Genetic Susceptibility to Autoimmune Thyroid Disease: Past, Present, and Future

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Background: Autoimmune thyroid diseases (AITD), including Graves' disease and Hashimoto's thyroiditis, arise due to complex interactions between environmental and genetic factors. There are sound data coming from epidemiological, family, and twin studies demonstrating a strong genetic influence on the development of AITD. In this review we summarize the new findings on the genetic susceptibility to AITD focusing on emerging mechanisms of susceptibility.

Summary: Candidate gene analysis, whole-genome linkage screening, genome-wide association studies, and whole-genome sequencing are the major technologies that have advanced this field, leading to the identification of at least seven genes whose variants have been associated with AITD. One of the major ones is the *HLA-DR* gene locus. Recently, it was shown that substitution of the neutral amino acids Ala or Gln with arginine at position beta 74 in the *HLA-DR* peptide-binding pocket is key to the etiology of both Graves' disease and Hashimoto's thyroiditis. Several other genes have also been shown to confer susceptibility to AITD. These can be classified into two groups: (i) immune regulatory genes (cytotoxic T lymphocyte-associated protein 4, *CD40*, protein tyrosine phosphatase-22, and *CD25*) and (ii) thyroid-specific genes (thyroglobulin and thyrotropin receptor genes). The influence of individual genes on the development of AITD when assessed in a population appears to be weaker than would be expected from the data showing strong genetic susceptibility to AITD. Two possible mechanisms explaining this discrepancy are gene–gene interactions and subset effects.

Conclusions: Significant progress has been made in our understanding of the immunogenetic mechanisms leading to thyroid autoimmunity. For the first time we are beginning to unravel these mechanisms at the molecular level. It is hoped that these new data will be translated into novel therapies and prevention strategies in AITD, such as costimulatory blockade.

Introduction

AUTOIMMUNE DISEASES (AITD) AFFECT many endocrine organs such as the thyroid, pancreatic islet beta cells, adrenal, and pituitary. However, by far the commonest autoimmune endocrine diseases are AITD (1). In fact, according to one series AITD are the commonest autoimmune diseases in the United States (2). Intriguingly, the autoimmune attack on the thyroid results in two opposing clinical syndromes, Hashimoto's thyroiditis (HT) and Graves' disease (GD). In HT the lymphocytic infiltration of the thyroid gland leads to apoptosis of thyroid cells and hypothyroidism (3). In contrast, in GD the lymphocytic infiltration of the thyroid leads to activation of thyrotropin receptor (TSHR)–reactive B-cells that secrete TSHR-stimulating antibodies causing hyperthyroidism (4). The etiology of HT and GD involves common path-

ways in which thyroid reactive T-cells escape tolerance and infiltrate the thyroid, and unique pathways in which these thyroid-reactive T-cells either cause thyroid cell death (in HT) or stimulation (in GD). Thus, it is not surprising that the genetic susceptibility to HT and GD involves shared genes, as well as unique genes (1). This review will summarize the major recent advances in our understanding of the genetic susceptibility to AITD.

The Evolution of Genetic Studies in AITD

Advances in genetic methods in the past 15 years enabled significant progress in the identification of complex disease genes. In fact the field was driven forward by the unprecedented rapid advances in the methods available for gene mapping and identification in complex diseases. The quest to

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identify complex disease genes, such as AITD, evolved in four phases: Phase 1, candidate gene analyses; Phase 2, whole-genome linkage studies; Phase 3, genome-wide association studies (GWAS); Phase 4, whole-genome sequencing.

Phase 1: candidate gene analyses

Candidate genes are genes that, based on their biological function, are believed to play a role in the genetic susceptibility to complex diseases. For example, the insulin gene is a candidate gene for type 1 diabetes (T1D) since T1D is characterized by antiinsulin immune response. Likewise, the major thyroid autoantigenes, *TSHR*, thyroid peroxidase, and thyroglobulin (Tg), are excellent candidate genes for thyroid autoimmunity. The first candidate gene to be tested in AITD was the *HLA-DR* gene. Indeed, *HLA-DR3* was shown to be a major susceptibility gene for GD and HT. Additional AITD susceptibility genes identified by the candidate gene approach are the cytotoxic T-lymphocyte-associated protein 4 (*CTLA-4*) gene and the *TSHR* gene (see below).

