRL3 and detected the association of neighboring FCRL5 with GD (30). Simmonds et al. used tag SNP screening of FCRL5 in an enlarged data set and confirmed the association of this gene with GD. Although the association at FCRL5 was shown to be secondary to the association at FCRL3, it does not prove that FCRL3 contains the etiological variant represented by these associations. It is tempting to embark upon a functional analysis of rs11264798 (the most strongly associated FCRL3 tag SNP) but haplotype analysis suggests that this might not be the etiological variant, as the high-risk G allele is associated only when present with the rs10489678 C allele (not the rs10489678 T allele),

suggesting the etiological variant could be located elsewhere within this region (31).

3.3. Chromosome 5q31–q33

Chromosome 5q31–q33 encodes a large variety of cytokines, inflammatory mediators and other immune modulators [interleukin (IL)-3, -4, -5, -9 and -13] and represents a region of linkage that has generated much interest (32). There is strong evidence that the Chr.5q31-33 region is linked to autoimmune thyroid disorders in Chinese and Japanese populations. Linkage of microsatellite marker D5S436 located within chromosome 5q31–33 was first detected in a Japanese GD genome-wide screen (33) and replicated later in a Han Chinese GD dataset along with other more strongly associated microsatellite markers, including D5S2090 (34) and in a second Japanese GD cohort associated with an extended 5q12–q33 region (35). Although no Caucasian GD genome-wide microsatellite screen has reported linkage to 5q31–33 (36–39), some evidence for linkage to a separate region of chromosome 5, 5q11.2–5q14.3, has been reported (37). Several genes within 5q31–33 have been proposed to explain the association of this region with GD, including beta-2-adrenergic receptor (ADRB2) (40), IL-12b (41), interferon regulatory factor 1, IL-13 and IL-4, although replication of these loci has proved difficult. More recently, two independent studies designed to narrow down the association within this region have identified two distinct GD susceptibility loci approximately 15 Mb apart, which could potentially explain linkage to GD in this region (32).

3.4. The HLA gene

The major histocompatibility (MHC) complex region encoding the HLA glycoproteins is a highly polymorphic genetic region that includes many genes and is located on chromosome 6p21. It is subdivided into (1) the class I region, which encodes HLA antigens A, B and C, (2) the class II region, which encodes HLA antigens DR, DQ and DP, each with one or more α and β chains and (3) the class III region, which encodes several immune-regulatory molecules, including complement components, heat shock protein 70 (HSP70) and TNF (42). Initial studies analyzed the major HLA class II alleles in AITD (43).

Grumet *et al.* were the first to show the association between GD and the alleles of MHC class I, with a higher frequency of HLA-B8 in GD patients (47%) compared to the controls (21%). However, a stronger association of GD was found with the MHC class II allele HLA-DR3, which is in strong linkage disequilibrium with HLA-B8. In non-white populations, GD has been found to be associated with different HLA alleles; HLA B35, B46, A2 and DPB1*0501 in Japanese and B46, DR9, DRB1*303 and DQB1*0303 in Hong Kong Chinese (42).

Recent studies have shifted the focus from the association of the major HLA-DR allele groupings with AITD to the molecular structure of the peptide-binding pocket and its association with disease (44). This mechanistic-based approach has proved to be very fruitful. Because T-cells recognize and respond to peptide antigens when presented by APCs bound to HLA class II pockets, it was hypothesized that certain HLA-DR alleles permit autoantigenic peptides to fit into the peptide-

binding pocket and to be presented more efficiently to T-cells (45). We recently identified arginine at position 74 of the HLA-DRb1 chain (DRb-Arg74) as the crucial DR amino acid conferring susceptibility to GD. By contrast, the presence of glutamine at position 74 of the DRb1 chain is protective. These data were replicated in an independent dataset (46). Similarly, a pocket HLA-DR amino acid signature was identified that confers strong risk for Hashimoto's thyroiditis (HT) (44). As in GD, the key pocket amino acid was DRb-Arg74. Structural analysis demonstrated that this pocket amino acid signature has a unique pocket structure that is likely to influence pathogenic peptide binding and presentation to T-cells. Further studies identified the Tg peptides that could be presented by HLA-DR pockets containing arginine at position beta 74 (47). Thus, the peptide-binding pocket structure has a major role in the etiology of AITD (48).

