Chapter

Graves' Disease: Pathophysiology, Genetics and Management

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Abstract

Graves' Disease is an autoimmune disorder in which hyperthyroidism (over active thyroid) is caused by the autoantibodies against the TSH receptor. It is mainly characterized by the appearance of goiter. The symptoms are wide ranging as thyroid hormone affects many body systems. It is common in women and in people with age below than 40. Graves' Disease is caused by a combination of genetic and environmental factors while genetics being the main cause. Graves' Disease is not a single gene defect but has a complex pattern of inheritance. Today it is clear that genetic predisposition to Graves' Disease is caused by multiple genes. HLA gene is one the most studied gene predisposing to Graves' Disease. Lot of polymorphisms in this gene has been to be associated with the disease. Lymphoid tyrosine phosphatase encoded by the gene PTPN22 has been found to increase the risk of many autoimmune diseases including Graves' Disease. The best documented association of *PTPN22* variants to autoimmune disorders including GD is rs2476601 (C1858T). Other genes associated with the risk of GD are thyrotropin receptor (TSHR), thyroglobulin gene, FCRL3, SCGB3A2, and CTLA4. This chapter will discuss in detail the genetics, pathophysiology, diagnosis and treatment of Graves' hyperthyroidism.

Keywords: Graves' Disease, GD, HLA region, PTPN22, CD40, CTLA4, CD152, TSHR, Tg, FCRL3, SCGB3A2

1. Introduction

Graves' Disease (GD) was named after *Robert J. Graves,* who first recognized this disease in the 19th century as a syndrome with enlarged and overactive thyroid gland (hyperthyroidism due to circulating autoantibodies), an high heart rate, and eye abnormalities (**Figure 1**). On the quality of life, GD has unpropitious effects [1], as a consequence of somatic and psychiatric symptoms, an inability to work and is connected with an increased risk of death [2]. The autoimmune basis of the GD results from complex interactions between different factors which include genetic, endogenous and environmental factors, and this is compulsory for current understanding of this disease [3, 4]. The circulating antibodies (IgG) that binds and activates the G-protein–coupled thyrotropin receptor leads to hyperthyroidism in this disease [5]. In this disease the G-protein–coupled thyrotropin receptor after getting activated stimulates follicular cell growth and excessive development which results in the enlargement of thyroid gland and also increase in thyroid hormone production and the fraction of triiodothyronine (T3) relative to thyroxine (T4) in

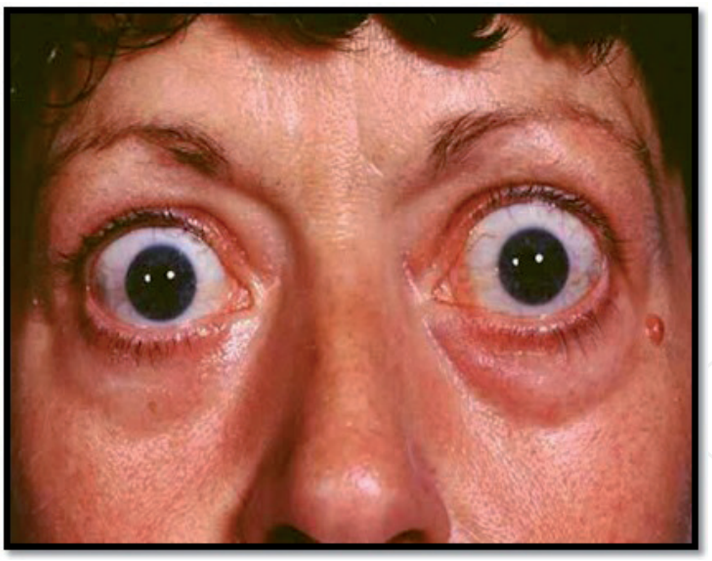

Figure 1.
Graves' Disease.

thyroid secretion [6]. In GD the suppressed serum thyrotropin level and elevated levels of serum T4 and T3 are revealed by the thyroid functioning tests. A term known as *subclinical hyperthyroidism* is referred when serum thyrotropin level is low as compared to normal serum levels of T4 and T3 [7].

2. Epidemiology

GD with an annual incidence of 20 to 50 cases per 100,000 persons is the most common cause of hyperthyroidism [8]. The incidence of GD peaks between 30 to 50 years of age, but people can be affected at any age. The lifetime risk for women is 3% and for men it is 0.5%. The risk of GD is not influenced by Long-term variations in iodine intake, but rapid repletion can transiently increase the incidence. The GD–associated incidence of ophthalmopathy is 16 cases per 100,000 in women and in men it is 3 cases per 100,000 annually. It is more common in whites than in Asians [9]. Older men develop severe ophthalmopathy more likely than younger persons [10]. Subtle abnormalities are revealed in 70% of patients by orbital imaging with GD [11]. In up to 50% of patients in specialized centres, clinically consequential ophthalmopathy is detected with GD, and as a consequence of corneal breakdown or optic neuropathy in 3 to 5% of such patients, sight is threatned [12]. The thyroid levels remain normal or autoimmune hypothyroidism develops either in 10% of the persons with ophthalmopathy [10–12]. In the *Whickham study* a population-based survey in England, the annual incidence of Graves' Disease was approximately 80 per 100,000 women, with most other surveys reporting incidence rates ranging from 15 to 50 per 100,000 persons per year while as the annual incidence in English men was approximately eight to ten fold lower than women (10 per 100,000) in keeping with gender differences seen in other thyroid diseases [13]. The incidence of Graves' hyperthyroidism in area of particularly high iodine intake (Japan), has been reported to be as high as 200 cases per 100,000 general population [14]. Similarly, following the introduction of iodine supplementation, an increases in the apparent incidence of GD have been reported, although in an area of mild to moderate iodine deficiency (Switzerland), a 33% reduction in the incidence of GD was associated with iodine supplementation [15].