Phase 2: whole-genome linkage screening

Whole-genome linkage screening is a powerful tool, as it enables scanning the entire human genome for a disease gene without any prior assumptions on disease pathogenesis. Whole-genome linkage screening is performed by testing a panel of markers that span the entire human genome for linkage with a disease in a dataset of families in which the disease aggregates.

Linkage analysis. The principle of linkage analysis is based on the premise that the likelihood of recombination between two genes is a direct function of the genetic distance between them. In fact, based on this assumption the distance between genes in linkage maps of the human genome is measured in recombination fraction units. Therefore, if two genes or polymorphisms are close together on a chromosome, they will cosegregate in families, since recombination between them will rarely occur. Similarly, if a polymorphic marker is close to a disease susceptibility gene, its alleles will cosegregate with the disease in families. The measure of the likelihood of linkage between a disease and a genetic marker is the logarithm of odds (LOD) score (5). 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 (6).

Polymorphic markers used in whole-genome linkage studies. The major genetic polymorphisms used in whole-genome linkage studies are microsatellites and single-nucleotide polymorphisms (SNPs). Microsatellites are dinucleotide repeats such as (AC)_n. They are highly polymorphic because there are many possible number of repeats. They are genotyped by PCR amplification and size separation. For screening the entire human genome for linkage, 400 microsatellites provide enough density. New technologies enabled automation and rapid screening of the entire human genome for linkage with sets of microsatellite markers. SNPs are single-base positions in genomic DNA where two alleles exist, such

as A/C. SNPs are very abundant and on the average there is a SNP every 300 bp. Since SNPs have two alleles, unlike microsatellites that have 5–10 alleles, more SNPs are required to screen the entire human genome for linkage with a disease, usually about 4000 SNPs are required.

Performing whole-genome linkage studies. To perform a whole-genome linkage study one needs to collect multiplex families (families in which more than one member is affected by the disease) and genotype all members for the panel of microsatellites or SNPs covering the entire human genome (7). If one or more markers in a certain locus show evidence for linkage with the disease, this locus may harbor a susceptibility gene for the disease studied. Linked regions can then be fine mapped and the genes identified (8). Many loci have been identified as linked with AITD in whole-genome linkage studies. In our own whole-genome linkage screens we have identified 7 AITD susceptibility loci on chromosomes: 2q, 6p, 8q, 10q, 12q, 14q, and 20q. Some of these loci have been 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.

Phase 3: GWAS

Association analysis. Association analysis is highly sensitive and may detect genes contributing $<5\%$ of the total genetic contribution to a disease. Association analyses are performed by comparing the frequency of the allele studied (e.g., *HLA-DR3*) in a dataset of patients and in ethnically matched control subjects. If the allele tested is associated with the disease, it will appear significantly more frequently in patients than in control subjects. The probability of having the disease in an individual positive for the allele compared with an individual negative for the allele is estimated by the relative risk (9). There are at least two possible explanations for the existence of an association between an allele and a disease: (i) the associated allele itself is the genetic variant causing an increased risk for the disease, and (ii) the associated allele itself is not causing the disease but rather a gene in linkage disequilibrium (LD) with it (10).

Performing a GWAS. Up until recently, whole-genome scanning was possible only using linkage because the linkage intervals needed between markers are 10–20 Mb, whereas for genome-wide screening by association analysis one would need to employ $\sim 500,000$ markers at much shorter distances ($\sim <50$ kb). The completion of the HapMap project (11) has made whole-genome scanning by association studies feasible (12). The HapMap project genotyped more than 1 million SNPs spanning the entire human genome in four ethnically distinct human populations and tested these SNPs for LD (11). The HapMap analysis demonstrated that the human genome is highly organized into discrete LD blocks that are flanked by recombination hot spots, or areas at which recombinations are much more likely to occur. Recombinations are much rarer at the LD blocks with all the markers in each block in tight LD. 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 enabled the typing of up to 500,000 SNPs in a single experiment. Thus, today it is possible to scan the entire human genome using densely spaced SNPs.