3.5. CTLA-4

The CTLA-4 gene is an important negative regulator of T-cell activation (49). Because CTLA-4 controls normal T-cell responses by suppressing T-cell activation, it was postulated that CTLA-4 polymorphisms that reduce its expression and/or function might predispose to autoimmunity by creating over-reactive T-cells.

The first demonstration of an association between CTLA-4 and GD was reported by DeGroot *et al.* (50). They showed a significant association between a microsatellite in the 3′UTR of CTLA-4 and GD, which was the first report of an association between CTLA-4 and an autoimmune condition. CTLA-4 is now established as an autoimmunity gene linked to and/or associated with various autoimmune conditions, including both GD and HT (51–56), as well as the production of thyroid antibodies alone without clinical disease (57–59). The association has been shown to be consistent across ethnic and geographic groups (50,53,60–63).

Recent work by Vieland *et al.* showed that the involvement of CTLA-4 in the genetic susceptibility to AITD is more complex than originally thought. CTLA-4 alone predisposes to the development of thyroid antibodies, but it could have a role in the susceptibility to high levels of thyroid antibodies and clinical AITD when interacting with other loci (64). Moreover, both the G allele (reported to be associated with AITD) and the A allele (reported to be protective) of the A/G49 SNP of CTLA-4 might predispose to AITD when interacting with different loci (64).

It is not known which CTLA-4 variant is the causative variant or by what mechanism it confers susceptibility to autoimmunity. Three main CTLA-4 variants have been studied: an AT-repeat microsatellite at the 3′UTR of the CTLA-4 gene (50,53); an A/G SNP at position 49 in the signal peptide resulting in an alanine/threonine substitution (A/G49) (52,63); and an A/G SNP located downstream and outside of the 3′UTR of the CTLA-4 gene. Further studies are needed to determine which one is causative (65).

3.6. Regulatory T-cell genes

Regulatory T-cells (Tregs) are an important subset of T-cells that regulate T-cell activation (66) and Tregs are known to have an important role in immune
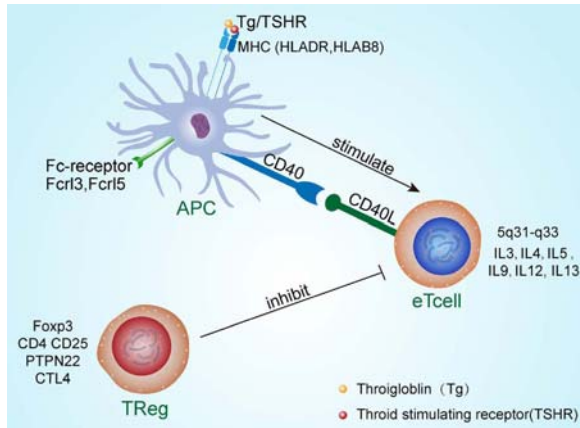
Figure 1. The susceptibility genes involved in Grave's disease: antigen presenting cells (APCs) capture the self-antigen thyroid globin and/or thyroid stimulating receptor and up-regulate CD40, the Fc-receptor and activating T-cells. The activated T-cells express cytokines IL-3, -4, -5, -9, -12 and -13. The Treg cell is a negative regulator of the immune response; it inhibits T-cell activation via up-regulation of Foxp3, CD25, PTPN22 and CTL4.

regulation. In a murine model of GD, Tregs appear to be crucial in the pathogenesis of GD (31). Moreover, an apoptosis-induced decrease in the proportion of intrathyroidal Tregs in patients with AITD is reported (67) .