3. Pathophysiology of Graves' Disease

In GD, four standard thyroid antigens: thyroglobulin, thyroid peroxidase, sodium-iodide symporter and the thyrotropin receptor are recognized to direct B and T lymphocyte-mediated autoimmunity. However, the primary auto antigen of GD is the thyrotropin receptor itself and is responsible for the manifestation of hyperthyroidism. In this disease, the antibody and cell-mediated thyroid antigen-specific immune responses are properly defined. The development of hyperthyroidism in healthy subjects by transferring thyrotropin receptor antibodies in serum from patients with GD and the passive transfer of thyrotropin receptor antibodies to the foetus in pregnant women are the direct proof of an autoimmune disorder that is mediated by means of autoantibodies. By circulating autoantibodies against the thyrotropin receptor, the thyroid gland is under continuous stimulation, and because of the increased production of thyroid hormones pituitary thyrotropin secretion is suppressed [16]. In the immunoglobulin G1 subclass, the stimulating activity of thyrotropin receptor antibodies is found mostly. The release of thyroid hormone and thyroglobulin that is mediated via 3,'5′-cyclic adenosine monophosphate (cyclic AMP) are caused by these thyroid-stimulating antibodies, and they also stimulate iodine uptake, protein synthesis, and thyroid gland growth. In the etiology of hyperthyroidism in GD the anti-thyroglobulin, anti-sodium-iodide symporter, and anti-thyroid peroxidase antibodies seem to have a very little role. However, against the thyroid, these are markers of autoimmune disease. In persons with autoimmune thyroid disease, intrathyroidal lymphocytic infiltration is the initial histologic abnormality which has a direct correlation with thyroid antibodies' titer [17, 18]. In addition to autoantigens, the cells of thyroid produce specific immune mediators such as cytokines and Fas which are involved in various immune process including complement legislation and T cell adhesion. Those individuals who are suffering from Graves' Disease have lesser percentage of CD4 lymphocytes in thyroid as compared to their peripheral blood. In addition, the CD4 reduction in these patients may also be related to the elevated Fas expression in intrathyroidal CD4 T lymphocytes. *CD40, CTLA-4, thyroglobulin, TSH receptor,* and *PTPN22* are several autoimmune thyroid disease susceptibility genes that have been identified. Either to GD or *Hashimoto thyroiditis*, some of these susceptibility genes are unique, while others confer susceptibility to both conditions. With environmental factors or activities to precipitate the onset of GD the genetic predisposition to thyroid autoimmunity might also interact [17–19]. The RNASET2-FGFR1OP-CCR6 region at 6q27 and an intergenic region at 4p14 are two new susceptibility loci that had been found [20]. Moreover, thyroid-stimulating hormone receptor and major histocompatibility complex class II versions have strong associations with thyroid stimulating hormone receptor autoantibodies (TRAb)-positive GD [21]. Compared with healthy controls, GD patients have higher rate of peripheral blood mononuclear cell conversion into $CD34^+$ fibrocytes. The production of inflammatory cytokines like Inter-leukin 6 (IL-6) and TNF-alpha by these cells after piling up in orbital tissues also contribute to the pathophysiology of thyroid eye disease (opthalmopathy) [22]. In a whole genome association study of more than 1500 individuals suffering from Graves' Disease and equal controls, six susceptible loci which are (*CTLA4; cytotoxic T-lymphocyte-associated protein 4, MHC; major histocompatibility complex, FCRl3; Fc receptor-like protein 3, TSHR; thyroid stimulating hormone receptor, RNASET2-FGFR1OP-CCR6 region at 6q27, and an intergenic region at 4p14*) have been discovered to be associated with GD. **Figure 2** describes the pathophysiology of Graves' Disease [23].

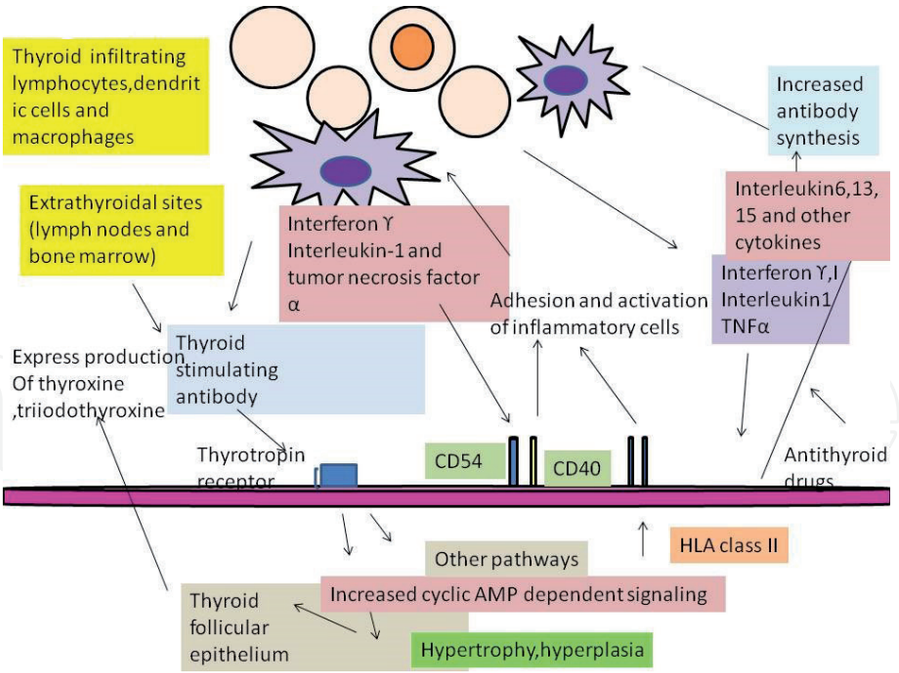

Figure 2.
Pathophysiology of Graves' Disease.

4. Genetics of Graves' Disease

GD is a complex autoimmune disorder which affects the functioning of the thyroid gland, which is the butterfly shaped gland in the lower neck. Specific antibodies targetting the thyrotropin receptor are found in about 95% of patients with GD. GD is thought to result from a combination of environmental and genetic factors most of which are unknown. A number of genes predispose to the GD which include *HLA region*, *protein tyrosine phosphatase-22 (PTPN22)*, *cluster of differentiation 40(CD40)*, *the cytototoxic T lymphocyte- Associated factor4 (CTLA4 or CD152), thyrotropin receptor (TSHR), thyroglobulin (Tg), FCRL3 (FC receptor-like-3), Secretoglobulin 3A2 (SCGB3A2) gene* encoding secretory uteroglobin- related protein 1 (UGRP) and many others. The role of these genes in the pathophysiology of GD is discussed below:

4.1 HLA region

Human leukocyte antigen (HLA) region (6p21) within the human genome codes for 252 expressed loci including numerous key immune response genes is the most gene dense region [24]. This region contains the highest degree of polymorphism within the genome and is divided into different classes which includes the extended class I, classical class I, classical class III, classical class II and extended class II [24] (**Figure 3**). The densest linkage disequilibrium (LD) is also shown by this gene, extending up to 540 kb [25], which compares with distances of between 1 and 173 kb seen in the rest of the genome [26]. When trying to tease out the exact site of etiological variants, the degree of LD within the region is challenging. Most studies highlights the importance on the role of HLA class II encoded HLA-DR and –DQ molecules, which present exogenous antigens for recognition by CD4+ T helper (Th) cells. Including GD, strong associations of *HLA* with almost all autoimmune disorders have been detected. Many studies regarding association of *HLA* alleles with GD have been done. Among different ethnic populations, association of *HLA* alleles with GD varies like *HLA-B*08, DR3* and *DQA1*05:01* are associated with a

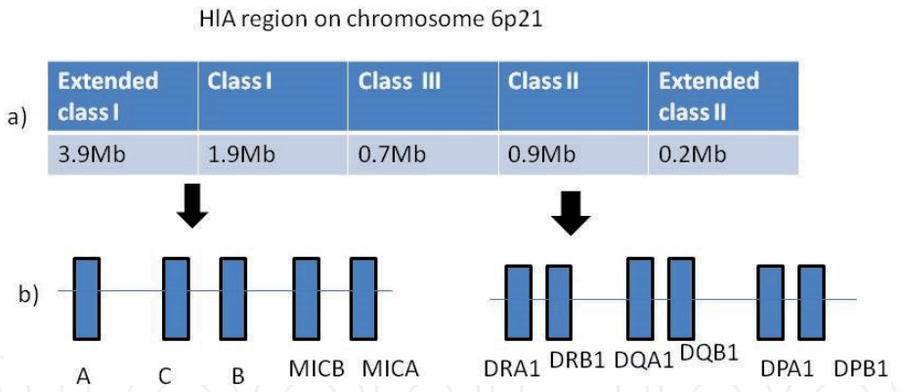

Figure 3.
HLA region on chromosome 6p21. a) nucleotide length of genes of HLA, b) regions of HLA genes.

