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Genetic Markers of Graves' Disease: A Historical View and Up-date

Dimitry Chistiakov[1*]

[1]*Chair of Medical Nanobiotechnology, N. I. Pirogov Russian State Medical University, Ostrovityanov St. 1, Moscow 117997, RUSSIA.*

Review Article

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ABSTRACT

Two decades of intensive but quite chaotic and decentralized population studies on susceptibility to Graves' disease (GD) provided a bulk of inconsistent data resulted in finding of proven association only for the HLA class II region that exerts a major effect in the genetics of GD. Using low-resolution microsatellite-based human genome-wide scans revealed several regions of linkage harboring putative susceptibility variants. Further, high throughput genotyping of large population cohorts with help of high dense panels of single nucleotide polymorphisms (SNPs) and application of advanced tools for analysis of extended blocks of linkage disequilibrium within a candidate gene (SNP tagging, etc.) revealed the presence of several susceptibility genes in the regions of linkage on chromosome 2q (CTLA-4), 8q (Tg), 14q (TSHR), 20q (CD40), 5q (SCGB3A2/UGRP1) and, probably, Xp (FOXP3). The list of GD-predisposing loci was then extended with three more genes (PTPN22, IL2RA/CD25, and FCRL3). In the nearest future, implementation of even more robust technology such as whole-genome sequencing is expected to catch any disease-associated genetic variation in the patient's individual DNA. In this review, the historical development of our knowledge on genetic factors predisposing to GD is considered, with special emphasis on the functional significance of observed associations and discussion of possible mechanisms of their contribution to GD pathogenesis.

Keywords: Autoimmune thyroid disease; Graves' disease; thyroid autoimmunity; genetic susceptibility; association; polymorphism;

**Corresponding author: Email: dimitry.chistiakov@lycos.com;*

ABBREVIATIONS

AITD- autoimmune thyroid disease; APCs- antigen-presenting cell; C/EBPα-CCAAT/enhancer-binding protein alpha; CTLA-4-cytotoxic T-lymphocyte-associated protein 4; DZ -dizygotic;FCRL3-Fc receptor-like protein 3; flCTLA-4- full-length CTLA-4; FOXP3-forkhead box P3; GD- Graves' disease; GWAS- genome wide association study; HLA-Human Leukocyte Antigens; IFIH1-interferon-induced helicase Cdomain-containing protein 1; IL2-interleukin-2; IL2RA-IL-2 receptor, alpha subunit; JIA-juvenile idiopathic arthritis; LD-linkage disequilibrium; LYP-lymphoid phosphatase; MAF- minor allele frequency; MARCO-macrophage scavenger receptor with collagenous structure; MS- multiple sclerosis;MZ-monozygotic; NFκB- nuclear factor kappa B; OR- odds ratio; PTPN22- protein tyrosine phosphatase, non-receptor type 22 (lymphoid); RA- rheumatoid arthritis; SCGB3A2-secretoglobin 3A2; sCTLA-4- soluble CTLA-4; sIL- 2RA-soluble IL2RA; SLE- systemic lupus erythematosus; SNP-single nucleotide polymorphism; T1D- type 1 diabetes mellitus; TBII-TSHR-binding inhibitory antibodies; TCR-T-cell receptor; Tg- thyroglobulin; TSAb- TSHR-binding stimulating antibodies; TSH- thyroidstimulating hormone; TSHR- thyroid stimulating hormone receptor; Tregs- regulatory T cells;

1. INTRODUCTION

Graves' disease (GD) belongs to autoimmune thyroid disease (AITD) characterized by self-antibodies-mediated stimulation of the thyroid stimulating hormone (TSH, thyrotropin) receptor (TSHR) that causes a hyperfunction of the thyroid gland. The thyroid activation leads to follicular hypertrophy and hyperplasia causing thyroid enlargement and increasing thyroid hormone production. GD diagnosis requires identification of suppressed TSH levels and elevated levels of the free thyroid hormone [i.e., thyroxine (T4) and/or triiodothyronine (T3]. GD affects ~0.5-2% of Western populations and accounts for the majority of cases of the hyperthyroidism. GD exhibits a clear sex-related bias in its frequency occurring 10-fold more often in females than in males.

The familial clustering of autoimmune thyroid disease (AITD) has been known since the middle of the last century, with ~50% of patients reporting a family history of disease (Bartels et al., 1941). Furthermore, a whole variety of thyroid abnormalities have been reported in relatives of patients with thyroid disease, with thyroid autoantibodies, for example, being present in over 50% of children of patients with GD (Desai and Karandikar, 1999). Perhaps, the most convincing evidences for a genetic predisposition to a disease are provided by twin studies. While in monogenic diseases there is a full concordance among monozygotic (MZ) twins, in disorders with complex inheritance, the concordance is incomplete, but still higher compared to dizygotic (DZ) twins. Twin data have confirmed, with remarkable clarity, the presence of a substantial inherited susceptibility to GD. Several large twin studies have reported a higher concordance rate of AITD in monozygotic (MZ) twins compared to dizygotic (DZ) twins (Tomer and Davies, 2003). Concordance rates were 35% in MZ twins and 3% in DZ twins for GD. Model-fitting analysis of these data showed that 79% of the predisposition to the development of GD is attributable to genetic factors, whereas individual-specific environmental factors not shared by the twins could explain the remaining 21% (Brix et al., 1998; 2001). The sibling risk ratio that is the ratio of the prevalence of the disease in siblings of affected individuals compared to the prevalence of the disease in the general population serves as a good estimate of disease heritability, with a ratio of > 5

considered significant. For AITD, the sibling risk ratio calculated for US Caucasians exceeds 16.0 thereby suggesting for a strong genetic influence on the pathogenesis of this disease (Jacobson et al., 2008).

Results from twin studies are informative and helpful but they should be analyzed with caution bearing in mind the bias they often have. In many twin studies, it is likely that at least two types of bias operate in the selection of twin pairs for inclusion in the sample from all possible twins in the population who meet the criteria for the study. One such bias is concordance-dependent ascertainment, where the probability of twin pairs being included in a study of a particular trait is dependent on whether they are concordant or discordant for that trait. Such a bias can occur in a number of ways, even when a voluntary recruitment procedure is adopted. Another bias that may occur is that of non-independent ascertainment, where ascertainment probability depends on the combination of within-pair similarity and the type of relative (e.g. MZ or DZ twins); for example, it may happen that concordant MZ twins are more likely to be included in a particular study than are concordant DZ twins.

Familial clustering of GD and twin studies showed that this disease does not occur because of a single gene defect and does not follow a simple pattern of Mendelian inheritance (Farid et al., 1981). To date, it is known that genetic susceptibility to GD is accounted by multiple genes, with the most of those exhibiting a rather modest effect, with Odds Ratio (OR) not exceeding 1.5 (Tomer, 2010). In this review, we will consider major findings in the genetics of GD from the evolutionary-historical point of view by focusing on the characterization of advances achieved with help of four major strategies in genetic analysis including candidate gene approach, whole-genome linkage screening, genome-wide association studies (GWAS), and whole-genome sequencing.

2. METHODOLOGY TO FIND GENETIC VARIANTS CONFERRING SUSCEPTIBILITY TO GD

2.1 Candidate Gene Studies

Functional candidate genes may be selected from a bulk of human genes on the basis of their functional significance. For example, due to the major role of autoimmune mechanisms in the pathogenesis of GD mediated by self-reactive T-cells, a variety of immune-related genes such as Human Leukocyte Antigens (HLA), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), and many others could be considered as candidates for GD susceptibility. Since GD is characterized by the presence of several major self-antigens such as TSHR, thyroid peroxidase, and thyroglobulin (Tg), their genes could be chosen as attractive candidates for thyroid autoimmunity.