Tregs have a major role in the peripheral tolerance to self-antigens. Indeed, up-regulation of Tregs suppressed experimental autoimmune thyroiditis in mice (68) and depletion of Tregs in mice increased their susceptibility to experimental GD (69). Several subtypes of Tregs have been identified. One subtype, the natural Tregs, are characterized by constitutive expression of CD25, CTLA-4 and glucocorticoid-induced tumor necrosis factor receptor. In addition, their development is regulated by the FOXP3 gene And, interestingly, both FOXP3 and CD25 have been found to be associated with GD.

The FOXP3 gene, which is the key to the differentiation of T-cells into natural Tregs, has been examined in a U.S. Caucasian and a Japanese cohort of AITD patients The results demonstrated an association of a microsatellite inside the FOXP3 gene with AITD in the Caucasian but not the Japanese patients, demonstrating ethnic differences in disease susceptibility (70). Further analysis found the association of FOXP3 with AITD was mostly in patients with juvenile GD (71).

3.7. The protein tyrosine phosphatase-22 gene

Lymphoid tyrosine phosphatase, encoded by the protein tyrosine phosphatase-22 (PTPN22) gene, is a negative regulator of T-cell activation. The PTPN22 gene is associated with GD and the causative SNP is a tryptophan/arginine variant at position 620. The disease-associated variant is a gain-of-function variant and, therefore, the mechanism by which it predisposes to autoimmunity is not trivial because it would be expected to

suppress T-cell activation. It is possible that the decreased activation of T-cells enables self-reactive T-cells to escape the thymic central tolerance mechanisms, but this possibility awaits experimental confirmation (65).

3.8. Thyroid-specific genes
3.8.1. Thyroglobulin (Tg)

Tg is one of the main targets of the immune response in GD (72) and, therefore, an obvious candidate gene for GD. Indeed, a whole-genome linkage study done by Tome *et al.* identified the Tg gene as an important GD susceptibility gene. These findings were replicated by several other studies of several ethnic groups (73,74). Sequencing the Tg gene identified A734S, V1027M and W1999R as amino acid variants associated with GD. Mechanistically, it is possible that the Tg variants predispose to disease by altering Tg degradation in endosomes, resulting in a pathogenic Tg peptide repertoire.

3.8.2. TSHR

GD is defined by the presence of stimulating TSHR antibodies. Not surprisingly, TSHR was the first gene (after HLA) to be tested for association with GD. Earlier studies that tested three non-synonymous SNPs in the TSHR gene for association with GD, D36H, P52T and D727E gave mixed results (75). However, studies from Japan consistently reported association of TSHR with GD in Japanese (61). Finally, it was found more recently that non-coding intronic SNPs in TSHR are associated with GD (76). The most consistent association in Caucasians is with an intron 1 SNP (77). Mechanistically, intron 1 SNPs in TSHR can alter its splicing; Indeed, several splice variants of TSHR are known. The major splice variant of TSHR identified by us in 1992 is a 1.3 kb variant that includes most of the extracellular domain of TSHR. Other minor splice variants have been reported (78).

4. CONCLUSION

It is widely agreed that complex diseases are not controlled by an individual gene or DNA variation but by a combination of genes or DNA variations; however, knowledge of individual susceptibility genes in GO might lead the way to fully understanding the mechanism underlying GD. As we showed earlier, the self-antigens are captured by the APC cells and this activates the effector T-cells and releases a number of cytokines and chemokines. As a negative regulator, the Treg cell might suppress this response by inhibiting the effector T-cells. There are quite a number of genes known to be involved (Figure 1) but the details of this complex process remain to be elucidated. A better understanding of the susceptible genes and their interactions might provide a novel therapeutic tool in the near future.