So far, no full GWAS have been reported in AITD. However, one limited nonsynonymous SNP GWAS was reported in GD (13). Nonsynonymous SNPs are SNPs that cause amino acid changes in the protein coded for by genes. In this study 14,500 nonsynonymous SNPs were genotyped in 900 AITD patients and 1466 control subjects. The study confirmed the *TSHR* gene as a susceptibility gene for GD and identified the *FCRL3* as an additional putative susceptibility gene for GD (13).

Phase 4: whole-genome sequencing

Very recently, the field of complex disease genetics was revolutionized again by the development of rapid and affordable platforms for sequencing the entire human genome. While the price is still high, it is expected that sequencing the entire human genome will cost around \$1000 per sample within the next few years making whole-genome sequencing a feasible approach to identify complex disease genes. Whole-genome sequencing has already been utilized successfully in two patients, one with Carcot-Marie-Tooth disease (14) and the other with acute myeloid leukemia (15). 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. However, once the sequence changes responsible for susceptibility to various complex diseases are mapped, it will be possible to offer to individuals a genetic risk assessment whereby a whole-genome sequence of the individual will provide a panel of genetic risks for the individual prompting prophylactic and life style interventions. Moreover, the availability of whole-genome sequencing will lead to better predictions of responses to medications, including idiosyncratic responses such as agranulocytosis from antithyroid medications. This will truly lead to a personalized approach to the treatment of complex diseases such as AITD.

AITD Susceptibility Genes Identified

Several recent reviews summarized thoroughly the data on susceptibility genes for thyroid autoimmunity (1,16–18). Therefore, here we will give only a brief overview of the major susceptibility genes for AITD.

Immunological synapse genes

The immunological synapse is the interface between antigen-presenting cells (APCs) and T-cells that is formed during T-cell activation. The immunological synapse is a complex interface involving peptide antigen bound to an HLA class II molecule and to the T-cell receptor, costimulatory molecules, and receptors on the APC and T-cells, integrins, and other molecules (19). Interestingly, several of the AITD susceptibility genes participate in the immunological synapse, suggesting that abnormalities in antigen presentation are important mechanisms leading to AITD.

The *HLA-DR* gene. The major histocompatibility complex region, encoding the HLA glycoproteins, is a highly

polymorphic genetic region that includes many genes and is located on chromosome 6p21 (16). Initial studies analyzed the major HLA class II alleles in AITD (16). These studies demonstrated a significant association of GD with *HLA-DR3* (20,21). However, data on HLA alleles in HT have been less definitive than in GD, with several *HLA-DR* alleles reported to be associated (22–26).

Recent studies shifted the focus from the association of the major *HLA-DR* allele groupings with AITD to the molecular structure of the peptide binding pocket and its association with disease (27,28). This mechanistic-based approach proved very fruitful. Since T-cells recognize and respond to peptide antigens when presented by APCs bound to HLA class II pockets, it was hypothesized that certain *HLA-DR* alleles may permit autoantigenic peptides to fit into the peptide binding pocket and to be presented more efficiently to T-cells (29). Indeed in several autoimmune diseases, most notable T1D, this hypothesis was confirmed (30). In T1D it was found that the amino acid residue at position 57 of the DQ β chain plays a key role in the genetic susceptibility to T1D (31,32). We have discovered that a similar mechanism is key to the etiology of AITD. We recently identified arginine at position 74 of the *HLA-DR* β 1 chain (DR β -Arg74) as the critical DR amino acid conferring susceptibility to GD (27). In contrast, the presence of glutamine at position 74 of the DR β 1 chain was protective. These data were replicated in an independent dataset (33). Similarly, we have identified a pocket *HLA-DR* amino acid signature that conferred strong risk for HT (28). As in GD, the key pocket amino acid was also DR β -Arg74. Structural analysis demonstrated that this pocket amino acid signature resulted in a unique pocket structure that is likely to influence pathogenic peptide binding and presentation to T-cells. Further studies enabled us to identify those Tg peptides that could be presented by *HLA-DR* pockets containing arginine at position beta 74 (34). Thus the peptide binding pocket structure plays a major role in the etiology of AITD, as has been shown in T1D (35).