The analysis of a limited number of DNA variants, often single nucleotide polymorphisms (SNPs), within a gene of interest in a relatively small number of cases and controls have been common place with journals reporting a longline of positive and negative results. Due to the significant inconsistency of produced results and underpowered character of most studies, association analyses resulted in the identification of only four susceptibility genes including HLA, CTLA-4, TSHR, and PTPN22 (encodes protein tyrosine phosphatase, non-receptor type 22, also known as LYP – lymphoid phosphatase). The role of these genes in etiology of GD will be considered below.

In early association studies, one or several polymorphisms within a gene of interest have been typically analyzed. Since a human gene usually contains dozens or even hundreds of SNPs, some genes have been erroneously considered as lacking association with GD on the basis of the analysis of only a few SNPs. In human genome, regions of extended linkage disequlibrium (LD) have been considered as problematic for precise identification of an etiological variant. More recently, however, using this strong LD, a single SNP can be chosen which will give a good representation of the associated LD block allowing a more comprehensive coverage of the gene region of interest. This allows for an estimation of a large number of genotypes by only typing a few that catch, or tag, a block of LD (Johnson et al., 2001). The tagging SNP approach makes the analysis of a gene more comprehensive and cost-effective since provides the possibility to find an etiological variant within an LD block without genotyping every SNP in a chromosomal region.

2.2 Whole-Genome Linkage Screening

Linkage analysis is based on study of affected families or a pedigree allowing the evaluation of co-segregation of a genetic variant with disease. If a tested marker is close to an etiological variant, the frequency of recombination between those may be significantly reduced to cause a preferential inheritance of the marker alleles among affected individuals, even though the marker itself is not involved in the disease pathogenesis. The measure of the likelihood of linkage between a disease and a genetic marker is the logarithm of odds (LOD) score (Ott, 1999). The LOD score is the base-10 LOD ratio in favor of linkage. According to widely accepted guidelines, in complex diseases an LOD score of >1.9 is suggestive of linkage, while an LOD score of >3.3 indicates significant linkage in studies using the parametric approach. Linkage is confirmed if evidence for linkage is replicated in two separate data sets (Lander and Kruglyak, 1995).

In a typical genome-wide approach, a set of ~300-400 microsatellite markers is sufficient to cover a whole genome. Compared to microsatellites, SNPs are less polymorphic since they typically represent biallelic markers. However, SNPs are very abundant and on the average there is a SNP every 300 bp. Therefore, to screen the entire human genome for linkage with a disease, more SNPs are required. Usually, at least 10,000 SNPs located across the genome are needed to provide a reasonable resolution to find a disease-associated variant.

The linkage analysis showed its proven robustness in the analysis of Mendelian traits caused by genetic alterations. However, the suitability of this approach is limited for dissecting complex disorders such as GD by the requirement for multiplex families and low power to detect susceptibility loci with weak genetic effects. Another limitation of linkage analysis is the low resolution, which makes it usually impossible to distinguish effects of loci within a distance of 2-3 Mb. Since the association analysis has a much profound sensitivity to detect genetic association for a set of polymorphisms located within the limited chromosomal region, this technique is applicable for further fine mapping of an etiological variant(s) within the region of linkage. Such an approach called ‘positional cloning’ allows narrowing the chromosomal region of the location of a putative causal variant down to the identification of a true etiological disease marker (Kennedy, 2003).

In microsatellite-based whole-genome linkage studies, several loci have been identified as linked with GD (Tomer et al., 1999, 2003; Sakai et al., 2001). However, only few regions of linkage discovered in early genome-wide screens were replicated in the last whole-genome analysis involved 1,119 AITD families (Taylor et al., 2006). Some of these loci have been then fine mapped and the genes identified. The AITD susceptibility gene on 2q is the CTLA-

4 gene (also identified by the candidate gene approach), the susceptibility gene on 8q is Tg, on 14q the TSHR (also identified by the candidate gene approach), and on 20q the CD40 gene.

2.3 Genome-Wide Association Studies

The completion of the HapMap project has made whole-genome scanning by association studies feasible. Besides genotyping over 1.0 million SNPs spanning the whole human genome, HapMap revealed the complex architecture of the human genome organized into discrete LD block, with limited recombination rate between markers located within the every LD block due to the tight pair-wise intermarker LD (Altshuler et al., 2005). This enabled the utilization of tag-SNPs (each SNP representing an entire LD block) to test the entire human genome for association with disease. Moreover, microarray-based genotyping technology using high-density genome-wide SNP platforms enabled the typing of up to 1,000,000 or even more SNPs in a single experiment (Distefano and Taverna, 2011).

Despite the unquestionable value and extraordinary high throughput capacity, GWAS have limitations such as a potential for false positive results, which necessitates very large sample sizes, genotyping errors or insensitivity to structural variants (Pearson and Manolio, 2008). Current GWAS usually take into consideration common SNPs, with minor allele frequency (MAF) of 5%. However, there is an increasing number of evidences showing that the disease risk may be significantly influenced by rare (MAF<5%) or very rare genetic variants (MAF<1%). For example, Nejentsev et al. (2009) found four rare (MAF=0.5-2%) functionally relevant variants of interferon-induced helicase C domain-containing protein 1 (IFIH1), which contributed to the risk of type 1 diabetes (T1D) more significantly than common non-synonymous SNPs within this gene. The genetically powered identification of association of such rare polymorphisms with a complex disease through implementation of GWAS requires enormously extended population sample sizes up to 100,000 cases whose recruitment and genotyping would be too laborious and expensive.

Three GWAS for AITD susceptibility have been performed. The first involved over 500,000 SNPs typed in seven common diseases each with 2,000 samples and a common control cohort of 3,000 samples (WCCT, 2007a) The second involved four disease states including AITD in which 14,500 non-synonymous SNPs (e.g. SNPs causing an amino acid substitution) have being typed in 900 AITD patients and 1,466 control subjects. The study confirmed the TSHR gene as a susceptibility gene for GD and identified FCRL3 and several other putative susceptibility genes for GD (WCCT, 2007b). The third GWAS recently performed in a Chinese cohort (over 1,500 GD subjects and over 1,500 controls) replicated four previously reported loci (HLA, TSHR, CTLA-4, and FCRL3) and discovered two more susceptibility loci located at 6q27 (the RNASET2-FGFR1-CCR6 gene region) and 4p14 (SNP rs6832151) (The China Consortium for the Genetics of AITD et al. 2011).

2.4 Whole-Genome Sequencing

While at present most interesting novel data on genetics of autoimmune diseases are coming from carefully designed GWAS, in the near future, this technology may be replaced by even more powerful approaches based on the next-generation DNA sequencing. Progress in this field makes it possible to sequence genomes or large parts thereof (such as an exome, i.e., all exons from a genome) at unprecedented speed. While the price is still high, it is expected that sequencing the entire human genome will cost around $1,000 per sample within the next few years making the whole-genome sequencing a feasible approach

to identify complex disease genes. The 1000 Genomes Project running now is focused on low-coverage whole-genome sequencing of 179 individuals from four populations, high-coverage sequencing of two mother-father-child trios, and exon-targeted sequencing of 697 individuals from seven populations (1000 Genomes Project Consortium, 2010). This project provides a wealth of information on rare polymorphic variants, copy number variations, genome-wide and local haplotype organization, and structural variants, most of which were previously undescribed. On average, each person is found to carry approximately 250 to 300 loss-of-function variants in annotated genes and 50 to 100 variants previously implicated in inherited disorders (1000 Genomes Project Consortium, 2010). Recently, whole-genome sequencing has been successfully utilized in two patients, one with Charcot-Marie-Tooth disease (Lupski et al., 2010) and the other with acute myeloid leukemia (Mardis et al., 2009). Finally, Thompson et al. (in press) reported a very promising cost-effective single-step strategy that provides a possibility to any gene can be captured and sequenced directly from the human genomic DNA without amplification, cloning, and using no proteins or enzymes prior to sequencing.