5. ACKNOWLEDGMENTS

Shanghai Municipal Science and Technology commission (NO.10DZ1975000 and 11DZ1972704)

6. REFERENCE

1. Bahn, R.S. and A.E. Heufelder, Pathogenesis of Graves' ophthalmopathy. *N Engl J Med* 329(20), 1468-75 (1993)

2. Heufelder, A.E., Pathogenesis of ophthalmopathy in autoimmune thyroid disease. *Rev Endocr Metab Disord* 1(1-2), 87-95 (2000)

3. Smith RS, Smith TJ, Blieden TM, Phipps RP. Fibroblasts as sentinel cells. Synthesis of chemokines and regulation of inflammation. *Am J Pathol* 151(2), 317-22 (1997)

4. Cao HJ, Wang HS, Zhang Y, Lin HY, Phipps RP. Activation of human orbital fibroblasts through CD40 engagement results in a dramatic induction of hyaluronan synthesis and prostaglandin endoperoxide H synthase-2 expression. Insights into potential pathogenic mechanisms of thyroid-associated ophthalmopathy. *J Biol Chem* 273(45), 29615-25 (1998)

5. Smith, T.J., Novel aspects of orbital fibroblast pathology. *J Endocrinol Invest* 27(3), 246-53 (2004)

6. Gerding MN, Terwee CB, Dekker FW, Koornneef L. Quality of life in patients with Graves' ophthalmopathy is markedly decreased: measurement by the medical outcomes study instrument. *Thyroid* 7(6), 885-9 (1997)

7. Burch, H.B. and L. Wartofsky, Graves' ophthalmopathy: current concepts regarding pathogenesis and management. *Endocr Rev* 14(6), 747-93 (1993)

8. Yamada, M., A.W. Li, and J.R. Wall, Thyroid-associated ophthalmopathy: clinical features, pathogenesis, and management. *Crit Rev Clin Lab Sci* 37(6), 523-49 (2000)

9. Bartalena, L., C. Marcocci, and A. Pinchera, Graves' ophthalmopathy: a preventable disease? *Eur J Endocrinol* 146(4), 457-61 (2002)

10. Bednarczuk T, Hiromatsu Y, Fukutani T, Jazdzewski K. Association of cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) gene polymorphism and non-genetic factors with Graves' ophthalmopathy in European and Japanese populations. *Eur J Endocrinol* 148(1), 13-8 (2003)

11. Jacobson, E.M. and Y. Tomer, The CD40, CTLA-4, thyroglobulin, TSH receptor, and PTPN22 gene quintet and its contribution to thyroid autoimmunity: back to the future. *J Autoimmun* 28(2-3), 85-98 (2007)

12. Bednarczuk T, Gopinath B, Ploski R, Wall JR. Susceptibility genes in Graves' ophthalmopathy: searching for a needle in a haystack? *Clin Endocrinol (Oxf)* 67(1), 3-19 (2007)

13. Bartalena, L., A. Pinchera, and C. Marcocci. Management of Graves' ophthalmopathy: reality and perspectives. *Endocr Rev* 21(2) 168-99 (2000)

14. Dustin, M L. T-cell activation through immunological synapses and kinapses. *Immunol Rev* 221, 77-89 (2008)

15. Ahmadi T, Flies A, Efebera Y, Sherr DH. CD40 Ligand-activated, antigen-specific B cells are comparable to mature dendritic cells in presenting protein antigens and major histocompatibility complex class I- and class II-binding peptides. *Immunology* 124(1), 129-40 (2008)

16. Armitage RJ, Macduff BM, Spriggs MK, Fanslow WC. Human B cell proliferation and Ig secretion induced by recombinant CD40 ligand are modulated by soluble cytokines. *J Immunol* 150(9), 3671-80 (1993)

17. Arpin C, Déchanet J, Van Kooten C, Merville P. Generation of memory B cells and plasma cells *in vitro*. *Science* 268(5211), 720-2 (1995)

18. Tomer Y, Barbesino G, Greenberg DA, Concepcion E, Davies TF. A new Graves disease-susceptibility locus maps to chromosome 20q11.2. International Consortium for the Genetics of Autoimmune Thyroid Disease. *Am J Hum Genet* 63(6), 1749-56 (1998)