CTLA-4. The *CTLA-4* gene is a major negative regulator of T-cell activation (36). 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.

The first demonstration of an association between *CTLA-4* and GD was reported by DeGroot and colleagues (37). In fact, their study showing a significant association between a 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. By now *CTLA-4* is established as an autoimmunity gene shown to be linked and/or associated with various autoimmune conditions. These include both GD and HT (38–43), as well as the production of thyroid antibodies (TAb) alone without clinical disease (44–46). The association has been consistent across ethnic and geographic groups (37,40,47–52).

Recent analysis by our group showed that the involvement of *CTLA-4* in the genetic susceptibility to AITD is more complex than originally thought. While *CTLA-4* alone predisposes to the development of TAb, *CTLA-4* may play a role in the susceptibility to high levels TAb and clinical AITD when interacting with other loci (53). Moreover, both the G allele

(previously reported to be associated with AITD) and the A allele (reported to be protective) of the A/G49 SNP of *CTLA-4* may predispose to AITD when interacting with different loci (53).

It is still not known which *CTLA-4* variant is the causative variant and by what mechanism it confers susceptibility to autoimmunity. Three main *CTLA-4* variants have been studied: an AT-repeat microsatellite at the 3'UTR of the *CTLA-4* gene (37,40); an A/G SNP at position 49 in the signal peptide resulting in an alanine/threonine substitution (A/G49) (39,51,54–56); and an A/G SNP located downstream and outside of the 3'UTR of the *CTLA-4* gene (designated CT60) (42). To identify the causative variant, functional studies are needed as all associated variants are in tight LD. Mechanistically, a polymorphism that reduced *CTLA-4* expression/function would be expected to augment T-cell activation leading to autoimmunity. Indeed, Kouki *et al.* (57) have shown an association between the G allele of the A/G49 SNP and reduced inhibition of T-cell proliferation, results that were later replicated by us (43). However, this association could be due to a direct effect of the A/G49 SNP on *CTLA-4* expression/function, or due to the effects of another variant in LD with the A/G49 SNP. Further studies did not support a role for the A/G49 SNP in reducing expression and function of *CTLA-4*. They suggested that another variant in LD with A/G49 is causative (58). At this point there are data to support either the CT60 (42) or the 3'UTR (AT)_n (59,60) as the causative variants, and further studies are needed to determine which one is causative.

CD40. *CD40* is expressed primarily on B-cells and other APCs (61), and plays a fundamental role in B-cell activation and antibody secretion (62,63). Our whole-genome linkage study identified a locus on chromosome 20q that was linked with GD (7). Fine mapping identified the *CD40* gene as the GD susceptibility gene in this locus, and further sequencing studies of the *CD40* gene demonstrated that a C/T SNP in the Kozak sequence of *CD40* was the likely causative variant (64–70). The CC genotype of this SNP was associated with GD (65,67–70). One study did not find the association, possibly due to ethnic differences among populations (71). However, a metaanalysis confirmed the association (69). Intriguingly, we have recently shown that the association of the CC genotype was stronger in a subset of GD patients who had persistently high levels of TAb (72). Mechanistically, the CC genotype, being located in the Kozak sequence of *CD40*, can alter *CD40* translation and expression. Indeed, we and others have shown that the C-allele of the polymorphism increased the translation of *CD40* mRNA transcripts by 20%–30% compared to the T-allele (73,74). Since *CD40* is expressed on B-cells (61) and on thyroid follicular cells (72,75,76), both involved in the development of GD, it is possible that increased *CD40* expression on B-cells and/or thyrocytes driven by the C-allele predisposes to disease (73). Thus, increased expression of *CD40* on B-cells can result in enhanced production of anti-TSHR-stimulating antibodies, whereas increased expression of *CD40* on thyrocytes can trigger an autoimmune response to the thyroid by resident T-cells. These mechanisms are not mutually exclusive and could both be operating.

Since *CD40* is a major APC and B-cell costimulatory molecule, it seems plausible that *CD40* will play a role in the genetic susceptibility to other autoimmune diseases. Indeed,

recent data have shown that *CD40* was associated and/or linked with high IgE levels in asthma (74), rheumatoid arthritis (77,78), systemic lupus erythematosus (79), and multiple sclerosis (80).