high risk of GD, and *HLA-DRB1*07:01* is a protective allele against GD in Caucasian populations [27]. *HLA* alleles have been shown to predispose certain groups of people to the disease and vary regionally. British Caucasians showed role of HLA class II alleles DRB1-0304, DQB1-02, and DQA1-0501 [28]. The *HLA* complex shows strong linkage disequilibrium. In Caucasians populations, It is found that there is strong linkage disequilibrium between the genes that codes DR and DQ molecules therefore the existence of a particular DRB1 variant to a larger degree determines DQA1 and DQB1 alleles. *DRB1*03:01-DQA1*05:01-DQB1*02:01 (DR17, DQ2)* and *DRB1*04:01- DQA1*03:01-DQB1*03:02 (DR4, DQ8)* are HLA haplotype combinations in GD sibling pairs. The maximum risk related to this disease is associated with *DR17, DQ2 while as the HLA-DRB1*07 (DR7)* is protective for Graves' Disease. The recessive inheritance of MHC related susceptibility is favored by the distribution of *HLA-B8* genotypes and it is in close accord with Hardy–Weinberg equilibrium proportions. The possibility that an individual will be affected with GD depends on sex, *HLA* genotype, and family history. 14.9% of DR3-positive women with an affected first-degree relative are liable to be affected [29]. *HLA Class I* is also linked with GD, the disease may be mainly associated with alleles of *HLA class I*, in particular *HLA-C*07* whereas *C*03* and *C*16* provides protection [30]. *HLA-DPB1*05:01* was the main gene predisposing to GD. Other alleles included *B*46:01, DRB1* 15:02* and *16:02* whereas *DRB1*12:02* and *DQB1*03:02* provide protection. The association of *DQA1*05:01* with GD was not supported by Linkage Disequilibrium patterns observed in Asians [31]. Peptides derived from TSHR are the cause of association with HLA and development of immune response. Other cause is owing to thymic selection affecting positive and negative selection of T cell clones with regulatory or effector functions. Moreover, impact on NK cell repertoire through interactions with killer immunoglobulin-like receptors (KIR) and/or serving directly as a source of auto antigens after misfolding and presentation by HLA class II molecules [30]. Development of Graves' Disease is related to HLA-DR3. The extracellular domain (ECD) of human TSH receptor (hTSH-R) is a crucial antigen in Graves' Disease. hTSH-R peptide 37 (amino acids 78-94) is an important immunogenic peptide [32]. HLA is the cause of many diseases, the disease occurred at an earliest age in HLA-DR3 positive patients and important link between exophthalmos and either exophthalmos and/or soft tissue modifications were found with DR3. HLA-DR3 positive patients were found to be more resilient to radioiodine therapy than patients negative for these antigens [33]. It has been hypothesized that arginine at position 74 of the HLA- DRB1 chain has role in GD pathogenesis. But the most common residues at position 74 of *DRB1*15:01* and *DRB1*16:02* reported in our association study are both alanine and it is considered to be neutral for GD risk [34]. On the other hand, HLA region is linked to GD susceptibility in both

Caucasian and Chinese Han populations [35]. The associated alleles vary from those in Causacians. *HLA-DPB1*05:01* is the major gene of GD in our population, *B*46:01, DQB1*03:02, DRB1*15:01* and *DRB1*16:02* were closely linked with GD [31]. As per the other meta-analysis study, the *HLA-B*46* allele is a risk factor for GD in Asian populations. The distribution of *HLA-B*46* and *HLA-B*08* vary between European and Asian populations. The allelic frequency of *HLA-B*08* is around 12%, while the allelic frequency is 0.3 to 0.5% in most Asian populations. By contrast, the allelic frequency of *HLA-B*46* is 3.9 to 8.6% in Asian populations and almost zero in Europe populations [35]. **Figure 4** depicts the classical HLA Class I and II pathways.

4.2 Protein tyrosine phosphatase-22 (PTPN22)

Protein tyrosine phosphatase, non-receptor type 22 (lymphoid) is also known as PTPN22. This in humans is encoded by the *PTPN22 gene* [36]. This gene has various variants. The mutations of this gene are associated with increase or decrease in the risk of autoimmune diseases. In many autoimmune diseases, this gene has been found strongly associated after HLA [37]. *rs2476601 (C1858T) is* the best known association of *PTPN22* variants to autoimmune condition including GD. This SNP (R620W) located in the P1 proline - rich motif of *PTPN22* binds with strong affinity to the SH3 domain of tyrosine kinase, Csk. This mutation disrupts the interaction between *PTPN22* and *Csk* [38] and also increases phosphatase activity which inturn suppresses the TCR signaling more efficiently than the wild type [39]. A role of *PTPN22* in T-cell regulation has been found by the results of knocking out the murine homolog of *PTPN22,* which lowered thresholds for T-cell-receptor signaling and inhibited production of IL-2 in these animals [40]. This *PTPN22 620 W* substitution, a gain of function mutation resulting in the reduction of phosphorylation of key signaling molecules and associated downregulation of TCR signaling which inturn leads to the inhibition of expansion of T cells, weakening of the positive selection in the thymus, and decreasing the antibodies' titer by reducing the activity of helper T lymphocytes [41]. Association between the GD and *PTPN22 620 W* polymorphism has been demonstrated in several studies among Caucasians

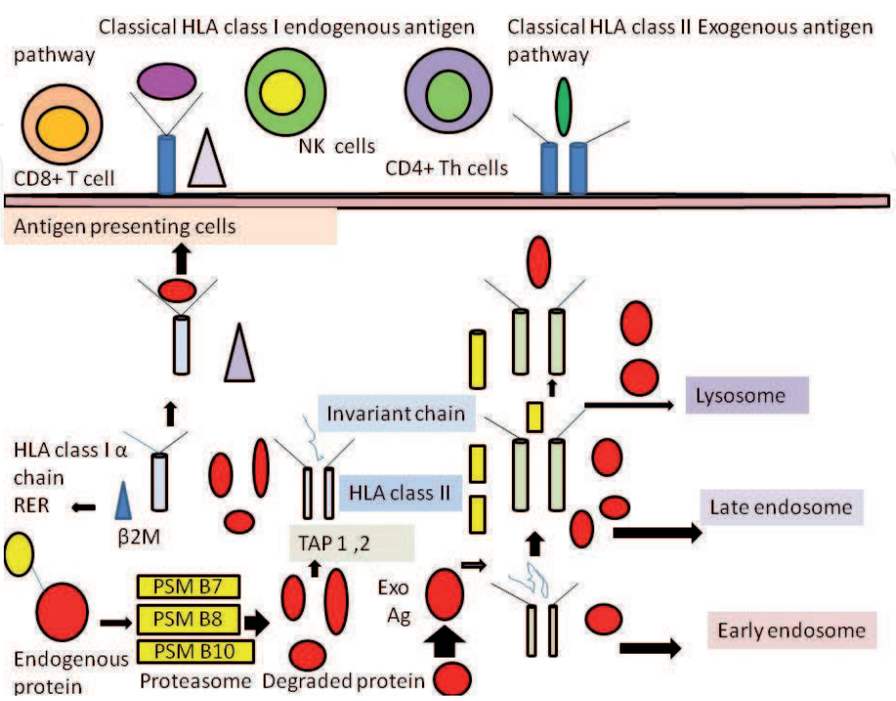

Figure 4.
Classical HLA class I and II pathways.

with odds ratio as OR 1.5-1.9 [42], which makes *PTPN22 620 W* polymorph one of the strongest known genetic factors influencing to autoimmune diseases. In polish population a gene dose-dependent effect of *PTPN22* 'T' allele on the age of onset of GD has been found [42] but that was not replicated in a cohort study done in UK [43]. The other available results have shown that the *PTPN22* locus contains other functionally important variants, particularly those conferring protection.