19. Pearce SH, Vaidya B, Imrie H, Perros P, Kelly WF, Toft AD. Further evidence for a susceptibility locus on chromosome 20q13.11 in families with dominant transmission of Graves disease. *Am J Hum Genet* 65(5), 1462-5 (1999)

20. Tomer, Y., E. Concepcion, and D.A. Greenberg, A C/T single-nucleotide polymorphism in the region of the CD40 gene is associated with Graves' disease. *Thyroid* 12(12), 1129-35 (2002)

21. Kim TY, Park YJ, Hwang JK, Song JY. A C/T polymorphism in the 5'-untranslated region of the CD40 gene is associated with Graves' disease in Koreans. *Thyroid* 13(10), 919-25 (2003)

22. Kurylowicz A, Kula D, Ploski R, Skorka A, Jurecka-Lubieniecka B. Association of CD40 gene polymorphism (C-1T) with susceptibility and phenotype of Graves' disease. *Thyroid* 15(10), 1119-24 (2005)

23. Mukai T, Hiromatsu Y, Fukutani T, Ichimura M. A C/T polymorphism in the 5' untranslated region of the CD40 gene is associated with later onset of Graves' disease in Japanese. *Endocr J* 52(4), 471-7 (2005)

24. Houston FA, Wilson V, Jennings CE, Owen CJ, Donaldson P. Role of the CD40 locus in Graves' disease. *Thyroid* 14(7), 506-9 (2004)

25. Jacobson EM, Huber AK, Akeno N, Sivak M, Li CW. A CD40 Kozak sequence polymorphism and susceptibility to antibody-mediated autoimmune conditions: the role of

CD40 tissue-specific expression. *Genes Immun* 8(3), 205-14 (2007)

26. Smith TJ, Sciaky D, Phipps RP, Jennings TA. CD40 expression in human thyroid tissue: evidence for involvement of multiple cell types in autoimmune and neoplastic diseases. *hyroid*. 9(8), 749-55 (1999)

27. Australia and New Zealand Multiple Sclerosis Genetics Consortium (ANZgene). Genome-wide association study identifies new multiple sclerosis susceptibility loci on chromosomes 12 and 20. *Nat Genet* 41(7), 824-8 (2009)

28. Davis, R.S., Fc receptor-like molecules. *Annu Rev Immunol* 25, 525-60 (2007)

29. Ehrhardt GR, Leu CM, Zhang S, Aksu G, Jackson T. Fc receptor-like proteins (FCRL): immunomodulators of B cell function. *Adv Exp Med Biol* 596, 155-62 (2007)

30. WTCCC and TASC, Association scan of 14,500 nsSNPs in four common diseases identifies variants involved in autoimmunity. *Nature Genetics* 39(11) 1329-1227 (2007)

31. Simmonds MJ, Brand OJ, Barrett JC, Newby PR, Franklyn JA, Gough SC. Association of Fc receptor-like 5 (FCRL5) with Graves' disease is secondary to the effect of FCRL3. *Clin Endocrinol (Oxf)* 73(5), 654-60 (2010)

32. Sakai K, Shirasawa S, Ishikawa N, Ito K, Tamai H. Identification of susceptibility loci for autoimmune thyroid disease to 5q31-q33 and Hashimoto's thyroiditis to 8q23-q24 by multipoint affected sib-pair linkage analysis in Japanese. *Hum Mol Genet* 10(13),1379-86 (2001)

33. Sakai K, Shirasawa S, Ishikawa N, Ito K, Tamai H, Kuma K. Identification of susceptibility loci for autoimmune thyroid disease to 5q31-q33 and Hashimoto's thyroiditis to 8q23-q24 by multipoint affected sib-pair linkage analysis in Japanese. *Hum Mol Genet* 10(13), 1379-86 (2001)

34. Jin Y, Teng W, Ben S, Xiong X, Zhang J, Xu S. Genome-wide scan of Graves' disease: evidence for linkage on chromosome 5q31 in Chinese Han pedigrees. *J Clin Endocrinol Metab* 88(4), 1798-803 (2003)