The main challenge of whole-genome sequencing is developing robust methods for analyzing the sequence data and sorting out normal variations between individuals from those that are responsible for disease susceptibility. When such a computer tool will be generated, this strategy becomes a truly personalized approach to the treatment of complex diseases such as AITD.

3. GD SUSCEPTIBILITY GENES

To date, proven records for association with GD have been produced for several immune-related genes such as HLA, CTLA-4, CD40, PTPN22, SCGB3A2/UGRP1, and FCRL3 and two thyroid-specific genes (TSHR and TG) (Fig. 1). Less consistent results have been obtained for IL2RA/CD25 and FOXP3, both are key regulators of natural Tregs.

3.1 HLA

HLA molecules as a part of the immunological synapse play a central role in the human immune system by binding fragments of processed antigens in the form of peptides and presenting them on the surface of an antigen-presenting cell (APC) to the T-cell receptor (TCR). HLA molecules are also involved in T-cell selection in the thymus (Splint and Kishimoto, 2001). Due to its crucial impact in the recognition of self- and foreign antigens and maintaining central immune tolerance, it is not surprisingly that the HLA locus is linked to a variety of autoimmune diseases including AITD and GD. The contribution of the HLA region in various autoimmune disorders is different. For example, in T1D, the HLA class II gene variants are the major susceptibility locus accounting for ~30-40% of genetic risk (Davies et al., 1994). In GD susceptibility, HLA does not play a major role accounting for the only ~10-20% of genetic predisposition (Vaidya et al., 2002). However, it should be stressed that these estimates have been made based on data produced in linkage analysis and before the discovery of several non-HLA susceptibility genes such as PTPN22, TSHR, and CD25.

In early studies, association between HLA and GD has been attributed to the HLA class I genes such as HLA-A and HLA-B, with ORs for GD ranging from 1.5 to 3.5 (Grumet et al., 1974; Farid et al., 1976) (Table 1). Further studies showed that association between the HLA class II genes and GD is stronger than that between the HLA class I and GD (Bech et al., 1977) and is a result of the strong LD between these loci within the entire HLA region

(Heward et al., 1998). Subsequently, among HLA class II genes, the strongest association has been shown for alleles DRB1*03 and DQA1*05 in various Caucasian populations, with a common susceptibility haplotype DR3 (DRB1*03-DQB1*02-DQA1*0501) (OR=3.1-3.8; Table 1) and a protective haplotype DR7 (DRB1*07-DQB1*02-DQA1*02) (Ban et al., 2002a; Simmonds et al., 2005). The frequency of DR3 in GD patients was generally 40–55% in GD patients, and ~ 15–30% in the general population, resulting in OR for people with HLA-DR3 of 3–4 (Jacobson et al., 2008).

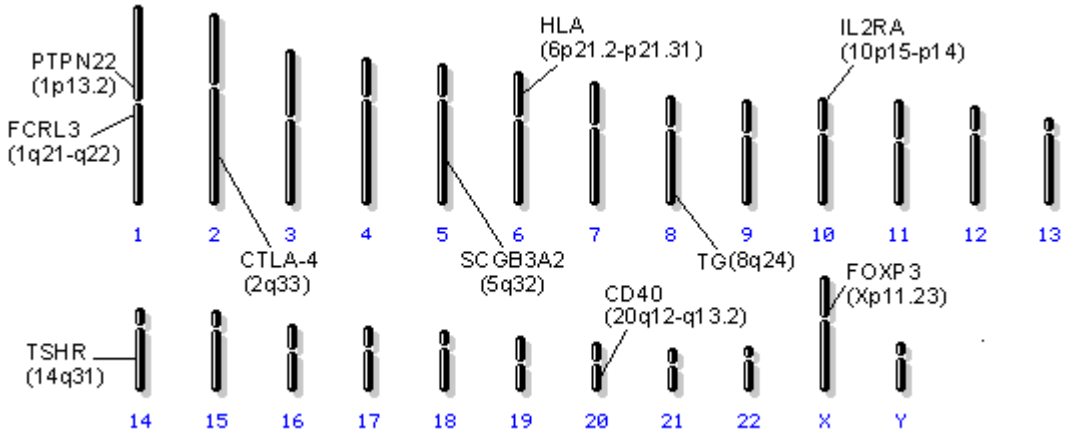

Figure 1: Location of genes contributing to susceptibility to Graves' disease in the human genome

Cytolocation of genes is shown in brackets. PTPN22: protein tyrosine phosphatase, non-receptor type 22 (lymphoid); FCRL3: Fc receptor-like protein 3; CTLA-4: cytotoxic T-lymphocyte-associated protein 4; CGB3A2: secretoglobin 3A2; HLA: Human Leukocyte Antigens; IL2RA: TG: thyroglobulin; IL-2 receptor, alpha subunit; TSHR: thyroid stimulating hormone receptor; CD40: antigen CD40; FOXP3: forkhead box P3.

Since T-cells recognize and respond to peptide antigens when presented by APCs bound to HLA class II pockets, it was proposed that that certain HLA-DR alleles may permit self-antigenic peptides to fit into the peptide-binding pocket and to be presented more efficiently to T-cells (Nepom et al., 1996). The hypothesis was confirmed in several autoimmune diseases, more notably in T1D. A key role of the amino acid residue at position 57 of the DQbeta chain has been found in the genetic susceptibility to T1D (Morel et al., 1988).

A similar molecular mechanism explaining the predisposing or protective role of HLA molecules has been identified for GD susceptibility (Menconi et al., 2008). The presence of arginine at position 74 of the HLA-DRbeta1 chain (DRbeta-Arg74) has been shown to be a critical factor for conferring DR-mediated susceptibility to GD (Ban et al., 2004). In contrast, the presence of glutamine at position 74 of the DRb1 chain provides the protective effect (Simmonds et al., 2005). Structural analysis showed the unique role of the position 74 in influencing peptide-binding properties of the HLA molecule and presentation to T-cells. This position encompasses several peptide-binding pockets within the peptide binding domain crucial for both T-cell receptor docking and antigen presentation (Chelvanayagam, 1997). Recently, Jacobson et al. (2009) found Tg peptides capable to be presented by HLA-DR pockets containing arginine at position beta 74. These findings indeed suggest that the peptide-binding pocket structure and conformation play a major role in the etiology of several autoimmune diseases including T1D and AITD (Todd et al., 1987; Jacobson et al., 2008).

Table 1: Some HLA association studies in GD performed in Caucasians

Country	No. of patients	HLA allele	RR/p-value	Reference
Denmark	86	B8 Dw3	2.8 3.94	Bech et al., 1977
Canada	175	B8 DR3	3.1 5.7	Farid et al., 1980
Sweden	78	B8 DR3	2.77 2.13	Dahlberg et al., 1981
Hungary	256	B8 DR3	3.48 4.8	Stenzsky et al., 1985
UK	127	B8 DR3	2.77 2.13	Kendall-Taylor et al., 1988
UK	101	DR3	2.1	Weetman et al., 1988
Germany	253	DR3	2.52	Schleusener et al., 1989
USA	65	DR3	3.38	Mangklabruks et al., 1991
USA	94	DQA1*0501	3.71	Yanagawa et al., 1993
UK	120	DQA1*0501	3.8	Barlow et al., 1996
UK	228	DRB1*0304 DRB1*0301 DQA1*0501	2.7 1.9 3.2	Heward et al. 1998
USA	92	DRB1*03 DRB1*08	2.6 3.2	Chen et al., 1999
Belgium	194	DRB1*0301	2.53	Zamani et al., 2000
Poland	228	DRB1*03	3.5	Bernarczuk et al., 2004
USA	160	DR3	3.8	Ban et al., 2004
UK, USA	871	DRB1*0301-5 DQB1*02 DQB1*04 DQA1*0501-2	2.98 2.56 2.88 2.53	Simmonds et al., 2005

RR: relative risk.