4.3 Cluster of differentiation 40 (CD40)

The Cluster of Differentiation (CD) are cell surface proteins with each of them assigned a specific number thereby allowing cell phenotypes to be recognized. Surface expression of a particular CD molecule is functional for the characterization of cell phenotypes. These molecules can act either as receptors or ligands. Some CD proteins though do not play role in cell signaling, but do have other functions, such as cell adhesion. CD for humans is numbered upto 371 with their specific functions. CD40 is a costimulatory protein found on the antigen- presenting cells and results in their activation. The binding of CD154 (CD40L) on helper T cells to CD40 activates antigen presenting cells and induces a variety of downstream effects. Deficiency can lead to Hyper-IgM syndrome type3. It is located on chromosome 20 in humans and chromosome 2 in mouse. Disruption of the CD40- CD40L co-stimulatory pathway has been found in many autoimmune diseases, including GD. on the basis of a genome-wide linkage study in GD, *CD40* has been associated with GD as a positional candidate which implicated 20q11 chromosomal region, designated GD-2, as harboring a susceptibility locus [44]. C/T polymorphism (rs1883832) located at position −1 relative to translation start site affects the initiation step of translation as it has a direct effect on kozak sequence. The C allele of *rs1883832* has been found to confer risk of GD among Caucasians whereas the results from in vitro transcription/translation system suggested that this allele predisposes to GD by increasing the efficiency of translation of CD40 mRNA [44]. There is a close association between GD and C variant of *rs1883832* as supported by studies in the Japanese population although in this population the effect may be constrained to patients with the late onset of disease [45] and/or to the CC and CT genotypes, signifying a dominant rather than a recessive model of inheritance [45]. Recently, siRNA mediated inhibition of CD40 expression was evaluated for potential to prevent development of GD in mice immunized with adenovirus expressing human TSHR A subunit. In spite of successful lowering of CD40 expression, no effect on the rate of disease induction was observed [46].

4.4 The cytotoxic T lymphocyte-associated factor 4 (CTLA4 Or CD152)

It is a protein receptor that functions as an immune checkpoint and down-regulates immune responses. It is constitutively expressed in regulatory T cells but only upregulated in conventional T cells after activation; a phenomenon which is particularly notable in cancers [47]. It is homologous to the T-cell co-stimulatory protein, CD28, and both molecules bind to CD80 and CD86, also called B7-1 and B7-2 respectively, on antigen-presenting cells. CTL4-4 binds CD80 and CD86 with greater affinity and avidity than CD28 thus enabling it to outcompete CD28 for its ligands. CTLA4 transfers an inhibitory signal to T cells, [48] whereas CD28 transmits a stimulatory signal [48]. CTLA4 is also found in regulatory T cells and thereby contributing to their inhibitory function. CTLA4 consists of four exons encoding different functional domains such as a leader sequence and extracellular, transmembrane as well as cytoplasmic domains. The most reliable associations with GD within *CTLA4* locus were found with three polymorphisms: the

AT-microsatellite polymorphism (ATn) at the 3'untranslated region (3'UTR) of the gene [49]. It has been proposed that this AT-repeat allele decreased the` stability of *CTLA4* mRNA thus dampening the inhibitory function of the protein and thus diminishing the control of T-cell proliferation [50]. The *second* polymorphism implicated was *rs231775 (A49G)* in the signal peptide causing a substitution of Thr to Ala [51]. This amino acid change could influence post-translational processing leading to inefficient glycosylation of the autoimmunity predisposing variant [52]. Another widely studied genetic polymorphism in *CTLA4 gene* is *rs3087243* (*CT60)* located downstream from the 3'UTR of the *CTLA4* [53]. After taking into account the CT60 genotype, Examination of full-length (flCTLA-4) and sCTLA-4 expression revealed a lower expression of sCTLA-4 in persons homozygous for the G allele [54]. But, in a larger Swedish study this result was not replicated which also did not find any association between concentration of serum sCTLA4 and disease status or CT60 genotype [54]. Lately, in Japanese patients a noteworthy association with CTLA4 CT60 was found for GD with OR=2.97. The present state of knowledge does not specify evidently the mechanism behind the association of *CTLA4* with GD. However, *CTLA4* polymorphism is consistently associated with thyroid autoimmune diseases in the majority of populations.

4.5 The thyrotropin receptor (TSHR)

The thyrotropin receptor (TSHR) responding to thyrotropin (thyroid-stimulating hormone, TSH) is a Gs-protein coupled receptor and stimulates the production of thyroxin (T4) and triiodothyronine (T3). It is primarily found on the surface of the thyroid epithelial cells [55], but also found on adipose tissue and fibroblasts. A G protein signal cascade is activated upon the binding of circulating ligand TSH which activates adenylyl cyclase that synthesizes cAMP from ATP and subsequently resulting in increased intracellular levels of cAMP. cAMP functions as a secondary messenger and activates all functional aspects of the thyroid cells which include thyroglobulin synthesis, iodine pumping, endocytosis, iodination, proteolysis, thyroid peroxidase activity and hormone release. *TSHR gene* is located on 14q31 [56] and consists of 13 exons [57]. The TSHR is among the susceptibility genes of GD because it encodes for a protein that is both responsible for the clinical manifestations of the disease and is the direct target of the autoimmune response in GD. The anti-TSHR antibodies in serum are the main serological manifestations of GD. Indeed, TSHR- stimulating antibodies (TSAbs) are present in nearly all cases of GD and severity of the disease correlates with TSAbs levels. One of the first non-MHC genes to be tested for association with the disease was TSHR. Three germline missence mutations (a substitution of aspartic acid (D) for histidine (H) in position 36 *(D36H)*; a substitution of a proline (P) for threonine (T) in position *52(P52T)*, and a substitution of aspartic acid (D) for glutamic acid (E) in position *727 (D727E)* were primarily described in individuals suffering from GD and proposed to be associated with the disease [3]. Of these three, *D36H* and *P52T* are the two mutations which are located in the putative ligand binding region of the extracellular domain of the TSHR, while the third one, *D727E* lies within the intracellular domain of the receptor.