35. Akamizu T, Hiratani H, Ikegami S, Rich SS, Bowden DW. Association study of autoimmune thyroid disease at 5q23-q33 in Japanese patients. *J Hum Genet* 48(5), 236-42 (2003)

36. Tomer Y, Barbesino G, Greenberg DA, Concepcion E, Davies TF. Mapping the major susceptibility loci for familial Graves' and Hashimoto's diseases: evidence for genetic heterogeneity and gene interactions. *J Clin Endocrinol Metab* 84(12), 4656-64 (1999)

37. Allen EM, Hsueh WC, Sabra MM, Pollin TI, Ladenson PW. A genome-wide scan for autoimmune thyroiditis in the Old Order Amish: replication of genetic linkage on

chromosome 5q11.2-q14.3. *J Clin Endocrinol Metab* 88(3), 1292-6 (2003)

38. Tomer Y, Ban Y, Concepcion E, Barbesino G. Common and unique susceptibility loci in Graves and Hashimoto diseases: results of whole-genome screening in a data set of 102 multiplex families. *Am J Hum Genet* 73(4), 736-47 (2003)

39. Taylor JC, Gough SC, Hunt PJ, Brix TH. A genome-wide screen in 1119 relative pairs with autoimmune thyroid disease. *J Clin Endocrinol Metab* 91(2), 646-53 (2006)

40. Chu X, Dong Y, Shen M, Sun L, Dong C, Wang Y, Wang B. Polymorphisms in the ADRB2 gene and Graves disease: a case-control study and a meta-analysis of available evidence. *BMC Med Genet* 10, 26 (2009)

41. Ikeda Y, Yoshida W, Noguchi T, Asaba K, Nishioka T. Lack of association between IL-12B gene polymorphism and autoimmune thyroid disease in Japanese patients. *Endocr J* 51(6), 609-13 (2004)

42. Sibarani, R.P., Genetics of Graves' disease: the lost concept. *Acta Med Indones* 41(1), 37-40 (2009)

43. Zamani M, Spaepen M, Bex M, Bouillon R, Cassiman JJ. Primary role of the HLA class II DRB1*0301 allele in Graves disease. *Am J Med Genet* 95(5), 432-7 (2000)

44. Menconi F, Monti MC, Greenberg DA, Oashi T, Osman R. Molecular amino acid signatures in the MHC class II peptide-binding pocket predispose to autoimmune thyroiditis in humans and in mice. *Proc Natl Acad Sci U S A* 105(37), 14034-9 (2008)

45. Faas, S. and M. Trucco, The genes influencing the susceptibility to IDDM in humans. *J Endocrinol Invest* 17(7), 477-95 (1994)

46. Simmonds MJ, Howson JM, Heward JM, Cordell HJ. Regression mapping of association between the human leukocyte antigen region and Graves disease. *Am J Hum Genet* 76(1), 157-63 (2005)

47. Jacobson EM, Yang H, Menconi F, Wang R, Osman R. Employing a recombinant HLA-DR3 expression system to dissect major histocompatibility complex II-thyroglobulin peptide dynamism: a genetic, biochemical, and reverse immunological perspective. *J Biol Chem* 284(49), 34231-43 (2009)

48. Todd, J.A., J.I. Bell, and H.O. McDevitt, HLA-DQ beta gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. *Nature* 329(6140), 599-604 (1987)

49. Teft WA, Kirchhof MG, Madrenas J. A molecular perspective of CTLA-4 function. *Annu Rev Immunol* 24, 65-97 (2006)

50. Yanagawa T, Hidaka Y, Guimaraes V, Soliman M, DeGroot LJ. CTLA-4 gene polymorphism associated with

Graves' disease in a Caucasian population. *J Clin Endocrinol Metab* 80(1), 41-5 (1995)