3.2 CTLA-4

CTLA-4 (also known as CD152) is another component of the immunological synapse. CTLA-4 molecule is responsible for negative regulation of TCR-mediated responses, and its function is opposite to the function of the CD28 costimulatory molecule that promotes T-cell activation (Walunas et al., 1996). CTLA-4 acts through delivering inhibitory signal through its cytoplasmatic domain, which can reverse the classic TCR-induced stop signal needed for physical interaction between T-cell and APC thus reducing adhesion periods between these cells that in turn decreases cytokine production and proliferation (Schneider et al., 2006).

A full-length CTLA-4 (flCTLA-4) consists of four exons each encoding functionally distinct portions of this protein such as the leader sequence and three structural domains (extracellular, transmembrane, and cytoplasmic). An alternatively splicing isoform, soluble CTLA-4 (sCTLA-4) lacking the transmembrane domain, also exists (Magistrelli et al., 1999). In addition to the cell intrinsic action mediated by the membrane-bound flCTLA-4, the sCTLA-4-dependent extrinsic model has been proposed (Qureshi et al., 2011). The extrinsic mechanism of CTLA-4 action may involve stimulation of regulatory T cells (Tregs) but may

be released through the removal of costimulatory ligands (CD86) from APCs via trans-endocytosis (Qureshi et al., 2011). Levels of sCTLA-4 were shown to be elevated in several autoimmune diseases including AITD (Oaks and Hallett, 2000).

Since CTLA-4 suppresses T-cell activation to control normal T-cell responses, it was postulated that CTLA-4 polymorphisms that reduce its expression and/or function might predispose to autoimmunity by creating overreactive T-cells (Chistiakov and Turakulov, 2003). The first evidence for association between CTLA-4 and GD was reported by Yanagawa et al. (1995). In fact, this study that found a significant association between a $(AT)_n$ microsatellite in the 3' untranslated region (3'UTR) of CTLA-4 and GD was the first report of an association between CTLA-4 and any autoimmune condition.

Except for the $(AT)_n$ microsatellite, several more functionally relevant polymorphisms at CTLA-4 have been widely evaluated for association with GD. It has been proposed that long AT-repeat allele of the $(AT)_n$ microsatellite decreases stability of CTLA4 mRNA blunting inhibitory function of the protein and thus reducing control of T-cell proliferation (Takara et al., 2003). Another polymorphism is an adenine-to-guanine change in codon 49 (A49G, rs231775) causing an amino acid substitution (Thr17Ala) at the signal peptide (Donner et al., 1997). Compared to the Thr17 allele, the predisposing Ala17 variant of CTLA-4 has been shown to have altered posttranslational processing resulting in insufficient glycosylation of this molecular variant (Anjos et al., 2002). Although studies in multiple ethnic groups showed strong association between this marker and GD (Heward et al., 1999; Vaidya et al., 1999; Park et al., 2000; Chistyakov et al., 2000), the evidence on CTLA-4 Thr17Ala as a causal variant for GD on chromosome 2q33 was positioned under question by Xu et al. (2002) who failed to show any significant influence of the codon 17 polymorphism on both the extrinsic and intrinsic actions of the recombinant human CTLA-4 transgene expressed in Jurkat T cells.

Among polymorphic sites located in the promoter region of the CTLA-4 gene, the C(-318)T polymorphism (rs5742909) showed the most consistent association with GD in various populations (Braun et al., 1998; Park et al., 2000; Chistiakov et al., 2006; Esteghamati et al., 2009). This nucleotide substitution alters the binding site sequence for the lymphoid enhancing factor 1 (LEF1) thereby affecting CTLA-4 expression (Ligers et al., 2001; Wang et al., 2002; Anjos et al., 2004; Chistiakov et al., 2006). Markers rs231775 and rs5742909 have been shown to contribute to GD susceptibility independently from the cluster of disease-associated SNPs situated at the genomic region downstream of the 3'UTR of CTLA-4 (Anjos et al., 2004; Chistiakov et al., 2006).

Using re-sequencing and fine mapping of all common variants within the CTLA4 gene, Ueda et al. (2003) reported the disease susceptibility locus located within a noncoding 6.1 kb region adjacent to the 3'UTR of CTLA-4. The susceptibility locus had four SNPs (CT60, J030, JO31, and JO27–1), which showed the strongest association with GD that was even stronger that an association between any other common SNP within the CTLA-4 gene including rs231775 and rs5742909. Surprisingly, the higher risk allele G of the marker CT60 (+6230 G/A, rs3087243) was associated with lower mRNA levels of sCTLA-4. The correlation between the carriage of the CT60 polymorphism and serum concentrations of sCTLA-4 has not been confirmed in other studies (Anjos et al., 2005; Mayans et al., 2007).

In a large-scale meta-analysis, Kavvoura et al. (2007) summarized data on 28 studies involved a total of 4,848 GD cases and 7,314 controls and reported significant association of the allele G of rs231775 and allele G of rs3087243 with higher risk of GD (OR=1.49 and

1.45, respectively). The modest association with increased GD risk was found for alleles G of markers of JO31 and JO30, but not for the $(AT)_n$ microsatellite and SNP C(-318)T or JO27-1 (Kavvoura et al. 2007). It is known that CTLA-4 polymorphisms are associated with production of thyroid self-antibodies in GD patients (Tomer et al., 2001; Zaletel et al., 2002), and may synergistically interact with GD-predisposing variants HLA-A*02 and -DPB1*05:01 in production of TSHR-blocking (TBII) antibodies (Takahashi and Kimura, 2010).

However, to date, the true etiological variant of CTLA-4 is still unknown. Perhaps, the predisposition to GD within the CTLA-4 locus is determined by the complex interplay between the clusters of disease-associated markers in 3' and 5'regions of CTLA-4 independently contributing to GD susceptibility. Interestingly, using commercial monoclonal antibodies againt the extracellular domain of CTLA-4, Tector et al. (2009) failed to find s-CTLA-4 itself in the CTLA-4 immunoreactive material isolated from the blood of patients with myasthenia gravis. These findings may reconcile the apparent discrepancy between reports of elevated levels of sCTLA-4 in plasma from patients with autoimmune disease and the report of decreased levels of the sCTLA-4 transcript among individuals with the CT60 allele of the CTLA-4 gene.

Like the HLA region, CTLA-4 belongs to general autoimmunity genes, for which association with the majority of autoimmune diseases has been found (Gough et al., 2005). The major role of this gene in thyroid-specific autoimmunity and other organ-specific and systemic autoimmune T-cell mediated disorders arises from the central role of CTLA-4 in controlling TCR-dependent activation of T-cells and maintaining peripheral immune tolerance (Riley and June, 2005).

3.3 CD40

CD40, which belongs to the family of tumor necrosis factor receptors, is primarily expressed on the surface of B-lymphocytes and other professional and non-professional APCs (Banchereau et al., 1994), and plays a fundamental role in B-cell activation and antibody production (Armitage et al., 1993). The physiological ligand for CD40 is the CD154 (CD40L) molecule that is expressed on the surface of activated T-helper cells (Hollenbaugh et al., 1992). In B-cells, CD40 ligation provides the necessary costimulatory signal for cell proliferation, immunoglobulin class switching, antibody secretion, prevention of apoptosis of germinal center B-cells, affinity maturation, and generation of long-lived memory cells (Chatzigeorgiou et al., 2009).