4.6 FCRL3 (FC receptor-like-3)

Fc receptor-like protein 3 is a protein that in humans is encoded by *FCRL3 gene* [58]. It is located on 1q23.1. This gene located on q arm of chromosome 1st is one of the several Fc receptors like glycoproteins which encode a member of the IR superfamily. The encoded protein plays a role in regulation of the immune system

and in its cytoplasmic domain it contains immunoreceptor-tyrosine activation motifs and immunoreceptor-tyrosine inhibitory motifs. Rheumatoid arthritis, autoimmune thyroid disease, and systemic lupus erythematosus have been associated in the mutation of this gene [58]. FCRL3 is a novel immunoregulatory gene believed to perform similar functions as FC gamma receptors due to high structural homology between them. FCRL3 gene polymorphism is related to the susceptibility to GD with regional and ethnic variability [59]. A SNP at the position *-169A/G (rs7528684)* in promoter of *FCRL3* gene has been found to be associated with GD. Correlation of this SNP to serum levels of FT3, FT4, TSH and TRAb, gender, age, TpoAb, TgAb, severity of goiter and presence or absence of exophthalmos in GD have been widely investigated [59]. There is an association of anther SNP *rs3761959* (tagging rs7528684) with GD. Overall the available data suggest that genetic polymorphism(s) modifying susceptibility for GD do exist in the *FCRL3* region but the primarily associated variant(s) remain(s) to be found [60].

4.7 Secretoglobin 3A2 (SCGB3A2) gene

This gene is present on chromosome 5 in humans and chromosome 18 in mouse. It is a homodimeric protein thought to play a role in the modulation of inflammation and tumorigenesis. SCGB3A2 is a member of secretoglobin superfamily, a family of small, secreted proteins found in animals exclusively of mammalian lineage. *SCGB3A2* mRNA is predominantly expressed in the lung with low levels of expression in the thyroid. Variants in the promoter of the *SCGB3A2* gene encoding secretory uteroglobin-related protein 1 (UGRP1) have been found associated with GD in an extensive study of a total of ~2500 patients and controls from the Chinese population who aimed to explain signals in the chromosomal region 5q12-q33 obtained in previous studies using linkage analysis [61–63]. Song *et al.* reported the strongest association of GD with *rs1368408 (112G/A, OR=1.28, P=1.43x10-6)* and *SNP75 (−623~−622 AG/−T, OR=1.32, P=7.62x10-5)* [60, 61]. Association between GD and the A allele of *rs1368408* (OR=1.18, P=0.007) was independently confirmed in a similarly sized UK cohort [64]. Recently, further evidence for association between rs1368408 and GD was provided by a study in a Russian cohort (~1500 cases and controls, OR=1.33, P=2.910-5) [60]. It should be noted that a relatively early study in a Chinese population did not observe the effect of *rs1368408,* although this might have been caused by low power due to limited numbers of subjects (~200 cases and controls) [62]. Till present it is still not clear how variants in *SCGB3A2* predispose to GD.

There are other genes which have strong association with GD such as cytokine genes: *IL3, IL4, IL5, IL9, IL13* and the *ADRB2* gene encoding beta-2-Adrenergic receptor [65–67] were all associated with GD.

5. Diagnosis of Graves' Disease

The diagnosis to confirm the cause of Graves' hyperthyroidism is based on the clinical and biochemical manifestations of hyperthyroidism and on the clinical and laboratory features. For the presence of hyperthyroidism, measurement of serum thyrotropin is a useful screening test, because the secretion of thyrotropin is reduced by very small increase in thyroid secretion, so by the measurement of serum free thyroxine the diagnosis of hyperthyroidism must be confirmed [68]. Patients may have only increased secretion of triiodothyronine in the earliest stage of Graves' hyperthyroidism; therefore, patients with normal serum free thyroxine concentrations and low serum thyrotropin concentrations, serum free

triiodothyronine should be measured. Because of the use of certain drugs and increase in thyroid hormone–binding proteins, measurements of serum total thyroxine and triiodothyronine are less reliable as it can cause high values [69]. In patients with hyperthyroidism and a diffuse goiter, the signs of ophthalmopathy or dermopathy are sufficient to confirm the diagnosis of GD. In patients with GD, other autoimmune disorders occur more frequently (Type 1 diabetes mellitus, Addison's disease, Vitiligo, Pernicious anemia Alopecia areata, Myasthenia gravis, Celiac disease) and their presence therefore supports this diagnosis. Occasionally, in patients with pre-existing nodular goiter, GD occurs which causes confusion. The presence of a high serum concentration of thyroid peroxidase antibody which is present in about 75 percent of patients with Graves' hyperthyroidism, or a thyroid radionuclide scan demonstrating a diffuse goiter provides evidence of GD, when the diagnosis is unclear clinically. Occasionally, to distinguish between Graves' hyperthyroidism and thyrotoxicosis caused by painless, destructive (autoimmune) thyroiditis, thyroid radionuclide studies may be indicated especially in women post-partum. Patients may have a small diffuse goiter with painless thyroiditis, like those with GD. However, it is very unlikely that thyrotoxicosis due to painless thyroiditis will last longer than two months [70].

5.1 Measurement of thyrotropin-receptor antibodies in serum

It is largely a matter of individual preference, whether serum thyrotropin-receptor antibodies should be measured in the differential diagnosis of GD, some argue that a test for the antibodies should be done routinely, and others that a diagnosis of GD can nearly always be inferred correctly on the basis of the clinical findings. The immunoglobulin-mediated inhibition of the binding of radiolabeled thyrotropin to thyrotropin receptors is most widely used assay for thyrotropin-receptor antibodies and is positive approximately in 80% of individuals suffering for Graves' hyperthyroidism [2]. Up to 99% sensitivity is shown by newer assays. Even though a positive result might specify the occurrence of either thyroid- stimulating antibodies or thyrotropin-receptor– blocking antibodies, it is rational to conclude that a positive test in the individual suffering from hyperthyroidism is owing to thyrotropin-receptor–stimulating antibodies. With time as the mechanism of the interactions of antibodies with the thyrotropin receptor improves, it would be possible to develop simple, precise immunoassays for thyroid-stimulating antibodies for routine use. The antibodies identified by bioassays that measure the synthesis of cAMP in retort to the stimulation of thyrotropin receptors are only thyroid-stimulating antibodies — for instance, in cells transfected with thyrotropin receptor— but such assays are relatively expensive and not widely available [71].

5.2 Computed tomography (CT) or magnetic resonance imaging (MRI)

CT and/or MRI of the orbits is indicated if there is any uncertainty about the cause of ophthalmopathy, particularly in a patient with unilateral exophthalmos, to rule out a retrobulbar tumor or arteriovenous malformation. Approaches used in assessing the activity of ophthalmopathy are very helpful in determining which individuals will be benefitted from immunosuppressive treatment. Measurement of the relaxation time for extraocular muscles on T2-weighted MRI, Clinical activity scores (CAS), and orbital scanning with indium In 111 pentetreotide [54] have all been suggested for this purpose but have not been fully assessed. These above tests are not needed for the majority of individuals, who have only mild or moderate Graves' ophthalmopathy [72].