51. Nisticò L, Buzzetti R, Pritchard LE, Van der Auwera B, Giovannini C. The CTLA-4 gene region of chromosome 2q33 is linked to, and associated with, type 1 diabetes. Belgian Diabetes Registry. *Hum Mol Genet* 5(7), 1075-80 (1996)

52. Donner H, Rau H, Walfish PG, Braun J, Siegmund T. CTLA4 alanine-17 confers genetic susceptibility to Graves' disease and to type 1 diabetes mellitus. *Clin Endocrinol Metab* 82(1), 143-6 (1997)

53. Kotsa, K., P.F. Watson, and A.P. Weetman, A CTLA-4 gene polymorphism is associated with both Graves disease and autoimmune hypothyroidism. *Clin Endocrinol (Oxf)* 46(5), 551-4 (1997)

54. Kouki T, Gardine CA, Yanagawa T, Degroot LJ. Relation of three polymorphisms of the CTLA-4 gene in patients with Graves' disease. *J Endocrinol Invest* 25(3), 208-13 (2002)

55. Ban Y, Davies TF, Greenberg DA, Kissin A, Marder B, Murphy B. Analysis of the CTLA-4, CD28, and inducible costimulator (ICOS) genes in autoimmune thyroid disease. *Genes Immun* 4(8), 586-93 (2003)

56. Ueda H, Howson JM, Esposito L, Heward J, Snook H, Chamberlain G. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature* 423(6939), 506-11 (2003)

57. Tomer Y, Greenberg DA, Barbesino G, Concepcion E, Davies TF. CTLA-4 and not CD28 is a susceptibility gene for thyroid autoantibody production. *J Clin Endocrinol Metab* 86(4), 1687-93 (2001)

58. Zaletel K, Krhin B, Gaberscek S, Pirnat E, Hojker S. The influence of the exon 1 polymorphism of the cytotoxic T lymphocyte antigen 4 gene on thyroid antibody production in patients with newly diagnosed Graves' disease. *Thyroid* 12(5), 373-6 (2002)

59. Zaletel K, Krhin B, Gaberscek S, Hojker S. Thyroid autoantibody production is influenced by exon 1 and promoter CTLA-4 polymorphisms in patients with Hashimoto's thyroiditis. *Int J Immunogenet* 33(2), 87-91. (2006)

60. Heward JM, Allahabadia A, Armitage M, Hattersley A, Dodson PM. The development of Graves' disease and the CTLA-4 gene on chromosome 2q33. *J Clin Endocrinol Metab* 84(7), 2398-401 (1999)

61. Akamizu T, Sale MM, Rich SS, Hiratani H, Noh JY, Kanamoto N.Association of autoimmune thyroid disease with microsatellite markers for the thyrotropin receptor gene and CTLA-4 in Japanese patients.*Thyroid* 10(10), 851-8 (2000)

62. Park YJ, Chung HK, Park DJ, Kim WB, Kim SW. Polymorphism in the promoter and exon 1 of the cytotoxic T lymphocyte antigen-4 gene associated with autoimmune thyroid disease in Koreans. *Thyroid* 10(6), 453-9 (2000)

63. Nithiyananthan R, Heward JM, Allahabadia A, Franklyn JA, Gough SC. Polymorphism of the CTLA-4 gene is associated with autoimmune hypothyroidism in the United Kingdom. *Thyroid* 12(1), 3-6 (2002)

64. Vieland VJ, Huang Y, Bartlett C, Davies TF, Tomer Y. A multilocus model of the genetic architecture of autoimmune thyroid disorder, with clinical implications. *Am J Hum Genet* 82(6), 1349-56 (2008)

65. Tomer, Y. Genetic susceptibility to autoimmune thyroid disease: past, present, and future. *Thyroid* 20(7), 715-25 (2010)

66. Paust, S. and H. Cantor, Regulatory T cells and autoimmune disease. *Immunol Rev* 204, 195-207 (2005)

67. Inoue N, Watanabe M, Morita M, Tomizawa R, Akamizu T, Tatsumi K. Association of functional polymorphisms related to the transcriptional level of FOXP3 with prognosis of autoimmune thyroid diseases. *Clin Exp Immunol*162(3), 402-6. (2010)