As a GD susceptibility gene, CD40 has been found by fine mapping within the GD-2 locus on chromosome 20q11 linked to the development of GD (Tomer et al., 1998; 2003; Pearce et al., 1999). In CD40, an etiological variant is presented by the functional SNP rs1883832 [C(-1)T] that is located at position -1 relative to the translation start and affects the Kozak sequence, which plays the major role in the initiation of the translation (Tomer et al., 2002a). The genotype C/C of rs1883832 showed association with higher GD risk, and this association has been widely replicated in Caucasian and Asian populations (Kim et al., 2003; Ban et al., 2006; Kurylowicz et al., 2007) except for two studies in the UK population (Heward et al., 2004; Houston et al., 2004). Overall, the meta-analysis of a total of 1,961 affected patients and 1,960 control subjects revealed significant but modest genetic effect of the allele C in GD susceptibility in Caucasians (OR=1.22) (Kurylowicz et al., 2007).
Functional analysis showed that, compared to the allele T, the higher risk allele C is associated with more efficient translation of CD40 reflected by a 20-30% gain in the production of CD40 in in vitro translation system (Jacobson et al., 2005; Park et al., 2007).

As mentioned above, CD40 is expressed in B-cells and non-professional APCs such as thyrocytes, i.e. in cell types involved in the pathogenesis of GD (Metcalfe et al., 1998). Therefore, increased expression of CD40 on B-lymphocytes can lead to enhanced production of anti-TSHR-stimulating antibodies (TSAbs), whereas increased expression of CD40 on thyrocytes can trigger an autoimmune response to the thyroid by resident T-cells. These mechanisms could be simultaneously operating in the thyroid thereby implicating in the etiology of GD. Finding of Jacobson et al. (2007) reported the stronger association of the CC genotype with GD in a subset of GD patients who had persistently high levels of thyroid antibodies provides the indirect evidence in support of the stimulatory effects of the C variant of CD40 on production of thyroid antibodies.

The association of CD40 with autoimmunity is not limited to GD only. Several studies showed that CD40 variants could be implicated in a set of autoimmune and proinflammatory conditions accompanied with activation of B-cells and propagation of B-cell autoreactive clones producing self-antibodies such as asthma (Metcalfe et al., 1998), rheumatoid arthritis (RA) (Raychaudhuri et al., 2008), systemic lupus erythematosus (SLE) (Gaffney et al., 2006), and multiple sclerosis (MS) (ANZgene, 2009).

3.4 PTPN22

The PTPN22 gene lies on chromosome 1p13 and encodes the immune regulatory phosphatase LYP, which triggers T-cells by inhibiting signal transduction and preventing activation through the interaction of LYP with several accessory molecules including protein tyrosine kinase Csk and Grb2 (Cloutier and Veillette, 1999). Initial reports of association of the C1858T polymorphism (rs2476601), causing an amino acid change of an arginine to tryptophan at residue 620 (R620W) of LYP, with T1D (Bottini et al., 2004; Smyth et al., 2004) were rapidly extended by finding positive associations not only with AITD (Smyth et al., 2004; Velaga et al., 2004) but also with SLE (Kyogoku et al., 2004), RA (Begovich et al., 2004), juvenile idiopathic arthritis (JIA), and Addison's disease (Lee et al., 2007). In many autoimmune diseases, PTPN22 represents a second most strongly associated locus after HLA, with OR typically ranging from 1.5 to 1.9 (Criswell et al., 2005; Vang et al., 2007).

The functional R620W polymorphism resides in the P1 proline-rich motif of LYP, which binds with high affinity to the Src homology 3 (SH3) domain of the tyrosine kinase, Csk, and hence affects binding properties of LYP with this partner molecule in an inhibitory complex that regulates key TCR signaling kinases (Lck, Fyn, ZAP-70) (Bottini et al., 2004). The W620 variant disrupts the interaction between PTPN22 and Csk (Begovich et al., 2004) and also increases the phosphatase activity, which in turn suppresses TCR signaling more efficiently than the wild-type protein (Vang et al., 2005; Rieck et al., 2007). In fact, the R620W polymorphism is a gain-of-function mutation, with 60% increase in the catalytic specific activity of the LYP 620W phosphatase compared to the LYP 620R variant (Vang et al., 2005). This results in enhanced down-regulation of TCR signaling followed by the inhibition of expansion of T-cells, weakening the positive selection in the thymus, and reduction of the antibody production through lowering activity of helper T-lymphocytes (Hasegawa et al., 2004). It is speculated that a lower T-cell signaling would lead to a tendency for self-reactive T-cells to escape thymic deletion and thus remain in the periphery. However, this theoretical possibility awaits experimental confirmation.

Recent experiments in mice expressing the LYP variant homolog Pep619W showed dramatic reduction in levels of the mutant (Pep619W) variant compared to the levels of the wild-type Pep619R protein due to the calpain 1-mediated proteolysis (Zhang et al., 2011).

Similarly, compared to the LYP 620R protein, human LYP 620W phosphatase was found to be sensitive to the calpain digestion in vitro that may explain less levels of the enzyme in T- and B-cells of the LYP 620W carriers. The reduced expression of LYP 620W was associated with lymphocyte and dendritic cell hyperresponsiveness, a mechanism by which LYP620W may increase risk for autoimmune disease. These data could be supported by observations of Zickerman et al. (2009) who reported the hyperactivation of CD45 E613R B-lymphocytes carrying the mutation E613R in the juxtamembrane wedge domain of the CD45 molecule and development of a B cell-driven, lupus-like disease in Pep-deficient mice. Therefore, the role of PTPN22 in autoimmunity is not restricted by altering function of T-lymphocytes, but also involves B-cells.

Interestingly, the capacity of human LYP to inhibit the activity of B-cell antigen receptor (BCR) has been reported (Rieck et al., 2007; Arechiga et al., 2009). Carriers of the autoimmunity-predisposing LYP 620W variant, have a decrease in memory B cells, which also exhibit impaired calcium flux upon BCR ligation, suggesting a B cell-intrinsic defect in individuals who express the LYP 620W variant (Rieck et al., 2007). It seems that the R620W polymorphism, by suppressing TCR and BCR signaling, globally alters maturation, selection, and function of both T- and B-lymphocytes that predisposes to inducing autoimmunity (Stanford et al., 2010).

The PTPN22 R620W polymorphism displays strong association with GD across multiple Caucasian populations as reflected by ORs ranging from 1.5 to 2.0 (Smyth et al., 2004; Velaga et al., 2004; Criswell et al., 2005; Skorka et al., 2005). However, this polymorphic variant is very rare or absent in Asian and African populations (Mori et al., 2005; Zhang et al., 2008). For example, SNP rs2476601 has not been found in Japanese AITD patients (Ban et al., 2005).

Whilst association of the rs2476601 SNP appears to be common to a number of autoimmune conditions, other independent associations within this gene region are being detected with different patterns of association emerging in individual diseases (Carlton et al., 2005; Onengut-Gumuscu et al., 2006; Heward et al., 2007; Michou et al., 2007). This includes disease-specific haplotypes providing both susceptibility to and protection from GD (Heward et al., 2007) suggesting that the mechanism by which PTPN22 confers susceptibility to GD may be different, for example, to T1D and RA.

3.5 IL2RA/CD25

Tregs are a unique population of T-lymphocytes involved in the regulation of T-cell activation (Paust and Cantor, 2005). Tregs are responsible for maintaining peripheral immune tolerance. Stimulation of Tregs results in inhibiting murine experimental autoimmune thyroiditis (Gangi et al., 2005). Depletion of Tregs in mice makes animals more prone to experimentally induced GD (Saitoh and Nagayama, 2006; Nagayama et al., 2007), while Tregs depletion in mice with induced GD causes switching the disease pathogenesis to a Hashimoto's-like phenotype (McLahlan et al., 2007). These findings suggests on the inhibitory role of Tregs against Graves' hyperthyroidism (Saitoh et al., 2007).