6. Therapy for Graves' Disease

According to age, severity of hyperthyroidism, goiter size, presence and degree of ophthalmopathy, as well as patient's personal preference, GD treatment should be tailored in each individual patient. Medical treatment with anti-thyroid medicine (thionamides) is usually suggested in all patients to revive euthyroidism at first. Once euthyroidism is achieved, the long strategy comprises many choices, including relatively long-term (usually twelve to twenty four months) course of anti-thyroid drugs, radioactive iodine or surgery. Beta-blockers, if not inadvisable are time and again used prior to restoration of euthyroidism to reduce the symptoms of thyrotoxicosis. The thyroid hormone formation is blocked by specific thionamides or antithyroid drugs (propylthiouracil and methimazole). These drugs prevent the thyroid hormone production by inhibiting iodine organification and coupling of iodotyrosines. Treatment of this disease with these drugs is usually well tolerated. Some of the side effects like skin rash and, hardly Granulopenia, Hepatits and Arteritis may occur. Methimazole is currently most well liked over propylthiouracil, owing to the proof of a lower prevalence of severe side-effects, particularly hepatitis [73], with the exception of the first trimester of pregnancy, when propylthiouracil is preferred due to the increased rate of congenitalmal formations, especially aplasia cutis, which has been reported with the utilization of methimazole [74]. Due to the high probability of recurrence of hyperthyroidism after the withdrawal of therapy, this method is not suggested to individuals having large goiters. Contrary, in individuals with thyroid eye disease (ophthalmopathy), we tend to like a surgical removal of all or part of the thyroid gland (thyroidectomy), radioiodine ablation, or both owing to the pathogenetic role of cross-reactive antigens between the thyroid and orbital tissues [75]. In some centers, the methimazole is used at higher doses than desirable amounts needed to correct hyperthyroidism in coalition with thyroid hormone for block and replacement treatment. This tactic relies on plausible immunosuppressive methimazole action, which still has not been incontestable in studies related to humans [76]. Radioiodine is ideally administered after the accomplishment of euthyroidism with the help of anti-thyroid drugs. Hypothyroidism is induced to attain a stable remission of GD, this is the goal of the treatment. To calculate the appropriate radioactive iodine dose to be given, 24 hour radioactive iodine uptake is usually performed before treatment and used together with gland volume. However, a fixed radioiodine dose may also be given without interfering with the outcome [77]. Radioiodine treatment, when using appropriate dose, within 1 to 6 months in the majority of patients (about 80%), it leads to hypothyroidism. Those Patients having large goiter the radioiodine therapy should not be used as it has low success rate unless repeated treatments are planned. It has been seen that radioiodine has acute side effects which are mild, well tolerated and generally self-limiting. Radioactive therapy for the treatment of hyperthyroidism sometimes causes a transitory pain and swelling of the neck and subsequently requires a treatment with oral glucocorticoids. During this process, for a short period of time the symptoms of thyrotoxicosis may exacerbate due to the release of preformed thyroid hormones. After radioiodine treatment, a transient worsening or more rarely, the fresh appearance of thyroid eye disease may occur, but it can be easily prevented by the administration of oral prednisone after radioiodine for 8–12 weeks. After radioiodine therapy in adults with Graves, there is no evidence of an increased risk of thyroid cancer and other solid tumors as well as of leukemia [78]. No major studies are available in children unfortunately. So, radioiodine treatment is not recommended before the age of 18–20 years. With the exception of a transitory decrease in testosterone levels in men, no effects on the reproductive system in male and female have been described [79]. A patient

with a large goiter has been indicated by Thyroidectomy. The surgical procedures most commonly recommended in patients with GD are near total thyroidectomy (NT) or total thyroidectomy (TT), consisting in the removal of most or all visible thyroid tissue, respectively. Both procedures result in hypothyroidism. Recurrence of hyperthyroidism is extremely rare. The rate of post-operative complications (e.g. surgical hypoparathyroidism, laryngeal nerve paralysis) is not increased compared with that observed using other less aggressive surgical procedures [80]. Briefly, the treatment of choice depends on the seriousness and activity of GD. In individuals with moderately severe thyroid eye disease, the usage of intravenous glucocorticoids is the first-line treatment and if this intravenous glucocorticoids treatment fails, orbital decompression is performed [81]. And this rehabilitative surgery (orbital decompression, muscle or eyelid surgery) must be considered when the eye disease is inactive. No major treatments are required for the majority of patients having a mild ophthalmopathy, and patients are given local measurement (e.g. eye lubricants, sun glasses) or, based on a recent study, selenium [82].

7. Conclusion

Since the incidence of Graves' Disease is increasing at pace and already told in the above chapter that it has an unpropitious effects on the quality of life as it causes weight loss, fatigue irritability, goiter (swelling in the thyroid gland) and much more. It also affects skin and eyes the conditions called Graves' dermopathy and Graves' ophthalmopathy respectively. As explained earlier this disease is thought to result from a combination of environmental and genetic factors most of which are unknown. So it is necessary to understand those factors, which will help us in the better management of this disease in future.

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References

[1] Kahaly GJ, Petrak F, Hardt, et al. Psychosocial morbidity of Graves' orbitopathy. Clinical Endocrinology. 2005; 63(4): 395-402.

[2] Terry JS and Laszlo H. Graves' Disease. N Engl J Med. 2016; 375 (16):1552-1565.

[3] Tomer Y. Mechanisms of autoimmune thyroid diseases: From genetics to epigenetics. Annual Review of Pathology: Mechanisms of Diseas.2014; 9: 147-156.

[4] Brix TH, Kyvik KO, Christensen, et al. Evidence for a major role of heredity in GD. A population-Based Study of Two Danish Twin Cohorts. J Clin Endocrinol Metab. 2001; 86(2): 930-934.

[5] Anthony PW. Graves' Disease. The New England Journal of Medicine. 2000; 343(17): 1236-1248.

[6] Girgis CM, Champion BL, Wall JR. Current concepts in GD.Therapeutic Advances in Endocrinology and Metabolism. 2011; 2(3): 135-144.

[7] Anthony. Subclinical hyperthyroidism. N Engl J Med. 2001; 345: 512-516.

[8] Zimmermann MB and Boelaert K. Iodine deficiency and thyroid disorders. The Lancet Diabetes and Endocrinology. 2015; 3(4): 286-295.

[9] Sagili VBR, Anu Jain, SB Yadav, et al. Prevalence of Graves' ophthalmopathy in patients with GD presenting to a referral centre in north India. Indian J Med Res. 2014; 139(1):99-104.

[10] Rebecca SB. Graves' Ophthalmopathy. N Engl J Med. 2010; 362(8):726-38.

[11] Villadolid MC, Yokoyama N, Izumi M. Untreated GD patients without clinical ophthalmopathy demonstrate a high frequency of extraocular muscle (EOM) enlargement by magnetic resonance. Journal of Clinical Endocrinology and Metabolism. 1995; 80(9): 2830-2833.

[12] Luigi B and Maria LT. Graves' Ophthalmopathy. N Engl J Med. 2009; 360 (10):994-1001.

[13] McIver B and Morris JC. The pathogenesis of GD. Endocrinology and Metabolism Clinics of North America. 1998; 27(1): 73-89.

[14] Sarıtas A, Sarıtas PU, Kurnaz MM et al. Spectrum and Prevalence of Thyroid Disorders in Patients Admitted to the Anaesthesiology Outpatient Clinic for Surgery. 2015;43(4): 240-245.

[15] Bg B and Lichiardopol C. Iodine Deficiency, Still a Global Problem. Curr Health Sci J. 2017; 43(2): 103-111.