The protein tyrosine phosphatase-22 gene. The lymphoid tyrosine phosphatase, encoded by the protein tyrosine phosphatase-22 (*PTPN22*) gene, is a negative regulator of T-cell activation (81). The *PTPN22* gene was found to be associated with AITD, including both GD (82) and HT (83), as well as with other autoimmune diseases (84–87). The causative SNP is a tryptophan/arginine variant at position 620. The disease associated variant is a gain-of-function variant and therefore, the mechanism by which it predisposes to autoimmunity is not trivial as it would be expected to suppress T-cell activation (88). It is possible that the decreased activation of T-cells enables self-reactive T-cells to escape the thymic central tolerance mechanisms, but this theoretical possibility awaits experimental confirmation.

Regulatory T-cells genes

Regulatory T-cells (Treg) are an important subset of T-cells that regulate T-cell activation (89). They play a major role in peripheral tolerance to self-antigens. Indeed, upregulation of Treg cells suppressed experimental autoimmune thyroiditis in mice (90). Moreover, depletion of Treg in mice increased their susceptibility to experimental GD (91). In another recent study, McLachlan *et al.* have demonstrated that Treg depletion in mice induced with experimental GD caused a switch in the mice disease to a Hashimoto's-like disease (92). Several subtypes of Treg cells have been identified. One subtype, the natural Treg cells, are characterized by expressing constitutively *CD25*, *CTLA-4*, and glucocorticoid-induced tumor necrosis factor receptor. In addition their development is regulated by the *FOXP3* gene. Interestingly, both *FOXP3* and *CD25* have been found to be associated with AITD.

FOXP3. The *FOXP3* gene is the key gene to the differentiation of T-cells into natural Treg cells. Indeed *FOXP3* knockout mice develop a fatal lymphoproliferative disorder (93). Therefore, the *FOXP3* is an excellent candidate gene for AITD. Moreover, *FOXP3* is located in a region on chromosome Xp11.23 that has been shown to be linked with AITD. Therefore, we have tested the *FOXP3* gene in two cohorts of AITD patients: U.S. Caucasians and a Japanese cohort. Our results demonstrated an association of a microsatellite inside the *FOXP3* gene with AITD in the Caucasians but not in the Japanese, demonstrating ethnic differences in disease susceptibility (94). Further analysis demonstrated that the association of *FOXP3* with AITD was mostly in the subset of patients with juvenile GD (95).

CD25. Natural Treg cells are characterized by constitutive expression of high levels of the alpha chain of the IL-2 receptor (*CD25*) (89). Recent studies have found association between the *CD25* gene and T1D (96), as well as with GD (97). However, the mechanisms by which *CD25* can predispose to autoimmunity are unclear since the levels of expression of *CD25* on Treg cells do not correlate with their suppressive activity (98).

Thyroid-specific genes

Thyroglobulin. Tg is one of the main targets of the immune response in AITD (99). Therefore, it was an obvious candidate gene for AITD. Indeed, our whole-genome linkage study identified the Tg gene as a major AITD susceptibility gene (7). These findings were replicated by several other studies in several ethnic groups (100–105). Sequencing of the Tg gene identified three amino acid variants that were associated with AITD, A734S, V1027M, and W1999R (106). Mechanistically, it is possible that the Tg variants may predispose to disease by altering Tg degradation in endosomes resulting in a pathogenic Tg peptide repertoire. Supporting this mechanism are our data showing a genetic interaction between HLA-DR β -Arg74 and one of the Tg variant, W1999R, resulting in a high odds ratio (OR) for GD (107). Further, we have now shown that only a small group of unique Tg peptides can bind to the HLA-DR β -Arg74 pockets (34), and one of these peptides was shown to represent a major T-cell epitope (108).