Several subtypes of Tregs have been detected. One subset, the naturally existing CD4+CD25+Tregs, constitutively express CD25, CTLA-4, and glucocorticoid-induced tumor necrosis factor receptor (Paust and Cantor, 2005). In GD patients, no alteration in the distribution of subpopulations of Tregs was found compared to the controls (Pan et al.,

2009). However, in GD, the function of CD4+CD25+ Tregs is impaired (Mao et al., 2011) and reduced (Wang et al., 2006).

Natural Tregs are characterized by high levels of the alpha chain of the interleukin-2 (IL-2) receptor (IL2RA; also known as CD25) on their surface (Burchill et al., 2007). Together with two other subunits, beta-chain (IL2RB, also known as CD122) and the common cytokine receptor gamma-chain (γc, also known as CD132), IL2RA/CD25 constitutes the IL-2 receptor molecule (Gaffen and Liu, 2004). IL-2 receptor mediates functional effects of IL-2, a cytokine that is vital in the regulation of the development of CD4+CD25+ Tregs (Chistiakov et al., 2008).

Using tagging SNP approach and multilocus test, Brand et al. (2007) showed significant evidence for association of the IL2RA/CD24 locus with GD (P=0.00045) in the British population. Findings of Brand et al. (2007) have been recently confirmed in a Russian dataset (Chistiakov et al., in press). We showed association of the haplotype AA compised by minor alleles of two SNPs, rs11594656 and rs41295061, located upstream the 5'promoter region of the IL2RA/CD25 gene, with increased risk of GD (OR=1.47). The carriage of the predisposing haplogenotype AA/AA correlated with elevated levels of the soluble IL-2RA (sIL-2RA) in sera of both GD patients and healthy controls. There is the first evidence of association between IL2RA/CD25 variants and serum concentrations of the soluble IL-2RA form.

In fact, IL2RA/CD25 may represent a general autoimmunity gene. Except for GD, association between this gene and several more autoimmune diseases including T1D (Vella et al., 2005), RA (Kurreeman et al., 2009), MS (Matesanz et al., 2007), and JIA (Hinks et al., 2009) has been reported. However, distinct polymorphic variants of IL2RA/CD25 contribute to the pathogenesis of different autoimmune disorders (Maier et al., 2009b). Likely, association of disease-associated markers at the IL2RA/CD25 region with serum levels of sIL-2RA could, at least partially, explain the contribution of this gene to autoimmunity.

Elevated concentrations of sIL-2RA have been detected in several autoimmune diseases including GD (Zwirska-Korczala et al., 2004; Jiskra et al., 2009) thereby suggesting for T-lymphocyte activation (Dedijca, 2001). Despite the lack of the transmembrane and cytoplasmic domains, sIL-2RA is able to bind IL-2 (Murakami, 2004). Indeed, elevated sIL-2RA could neutralize available IL-2, which is necessary for activation of CD4+CD25+ Tregs. On the other hand, increased production of sIL-2RA is associated with enhanced proliferation and expansion of responder CD4+ T cells (Maier et al., 2009a). Therefore, correlation between the carriage of disease-associated variants of IL2RA/CD25 and increased levels of sIL-2RA may be related to reduction in the inhibitory role of CD4+CD25+ Tregs and increase in the activity of responder CD4+ T-cells (including self-reactive clones of T-lymphocytes), and as a consequence, this imbalance will contribute to thyroid autoimmunity.

Since IL-2 inhibits its own production (Villarino et al., 2007), the level of sIL-2RA could influence this self-inhibitory feedback and therefore IL-2 production. There is a second putative mechanism by which increased sIL-2RA levels could promote thyroid autoimmunity. This finding could also at least partly explain reduced levels of IL-2 observed in sera of GD patients (Eisenstein et al., 1994; Ward and Fernandes, 2000).

3.6 FCRL3

FCRL3 (FC receptor-like-3, also known as CD307c) is a receptor containing immunoreceptor-tyrosine activation motifs and immunoreceptor-tyrosine inhibitory motifs in its cytoplasmic domain making it important in the regulation of the immune system. The FCRL3 molecule shares significant structural homology to classical receptors for immunoglobulin constant chains (Fc receptors) (Miller et al., 2002). RCRL3 is found mainly on B-cells but also on T-cells. Among B-cell subsets, this molecule is present on mature, germinal center, memory, plasma cells, and bone marrow immature B cells suggesting for its key role in the development, maturation, and function of B-lymphocytes (Matesanz-Isabel et al., 2011).

The first evidence for association of FCRL3 with GD has been obtained in Japanese (Kochi et al., 2005). The allele C of rs7528684 located at position –169 in the promoter of FCRL3 showed the strongest association with higher risk of GD (OR=2.15, P $=8.5x10^{-6}$). The disease-associated variant has been found to be functionally significant because it increased the affinity for the NFκB transcription factor and caused enhanced transcription activity of the FCRL3 promoter (Kochi et al., 2005). The association between different variants of FCRL3 and GD has been then replicated in the independent Japanese dataset (Kochi et al. 2005), Chinese population (Gu et al., 2010) as well as by several large-scale population studies in the UK Whites (Simmonds et al., 2006; 2010; WCCT et al., 2007b; Owen et al., 2007).

However, FCRL3 disease-associated variants in UK Caucasians were different from those found in Japanese. In Japanese, the susceptibility locus within the FCRL3 region has been mapped to the cluster of SNPs located in the 5' region of the gene. In contrast, in the UK datasets, implementation of the tag SNP approach and logistic regression that association of rs3761959 (that tagged rs7528684) with GD is secondary to rs11264798 and rs10489678 SNPs located in the LD block at the 3'region of FCRL3 (Owen et al., 2007). Further analysis revealed the primary contribution of the allele C of rs10489678 to GD susceptibility in the predisposing extendend haplotype of FCRL3, and this effect is independent on the impact of the SNP cluster at the neighboring FCRL5 gene (Simmonds et al., 2010a). 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) remains to be found.

Furthermore, the FCRL3 gene has been reported to contribute to several autoimmune diseases including GD, SLE, and RA (reviewed by Chistiakov and Chistiakov, 2007; Kochi et al., 2010). Again, compared to the Asian populations, other variants of FCRL3 are implicated in autoimmunity in Caucasians, since the marker rs7528684 associated with autoimmunity in Japanese repeatedly failed to show significant association with various autoimmune outcomes in Caucasian populations (Chistiakov and Chistiakov, 2007; Davis, 2007; Mao et al., 2010).

The pathogenic activation of FCRL3 expression is suggested to lead to the down-regulation of BCR-mediated signaling, incomplete induction of anergy and deletion in autoreactive B-cells, and, finally, to breakdown of B-cell tolerance (Kochi et al., 2009). Recently, a high expression of FCRL3 has been found on 40% of natural CD4+CD25+ $CD127^{low}$ Tregs that have a memory phenotype and decreased response to IL-2 stimulation (Nagata et al., 2010). These cells also had a reduced capacity to suppress the proliferation of effector T-cells (Swainson et al., 2010). Thus, FCRL3 could contribute to the loss of self-tolerance and inducing autoimmunity at least through two pathogenic mechanisms: by excessive inhibiting BCR signaling and the impairment of suppressing function of Tregs. Predisposing variants

FCRL3 and CD40 could cooperate in the breakage of B-cell tolerance since stimulation of CD40 was shown to result in the up-regulation of FCRL3 expression through the TRAF6-NF-κB1-mediated signaling pathway (Kochi et al., 2005).