[16] Morshed SA, Latif R, Davies TF. Characterization of thyrotropin receptor antibody induced signaling cascades. Endocrinology. 2009; 150(1):519-529.

[17] Jacobson EM and Tomer Y. The CD40, CTLA-4, thyroglobulin, TSH receptor, and PTPN22 gene quintet and its contribution to thyroid autoimmunity: Back to the future. Journal of Autoimmunity. 2007; 28 (2-3): 85-98.

[18] Iwama S, Ikezaki A, Kikuoka N, et al. Association of HLA-DR, –DQ genotype and CTLA-4 gene polymorphism with GD in Japanese children. Hormone Res. 2005; 63:55-60.

[19] Nagata K, Nakayama Y, Higaki K, et al. Reactivation of persistent Epstein-Barr virus (EBV) causes secretion of thyrotropin receptor antibodies (TRAbs) in EBV-infected B

lymphocytes with TRAbs on their surface.Autoimmunity. 2015; 48(5):328-335.

[20] Chu X, Pan CM, Zhao SX, et al. A genome-wide association study identifies two new risk loci for GD. Nature Genetics. 2011; 43(9): 897-901.

[21] Bell L, Hunter AL, Kyriacou A, et al. Clinical diagnosis of Graves' or non-Graves' hyperthyroidism compared to TSH receptor antibody test. Endocrine Connections. 2018; 7(4):504-510.

[22] Douglas RS, Afifiyan NF, Hwang CJ, et al. Increased generation of fibrocytes in thyroid-associated ophthalmopathy. Journal of Clinical Endocrinology and Metabolism. 2010; 95 (1):430-438.

[23] Rafał P, Konrad S, Tomasz B. The Genetic Basis of Graves' Disease. Curr Genomics. 2011; 12(8): 542-563.

[24] Horton R, Wilming L, Rand V, et al. Gene map of the extended human MHC. Nat Rev Genet. 2004; 5: 889-899.

[25] Shiina T, Inoko H, Kulski JK, et al. An update of the HLA genomic region, locus information and disease association. Tissue Antigens 2004; 64: 631-649.

[26] Simmonds MJ, Gough SC. Genetic insights into disease mechanisms of autoimmunity. Br Med Bull. 2004; 71: 93-113.

[27] Yanagawa T, Mangklabruks A, Chang YB. Human histocompatibility leukocyte antigen-DQA1*0501 allele associated with genetic susceptibility to Graves' disease in a Caucasian population. J Clin Endocrinol Metab. 1993; 76: 1569-1574.

[28] Joanne Heward, Amit Allahabadia, Jacquie Daykin, et al. Linkage Disequilibrium between the Human Leukocyte Antigen Class II Region of the Major Histocompatibility Complex and Graves' Disease: Replication Using a Population Case Control and Family-Based Study. *The Journal of Clinical Endocrinology & Metabolism. 1998; 83:3394-3397.*

[29] Stenszky V, Kozma L, Balazs C, et al. The genetics of Graves' disease. HLA and disease susceptibility. J Clin Endocrinol Metab. 1985; 61: 735-740.

[30] Gough SCL and Simmonds MJ. The HLA Region and Autoimmune Disease: Associations and Mechanisms of Action. Curr Genomics. 2007; 8(7):453-465.

[31] Chen PL, Fann CS, Chu CC, et al. Comprehensive genotyping in two homogeneous Graves' Disease samples reveals major and novel HLA association alleles. PLoS ONE. 2011; 6: e16635.

[32] Pearson TA and Manolio TA. How to interpret a genome-wide association study. JAMA. 2008; 299: 1335-1344.

[33] Ban Y , Davies TF , Greenberg DA , et al. Arginine at position 74 of the HLA-DR β1 chain is associated with Graves' disease. Genes & immunity. 2004; 5: 203-208.

[34] Brix TH, Kyvik KO, Christensen K, et al. Evidence for a major role of heredity in Graves' disease: a population-based study of two Danish twin cohorts. J. Clin. Endocrinol. Metab. 2001; 86: 930-934.

[35] Cohen S, Dadi H, Shaoul. Cloning and characterization of a lymphoid-specific, inducible human protein tyrosine phosphatase. Blood. 1999; 93: 2013-24.

[36] Lee YH, Rho YH, Choi SJ, Ji, et al. The PTPN22 C1858T functional polymorphism and autoimmune diseases--a meta-analysis. Rheumatology. 2007; 46: 49-56.

[37] Begovich, AB, Carlton VEH, Honigberg LA, et al. A missense

single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. Am J Hum Genet. 2004; 75: 330-337.

[38] Rieck M, Arechiga A, Onengut Gumuscu S, et al. Genetic variation in PTPN22 corresponds to altered function of T and B lymphocytes. J Immunol. 2007; 179: 4704-4710.

[39] Stanford SM, Mustelin TM, Bottini N. Lymphoid tyrosine phosphatase and autoimmunity: human genetics rediscovers tyrosine phosphatases. Semin Immunopathol. 2010; 32: 127-136.

[40] Hasegawa K, Martin F, Huang G, et al. Pest domain-enriched tyrosine phosphatase (PEP) regulation of effector/memory t cells. Science. 2004; 303: 685-689.

[41] Zhebrun D, Kudryashova Y, Babenko A, et al. Association of PTPN22 1858T/T genotype with type 1 diabetes, Graves' disease but not with rheumatoid arthritis in Russian population. Aging (Albany, NY). 2011 ; 3(4): 368-373.

[42] Zeitlin AA, Heward JM, Brand OJ, et al. Use of Tag single nucleotide polymorphisms (SNPs) to screen PTPN21: no association with GravesGÇÖ disease. Clin Endocrinol. 2006; 65: 380-384.

[43] Ban Y, Tozaki T, Taniyama M, et al. Association of the protein tyrosine phosphatase nonreceptor 22 haplotypes with autoimmune thyroid disease in the Japanese population. Thyroid. 2010; 20(8): 893-9.

[44] Ye F, Shi B, Wu, et al. Experience with lentivirus-mediated CD40 gene silencing in a mouse model of Graves' disease. J. Endocrinol. 2011; 208: 285- 291.

[45] Qureshi OS, Zheng Y, Nakamura K, et al. Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4. Science. 2011; 332: 600-603.

[46] Syn NL, Teng MW, Mok TS et al. De-novo and acquired resistance to immune checkpoint targeting. The lancet.Oncology. 2017; 18:731-741.

[47] Waterhouse P, Penninger JM, Timms E.et al. Lymphoproliferative disorders with early lethality in mice deficient in CTLA4. Science. 1995; 270: 985-8.

[48] Jermendy A, Szatmári, Laine AP, et al. The interferon-induced helicase IFIH1 Ala946Thr polymorphism is associated with type 1 diabetes in both the high-incidence Finnish and the medium-incidence Hungarian populations. Diabetologia. 2010; 53: 98-102.

[49] Enevold C, Oturai AB, Sorensen PS, et al. Multiple sclerosis and polymorphisms of innate pattern recognition receptors TLR1-10, NOD1-2, DDX58, and IFIH1. J. Neuroimmunol. 2009; 212: 125-131.