TSHR gene. GD is defined by the presence of stimulating TSHR antibodies (4). Not surprisingly, the TSHR was the first gene (after HLA) to be tested for association with GD. Earlier studies that tested three nonsynonymous SNPs in the TSHR gene for association with GD, D36H, P52T, and D727E, gave mixed results (109–114). However, studies from Japan consistently reported associations of the TSHR with GD in the Japanese (48,52). Finally, more recently, it was found that noncoding intronic SNPs in the TSHR are associated with GD (115,116). The most consistent association in Caucasians has been with an intron 1 SNP (117,118). Mechanistically, intron 1 SNPs in the TSHR can alter its splicing. Indeed, several splice variants of the TSHR exist. The major splice variant of the TSHR, identified by us in 1992 (119), is a 1.3 Kb variant, which includes most of the extracellular domain of the TSHR. Other minor splice variants have also been discovered (120).

From Genes to Mechanisms*How many susceptibility genes are needed to develop AITD?*

One of the many unexpected findings of genetic studies in complex diseases was that most genes identified had a very minor effects (121). Indeed, with the exception of the DR β 1-Arg74 HLA variant, which gave an OR for GD of >5, all other AITD genes gave very low ORs of <1.5 (17). How can we reconcile the overwhelming data on the strong genetic susceptibility to AITD, contributing about 80% of the liability to disease (122), with the very weak effects of most genes found? The dogma is that for there to be a strong genetic effect on disease, an individual needs to inherit many genes with small effect. Some have estimated that >20 different susceptibility variants need to be inherited to develop complex diseases such as AITD. However, as illustrated in Figure 1, the dogma is clearly wrong as it cannot explain the high prevalence of AITD in the general population. To demonstrate this we simulated five genetic variants predisposing to AITD, each giving an OR of 1.2 individually and each having a population frequency of 0.2 (20%). Therefore, the combined OR if an individual inherited all five variants, assuming an additive effect is $1.2 \times 1.2 \times 1.2 \times 1.2 \times 1.2 = 2.5$. The frequency in the population of the combined

Gene	OR	Combined OR	Prevalence	Combined Prevalence
Gene A	1.2	1.2	20%	20%
		↓		↓
Gene B	1.2	1.4	20%	4%
		↓		↓
Gene C	1.2	1.7	20%	0.8%
		↓		↓
Gene D	1.2	2.1	20%	1.6 : 1,000
		↓		↓
Gene E	1.2	2.5	20%	3.2 : 10,000

FIG. 1. This figure illustrates the inherent paradox with the dogma that numerous genes with small effect can cause an additive strong genetic effect on susceptibility to disease. We simulated five genetic variants predisposing to autoimmune thyroid diseases, each giving an odds ratio (OR) of 1.2 individually. Therefore, the combined OR if an individual inherited all five variants, assuming an additive effect is $1.2 \times 1.2 \times 1.2 \times 1.2 \times 1.2 = 2.5$. However, the calculated frequency in the population of the combined genotype including all five susceptible variants (assuming no linkage disequilibrium exists between them), assuming the frequency of each variant alone is 0.2 (20%) would be $0.2 \times 0.2 \times 0.2 \times 0.2 \times 0.2 = 0.00032$ or 0.032%. Such a low frequency of the combined genotype is not consistent with the strong genetic influence on highly prevalent complex diseases, such as autoimmune thyroid diseases.

genotype including all five susceptible variants (assuming no LD exists between them, and assuming the frequency of each variant alone is 0.2 [20%]) is $0.2 \times 0.2 \times 0.2 \times 0.2 \times 0.2 = 0.00032$ or 0.032% (Fig. 1). This is an extremely low frequency of the combined genotype and cannot explain the high frequency of AITD in the general population (up to 5%) (2). Therefore, our group has proposed two alternative mechanisms for the finding of very low ORs for most AITD genes identified: subset effect and gene–gene interactions.

Gene–gene interaction. According to this model two genes with weak effects (i.e., associated with low ORs) interact, biologically resulting in a combined OR that is significantly higher than the one expected with an additive effect alone. For example, two genes with ORs for disease of 1.2 when inherited together would give an OR of 1.44 (1.2×1.2) if there was only an additive effect. However, if there is an interaction between these two genes, the OR for disease will be significantly higher than 1.44. Indeed, we have shown suggestive evidence for interaction between the Tg gene and DR β 1-Arg74 when conferring susceptibility for GD (107).