3.7 SCGB3A2/UGRP1

The secretoglobin 3A2 (SCGB3A2) gene encoding secretory uteroglobin-related protein 1 (UGRP1) resides on chromosome 5q12-q33, a region that showed linkage with GD in two Asian populations (Sakai et al., 2001; Jin et al., 2003). Initially, studies of positional candidate genes located in the susceptibility locus on chromosome 5q12-q33 including SCGB3A2 failed to show association with GD in Chinese likely due to the small size of a population studied (Yang et al., 2005). However, using the extended dataset (over 2800 affected Chinese patients), Song et al. (2009) found association between two polymorphisms (-112G/A (rs1368408) and -623 ~ -622 AG/T) both located in the promoter region of SCGB3A2 (OR=1.28 and 1.32, respectively) with GD. Furthermore, these SCGB3A2 variants constituted two higher risk haplotypes associated with reduced SCGB3A2 gene expression levels in human thyroid tissue due to the lower transcriptional activity of disease-associated variants (Song et al., 2009). Association between rs1368408 and GD has been recently replicated in two Caucasian large cohorts including UK Whites (OR=1.18, P=0.007; Simmonds et al., 2010b) and Russians (OR=1.33, $P=2.9\times10^{-5}$; Chistiakov et al., 2011).

The higher risk allele A of the −112G/A variant of SCGB3A2 may potentially disrupt the binding site for CCAAT/enhancer-binding protein alpha (C/EBPα), which positively regulates transcription of SCGB3A2 (Tomita et al., 2008; Song et al., 2009). Consequently, compared to the allele -112G, the SCGB3A2 −112A variant displays a 24% decrease in the promoter activity (Niimi et al., 2002) that results in lower levels of SCGB3A2 mRNA in the thyroid issue and decreased concentrations of UGRP1 in sera of healthy subjects and individuals affected with GD (Chistiakov et al; 2011) and asthma (Inoue et al., 2008).

At present, it is unclear how SCGB3A2 variants predispose to GD. In humans, this protein is predominantly expressed in the lung although a low level expression was also found in thyroid and kidney (Niimi et al., 2002; Song et al., 2009). In lungs, UGRP1 is a ligand for macrophage scavenger receptor with collagenous structure (MARCO), an important member of the innate immune system of the lung where it binds inhaled particles including microbial pathogens and facilitates their clearance by the macrophage system (Areschoug and Gordon, 2009). Both MARCO and UGRP1 have been shown to play a key role in pulmonary inflammation including bronchial asthma and rhinosinusitis (Niimi et al., 2002; Thakur et al., 2009). Probably, the involvement of UGRP1 inGD may be a consequence of systemic effects originating from the respiratory system such as elevation in serum IgE, a hallmark of allergy. The correlation between the −112G/A polymorphism of SCGB3A2 and IgE concentrations in sera of healthy subjects have been observed (Chistiakov et al., 2011). A number of studies provide evidence that allergy-associated mechanisms can contribute to the pathogenesis of autoimmune diseases such as AITD (Tanda et al., 2009). However, further studies are needed to investigate a precise mechanism by which UGRP1 links allergic asthma and thyroid autoimmunity.

3.8 FOXP3

Additionally to CD25, the expression of the forkhead box P3 (FOXP3) is a molecular signature of natural Tregs. This gene acts as a key regulator of the development and

function of natural Tregs (Zhang and Zhao, 2007). Foxp3-deficient mice develop a fatal lymphoproliferative disorder (Brunkow et al., 2001). This gene resides in a region on chromosome Xp11.23 that has been shown to be linked with GD (Barbesino et al., 1998; Tomer et al., 1999). Therefore, the FOXP3 is an excellent positional and functional candidate gene for GD.

In US Caucasians, family-based analysis showed association of a microsatellite inside the FOXP3 gene with AITD in a subset of patients with juvenile GD (Ban et al., 2007; Tomer et al., 2007). No association between FOXP3 and AITD has been found in a population-based study in the UK (Owen et al., 2006) and Japanese cohorts (Ban et al., 2007). However, in a small independent Japanese dataset, an association of the -3279 C/A polymorphism (genotype AA) with GD in remission has been reported (Inoue et al., 2010). The marker 3279C/A is functional, with allele A related to the low translation of FOXP3. Defects in FOXP3 expression suppresses the regulatory function of Tregs and therefore should positively correlate with poor prognosis (relapse) of AITD (Mao et al., 2011). Thus, the obtained data on association between FOXP3 and GD are still inconsistent. Additional population studies and functional analyses are required to replicate findings on FOXP3 association with GD and emphysize a role of Tregs in thyroid autoimmunity.

3.9 TSHR

TSHR located on the surface of thyroid epithelial cells is a G_s-protein coupled receptor responding to thyrotropin (Akamizu et al., 1990). TSH is central to the regulation of thyroid gland. Since anti-TSHR antibodies circulating in the serum of affected subjects are the hallmark of GD, not surprisingly, that the TSHR became the first gene (after HLA) to be tested for association with GD. The TSHR resides on chromosome 14q31 and comprises 13 exons (Kakinuma and Nagayama, 2002). Initial studies have been focused on three germline non-synonymous SNPs in the TSHR: D36H and P52T, both located in the extracellular domain of the receptor, and D727E found in the intracellular portion of the molecule (Tonacchera and Pinchera, 2000). Despite the positive results of some studies (Cuddihi et al., 1995; Gustavsson et al., 1995; Chistiakov et al., 2002; 2004), subsequent case-control studies have largely rejected association with GD for either of these TSHR SNPs in Caucasians (de Roux et al., 1996; Kotsa et al., 1997; Allahabadia et al., 1998; Simanainen et al., 1999; Kaczur et al., 2000; Ban et al., 2002b).

Nevertheless, genome-wide linkage analysis subsequently suggested for a GD susceptibility locus in chromosomal region 14q31 (Tomer et al., 2003). This encouraged extension of the search for susceptibility loci to non-coding sequences within TSHR gene. In Japanese, polymorphic markers within intronic regions of TSHR consistently showed associations with GD including microsatellites (Akamizu et al., 2000) and haplotypes comprised of alleles of an SNP cluster in intron 7 (Hiratani et al., 2005).

In Caucasians, implementation of large population cohorts and a tagging SNP approach resulted in the identification of a higher risk haplotype (OR=1.7) spanning through two LD blocks and containing SNP rs2268458 (located in intron 1) as a marker that showed the strongest association with GD (OR=1.31) at the TSHR gene region (Dechairo et al., 2005). Further analysis of a panel of 98 SNPs (including rs2268458) encompassing a 800-kb genomic region with the TSHR gene, revealed two markers in intron 1 (rs179247 and rs12101255) with the strongest association with GD (OR=1.53 and 1.55, respectively) (Brand et al., 2009). Functional analyses showed association of both markers with reduced expression of the full-length TSHR mRNA relative to two truncated splice variants, which in

turn could lead to increase in shedding of a part of the TSHR receptor called the A-subunit (i.e., TSHR-A). The role of TSHR shedding in inducing thyroid autoimmunity is established (Chen et al., 2003; Chistiakov, 2003), and increase in TSHR-A levels should contribute to the pathogenesis of GD. Association of these two SNPs was recently confirmed in the extended dataset of Europeans, with the primary role of marker rs12101255 in conferring GD susceptibility (Ploski et al., 2010). An evidence supporting TSHR as a GD susceptibility gene was also produced in GWAS (WCCT, 2007b).

Disease-associated haplotypes found in intron 7 of TSHR in Japanese (Hiratani et al., 2005), are awaiting for replication in the independent cohort. Some data supporting association of intron 1 SNPs have been obtained in Asian populations including marker rs2268474 in Japanese (Hiratani et al., 2005) and rs2239610 in an ethnically mixed Asian population from Singapore (Ho et al., 2003).