68. Gangi E, Vasu C, Cheatem D, Prabhakar BS. IL-10-producing CD4+CD25+ regulatory T cells play a critical role in granulocyte-macrophage colony-stimulating factor-induced suppression of experimental autoimmune thyroiditis. *J Immunol* 174(11), 7006-13 (2005)

69. Saitoh, O. and Y. Nagayama, Regulation of Graves' hyperthyroidism with naturally occurring CD4+CD25+ regulatory T cells in a mouse model. *Endocrinology* 147(5), (2006)

70. Ban Y, Tozaki T, Tobe T, Ban Y, Jacobson EM, Concepcion ES. The regulatory T cell gene FOXP3 and genetic susceptibility to thyroid autoimmunity: an association analysis in Caucasian and Japanese cohorts. *Autoimmun* 28(4), 201-7 (2007)

71. Tomer Y, Menconi F, Davies TF, Barbesino G, Rocchi R, Pinchera A. Dissecting genetic heterogeneity in autoimmune thyroid diseases by subset analysis. *J Autoimmun* 29(2-3), 69-77 (2007)

72. Tomer, Y., Anti-thyroglobulin autoantibodies in autoimmune thyroid diseases: cross-reactive or pathogenic? *Clin Immunol Immunopathol* 82(1), 3-11 (1997)

73. Collins JE, Heward JM, Carr-Smith J, Daykin J, Franklyn JA, Gough SC. Association of a rare thyroglobulin gene microsatellite variant with autoimmune thyroid disease. *J Clin Endocrinol Metab* 88(10), 5039-42 (2003)

74. Tomer, Y. and D. Greenberg, The thyroglobulin gene as the first thyroid-specific susceptibility gene for

autoimmune thyroid disease. *Trends Mol Med* 10(7), 306-8 (2004)

75. Cuddihy, R.M., C.M. Dutton, and R.S. Bahn, A polymorphism in the extracellular domain of the thyrotropin receptor is highly associated with autoimmune thyroid disease in females. *Thyroid* 5(2), 89-95 (1995)

76. Ho, S.C., S.S. Goh, and D.H. Khoo, Association of Graves' disease with intragenic polymorphism of the thyrotropin receptor gene in a cohort of Singapore patients of multi-ethnic origins. *Thyroid* 13(6), 523-8 (2003)

77. Dechairo BM, Zabaneh D, Collins J, Brand O, Dawson GJ, Green AP. Association of the TSHR gene with Graves' disease: the first disease specific locus. *Eur J Hum Genet* 13(11), 1223-30 (2005)

78. Kakinuma, A. and Y. Nagayama, Multiple messenger ribonucleic acid transcripts and revised gene organization of the human TSH receptor. *Endocr J* 49(2), 175-80 (2002)

79. Wei B, Peng Q, Zhang Q, Li C. Identification of a combination of SNPs associated with Graves' disease using swarm intelligence. *Sci China Life Sci* 54(2), 139-45 (2011)

Abbreviations: GD: Graves' disease, GO: Graves' ophthalmopathy, HLA: human leukocyte antigen, CTLA-4: cytotoxic T-lymphocyte antigen-4, APCs: antigen-presenting cells, SNP: single nucleotide polymorphism, FCRL: Fc receptor-like, MHC: major histocompatibility complex, HSP70: heat shock protein 70, HT: Hashimoto's thyroiditis, Tregs: regulatory T-cells, PTPN22: protein tyrosine phosphatase-22

Key Words: Graves' disease, Graves' ophthalmopathy, CTLA-4, APCs, HSP70, Review

Send correspondence to: Hong Li, Department of Endocrinology, Long Hua Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai, China, Tel: 021-64385700-3922, Fax: 021-64385700-3922, E-mail: shanhongli2012@yeah.net