Subset effect (also called genetic heterogeneity). According to this model each of the genetic variants identified has a large effect resulting in a high OR in a subset of the AITD patients studied. However, when these variants are tested in the entire population of AITD patients, their effects are diluted, resulting in much smaller ORs. This model is illustrated in Figure 2. Indeed, we have shown that the CD40 gene was associated in all GD patients with an OR of 1.8, but when we divided the patients into the subset of those that had high

FIG. 2. The model of subset effects or genetic heterogeneity. To simulate the effects of subsets we simulated a dataset of Graves' patients in which four genetic subsets exist: Subset 1 was assumed to comprise 15% of the total Graves' disease (GD) population; subset 2, 35%; subset 3, 30%; and subset 4, 20%. Each subset is simulated to be influenced by one gene. We simulated the relative risks of the subset-specific genes as follows: Gene 1 was assumed to have a relative risk (RR) of 2.0 for GD in subset 1; gene 2 was assumed to have an RR of 2.5 in subset 2; gene 3 was assumed to have an RR of 3.5 in subset 3; gene 4 was assumed to have an RR of 3.0 in subset 4. Using the assumed percent of the population belonging to each

GD subset and the assumed relative risks contributed by each subset-specific gene, we calculated the relative risk contributed by the gene when testing the entire population of GD patients. The results showed that the relative risks decreased significantly when testing the gene in the entire dataset of GD patients; that is, the effect of the gene is diluted and the relative risk is reduced when the gene is tested in the entire GD population, most of which are not influenced by this gene.

antibody levels after treatment, the association became stronger and the OR was 2.5.

Putative mechanisms for the development of AITD

So far, seven confirmed AITD susceptibility genes have been identified. We still do not know the mechanisms by which they confer susceptibility to disease, but several putative mechanisms are conceivable.

Immunological synapse genes. The genes involved in the immunological synapse can predispose to disease by enabling presentation of self-antigens. It is likely that the DR β 1-Arg74, which is associated with GD and HT, predisposes to AITD by creating a peptide binding pocket that can more easily accommodate pathogenic Tg and/or TSHR peptides that can trigger T-cell response to thyroid antigens. Studies by DeGroot (123) and by us (34) support this hypothesis. The costimulatory molecules can enhance the activation of cells participating in the immunological synapse. The *CTLA-4* variants that are associated with autoimmunity are believed to reduce the inhibitory effect of *CTLA-4* on T-cell activation, resulting in uncontrolled activation of T-cells, which can predispose to autoimmunity in general (43,57,59). On the other hand, the *CD40* variant associated with disease increases its expression and function (73). Increased *CD40* expression on B-cells can enhance their capacity to produce antibodies. Alternatively, it is possible that thyroid-specific *CD40* expression can trigger thyroid autoimmunity by bystander mechanisms. The role of the *PTPN22* gene in autoimmunity is unclear since the variant associated with autoimmunity activates the gene that normally suppresses T-cell activation.

Treg genes. Since Tregs play a major role in peripheral tolerance, it seems likely that variants in *FOXP3* and/or *CD25* that can reduce Treg function could predispose individuals to autoimmunity. Indeed, a complete knockout of *FOXP3* results in a fatal lymphoproliferative disorder (93). Moreover, Tregs seem to play an important role in the mouse models of HT (90) and GD (92).

Thyroid-specific genes. Both the Tg and TSHR genes were found to be major susceptibility genes for AITD. It is conceivable that the variants in these genes associated with disease could influence the peptide profile generated by digestion of these proteins. Alternatively, the associated variants may influence Tg and TSH expression and splicing.

Conclusions

The AITD are complex diseases that are postulated to be caused by the combined effects of susceptibility genes and environmental triggers. Significant progress has been made in mapping the AITD susceptibility genes and understanding the mechanisms by which they confer risk for disease. The AITD susceptibility genes identified so far can be divided into three broad groups: (i) immunological synapse genes, (ii) Treg genes, and (iii) thyroid-specific genes. It is clear that additional genes contribute to the genetic susceptibility to AITD, as well as to the different phenotypes of AITD, disease severity, and, possibly, response to therapy. Ultimately, this interplay between susceptibility genes and environmental factors results in breakdown of self-tolerance leading to AITD. Understanding the mechanism of this interplay will be crucial for designing new, mechanism-based therapies for AITD.

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