[50] Daroszewski J, Pawlak E, Karabon L, et al. Soluble CTLA-4 receptor an immunological marker of Graves' disease and severity of ophthalmopathy is associated with CTLA-4 Jo31 and CT60 gene polymorphisms. Eur. J. Endocrinol. 2009; 161: 787-793.

[51] Anjos S, Nguyen A, Ounissi-Benkalha H, et al. A common autoimmunity predisposing signal peptide variant of the cytotoxic T-lymphocyte antigen 4 results in inefficient glycosylation of the susceptibility allele. J. Biol. Chem. 2002; 277: 46478-46486.

[52] Petrone A, Giorgi G, Galgani A, et al. CT60 single nucleotide

polymorphisms of the cytotoxic Tlymphocyte-associated antigen-4 gene region is associated with Graves' disease in an Italian population. Thyroid. 2005; 15: 232- 238.

[53] Mayans S, Lackovic K, Nyholm C, et al. CT60 genotype does not affect CTLA-4 isoform expression despite association to T1D and AITD in northern Sweden. BMC. Med. Genet. 2007; 8: 3.

[54] Takahashi M and Kimura A. HLA and CTLA4 polymorphisms may confer a synergistic risk in the susceptibility to Graves' disease. J. Hum. Genet. 2010; 55: 323-326.

[55] Brand OJ, Barrett JC, Simmonds MJ, et al. Association of the thyroid stimulating hormone receptor gene (TSHR) with Graves' disease. Hum Mol Genet. 2009; 18: 1704- 1713.

[56] Ploski R, Brand OJ, Jurecka-Lubieniecka B, et al. Thyroid stimulating hormone receptor (TSHR) intron 1 variants are major risk factors for Graves' disease in three European Caucasian cohorts. PLoS ONE. 2010; 5: e15512.

[57] Davis RS, Wang YH, Kubagawa H. Identification of a family of Fc receptor homologs with preferential B cell expression. Proc Natl Acad Sci USA. 2001; 98: 9772-7.

[58] Sgarbi JA, Kasamatsu TS, Matsumura LK. Parity is not related to autoimmune thyroid disease in a population-based study of Japanese-Brazilians. Thyroid. 2010; 20: 1151-1156.

[59] Chistiakov DA, Voronova NV, Turakulov RI, et al. The -112G > A polymorphism of the secretoglobin 3A2 (SCGB3A2) gene encoding uteroglobin-related protein 1 (UGRP1) increases risk for the development of Graves' disease in subsets of patients with elevated levels of immunoglobulin. E. J. Appl. Genet. 2011; 52: 201-207.

[60] Song HD, Liang J, Shi JY, et al. Functional SNPs in the SCGB3A2 promoter are associated with susceptibility to Graves' disease. Hum. Mol. Genet. 2009; 18: 1156-1170.

[61] Areschoug T and Gordon S. Scavenger receptors: role in innate immunity and microbial pathogenesis. Cell Microbiol. 2009; 11: 1160-1169.

[62] Jin Y, Teng W, Ben S, et al. Genome-wide scan of Graves' disease: evidence for linkage on chromosome 5q31 in Chinese Han pedigrees. J. Clin. Endocrinol. Metab. 2003; 88: 1798-1803.

[63] Simmonds MJ, Yesmin K, Newby PR, et al. Confirmation of association of chromosome 5q31.33 with United Kingdom caucasian graves' disease. Thyroid. 2010; 20: 413-417.

[64] Thakur SA, Beamer CA, Migliaccio CT, et al. Critical role of MARCO in crystalline silica-induced pulmonary inflammation. Toxicol. Sci. 2009; 108: 462-471.

[65] Zhu W, Liu N, Zhao Y, et al. Association analysis of polymorphisms in IL-3, IL-4, IL-5, IL-9, and IL-13 with Graves' disease. J. Endocrinol. Invest. 2010; 33: 751-755.

[66] Chu X, Dong Y, Shen M, et al. Polymorphisms in the ADRB2 gene and Graves disease: a casecontrol study and a meta-analysis of available evidence. BMC Medical Genetics. 2009; 10:26.

[67] Jacqueline J, Antonio CB, Andrew JB, et al. Guidelines for the Treatment of Hypothyroidism: Prepared by the American Thyroid Association Task Force on Thyroid Hormone Replacement. Thyroid. 2014; 24(12): 1670-1751.

[68] Garber JR, Cobin RH, Gharib H, et al. Clinical practice guidelines for hypothyroidism in adults: Cosponsored by the american association of clinical endocrinologists and the American thyroid association. Endocrine Practice. 2012; 18: 988-1028.

[69] Stan MH, Garrity JH, Bahn RS. The evaluation and treatment of graves Ophtalmologie. Med Clin North Am. 2012; 96(2): 311-328.

[70] Matthews DC and Syed AA. The role of TSH receptor antibodies in the management of GD. European Journal of Internal Medicine. 2011; 22: 213-216.

[71] Streetman DD and Khanderia U. Diagnosis and treatment of GD. Annals of Pharmacotherapy. 2003; 37: 1100-1109.

[72] Prummel MF, Bakker A, Wiersinga WM, et al. Multi-center study on the characteristics and treatment strategies of patients with Graves' orbitopathy: The first European Group on Graves' Orbitopathy experience. European Journal of Endocrinology. 2003; 148: 491-495.

[73] Orgiazzi J. Antithyroid drugs. Encyclopedia of Endocrine Diseases. 2018; 683-687.

[74] De Groot L, Abalovich M, Alexander EK, et al. Management of thyroid dysfunction during pregnancy and postpartum: An endocrine society clinical practice guideline. Journal of Clinical Endocrinology and Metabolism. 2012; 97: 2543-2565.

[75] Louvet C, De Bellis A, Pereira B, et al. Time course of Graves' orbitopathy after total thyroidectomy and radioiodine therapy for thyroid cancer. Medicine. 2016; 95: e5474.

[76] Menconi F, Marcocci C, Marinò M. Diagnosis and classification of GD. Autoimmunity Reviews. 2014; 13(4-5): 398-402.

[77] Leslie WD, Ward L, Salamon, EA, Ludwig S, Rowe RC, Cowden EA. A randomized comparison of radioiodine doses in graves' hyperthyroidism. Journal of Clinical Endocrinology and Metabolism. 2003; 88(3): 978-983.

[78] Bonnema SJ and Hegedüs. Radioiodine therapy in benign thyroid diseases: Effects, side effects, and factors affecting therapeutic outcome. Endocrine Reviews. 2012; 33(6): 920-980.

[79] Leo SD, Lee SY, Braverman LE. Hyperthyroidism. Lancet. 2016; 388(10047): 906-918.

[80] Nawrot I, Pragacz A, Pragacz K, et al. Total thyroidectomy is associated with increased prevalence of permanent hypoparathyroidism. Medical Science Monitor. 2014; 20: 1675-1681.

[81] Bartalena L, Baldeschi L, Dickinson A, et al. Consensus statement of the European Group on Graves' orbitopathy (EUGOGO) on management of GO. European Journal of Endocrinology. 2008; 158(3): 273-285.

[82] Marcocci C, Kahaly GJ, Krassas GE, et al. Selenium and the Course of Mild Graves' Orbitopathy. New England Journal of Medicine. 2011; 364(20): 1920-1931.