Yet undiscovered, an etiological variant in intron 1 of TSHR, which is in strong LD with rs12101255, is suspected to alter TSHR splicing. The major splice variant of the TSHR whose length is 1.3 Kb includes most of the extracellular domain of the TSHR (Graves et al., 1992). Other minor splice variants have been also discovered (Kakinuma, Nagayama, 2002).

3.10 TG

Tg, which is a major antigenic target for autoreactive antibodies in AITD, was considered as an excellent candidate gene for AITD. Early genome-wide linkage scans identified the region 8q24 harboring the Tg gene as a major AITD susceptibility locus (Tomer et al., 2003). These findings have been independently replicated in several ethnic groups including Caucasians (Tomer et al., 2002b; Collins et al., 2003) and Asians (Hsiao et al., 2007; 2008; Maierhaba et al., 2008). Further studies revealed three Tg non-synonymous amino acid substitutions (A734S, V1027M, and W1999R) associated with GD (Ban et al., 2003).

It was suggested that these Tg variants may be implicated in AITD susceptibility by altering Tg processing in endosomes causing production of the pathogenic Tg peptide repertoire. In support of this hypothesis, an evidence for gene-gene interaction between the predisposing variant HLA-DRb-Arg74 and W1999R polymorphism of Tg has been found resulting in a high OR of 6.1 for GD (Hodge et al., 2006). Subsequent immune-binding assays revealed only a small group of unique Tg peptides capable to bind to the HLA-DRb-Arg74 pockets (Jacobson et al., 2009). Specific binding of the peptide Tg.2098 to the HLA-DRβ1-Arg74 allele was able to stimulate T-cells from mice and humans with autoimmune thyroiditis therefore suggesting that this peptide is a major T-cell epitope (Menconi et al., 2010).

4. CONCLUSIONS

Genes whose variants are involved in the pathogenesis of GD could be functionally devided into several groups. Since GD is a thyroid autoimmune pathology, the contribution of two thyroid-specific genes such as Tg and TSHR to its etiology perfectly explains the organ specificity of this disease. The HLA, CTLA-4, and PTPN22 genes encode the members of the immunological synapse itself between an APC (presenting thyroid-specific antigens) and self-reactive T-helper cell and a component of a complex of signaling kinases/phosphatases primarily segregated with the TCR molecule (Fig. 2).

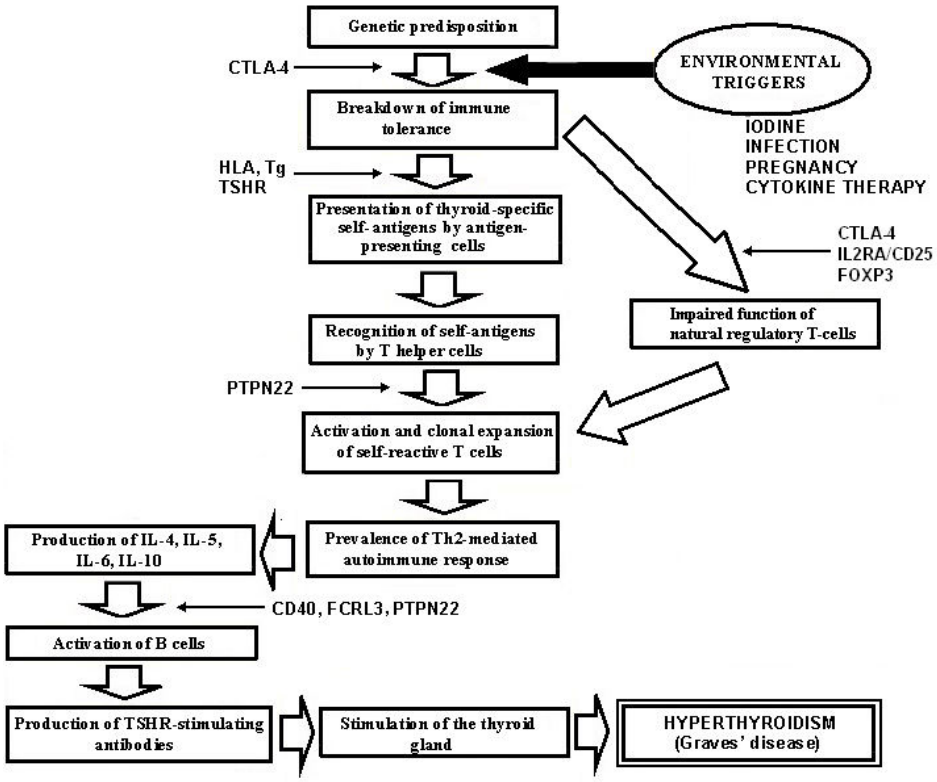

Figure 2: Putative genetic contribution at different stages of the development of thyroid-specific autoimmunity

Th2 autoimmune pathway mediated by self-reactive T helper cells leads to clinical hyperthyroidism, e.g. to Graves' disease (GD). When the clinical presentation of autoimmune thyroid disease is switched toward GD, the alternative way, leading to autoimmune hypothyroidism (Hashimoto's thyroiditis), is suppressed through antiapoptotic mechanisms by activated T cells, cytokines and thyroid-stimulating antibodies, promoting thyrocyte survival. Predisposing variants of the susceptibility genes could contribute on different stages of the pathogenesis of GD. Putative sites of their implication in the pathogenic mechanism of GD are marked by narrow arrows. Abbreviations: CD40: surface antigen CD40 (immune costimulator); CTLA-4: cytotoxic T lymphocyte associated protein-4; FCRL3: Fc receptor-like 3; FOXP3: forkhead box P3; HLA: Human Leucocyte Antigens; IL: interleukin, IL2RA: interleukin-2 receptor, alpha-subunit; PTPN22: lymphoid protein thyrosine phosphatase, member 22; Tg: thyroglobulin; TSHR: thyroid-stimulating hormone receptor.

Susceptibility variants of both CD40 and FCRL3 are involved in functional support of antibody-produced autoreactive B-cell clones. CD25 and FOXP3 are central in the development and functioning Tregs whose regulatory activity is impaired and/or reduced in GD. The position and functional significance of SCGB3A2/UGRP1 is this mosaic is waiting for its explanation. Despite this, functional groups of known GD susceptibility genes thoroughly capture all major players of an autoimmune process.

In the future, new susceptibility loci with less genetic effects on GD susceptibility are likely to be discovered. Genes detected in association studies as giving a low relative risk (risk ratios < 3–5) such as PTPN22 and CTLA-4 in AITD may contribute no more than 5% each to overall genetic susceptibility (Risch and Merikangas, 1996).Hence, 10–20 genes may be influencing the expression of AITD (Davies, 1998). Among the known GD-predisposing

variants, only the HLA-DR3 exhibits a very strong impact (OR > 5) in the genetics of GD while the individual contribution of each non-HLA locus to the risk of GD is significantly weaker and typically does not exceed OR of 2.0 (Pearce and Merriman, 2009). The genetic background has a very substantial influence on the etiology of GD accounting for 70-80% of the disease risk (Brix et al., 2001). The inheritance of multiple genes with small additive effects cannot explain the high prevalence of AITD in the general population. Therefore, co-inherited susceptibility variants should synergistically interact to each other resulting in a combined OR that is significantly higher than the one expected with an additive effect alone. Such an example of a synergism in gene-gene interaction was observed between the Tg gene and DRbeta1-Arg74 in GD susceptibility (Hodge et al., 2006). Another putative mechanism is genetic heterogeneity that increases the genetic effect of a particular susceptibility variant in a subset of GD subjects studied while in the whole population of GD patients this effect is diluted resulting in much smaller ORs.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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