Susceptibility genes in thyroid autoimmunity

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Abstract

The autoimmune thyroid diseases (AITD) are complex diseases which are caused by an interaction between susceptibility genes and environmental triggers. Genetic susceptibility in combination with external factors (e.g. dietary iodine) is believed to initiate the autoimmune response to thyroid antigens. Abundant epidemiological data, including family and twin studies, point to a strong genetic influence on the development of AITD. Various techniques have been employed to identify the genes contributing to the etiology of AITD, including candidate gene analysis and whole genome screening. These studies have enabled the identification of several loci (genetic regions) that are linked with AITD, and in some of these loci, putative AITD susceptibility genes have been identified. Some of these genes/loci are unique to Graves' disease (GD) and Hashimoto's thyroiditis (HT) and some are common to both the diseases, indicating that there is a shared genetic susceptibility to GD and HT. The putative GD and HT susceptibility genes include both immune modifying genes (e.g. HLA, CTLA-4) and thyroid specific genes (e.g. TSHR, Tg). Most likely, these loci interact and their interactions may influence disease phenotype and severity.

Keywords: *Gene, thyroid, Graves' disease, Hashimoto's thyroiditis, linkage, association*

Introduction

The autoimmune thyroid diseases (AITD) include a number of conditions which have in common cellular and humoral immune responses targeted at the thyroid gland. The AITD include Graves' disease (GD) and Hashimoto's thyroiditis (HT), both of which are characterized by infiltration of the thyroid by T and B cells reactive with thyroid antigens, production of thyroid autoantibodies, with the resultant clinical manifestations (hyperthyroidism in GD and hypothyroidism in HT) (reviewed in Weetman (1996) and Davies (2000)). There is abundant evidence for a major genetic influence on the development of AITD (reviewed in Tomer et al. (1997a) and Brix et al. (1998a)). Therefore, the current paradigm is that AITD are complex diseases in which susceptibility genes and environmental triggers act in concert to initiate the autoimmune response to the thyroid. In this review, we focus on the genes already found to contribute to AITD. While the proof that a gene causes AITD requires functional studies, the genes we will discuss are strong candidates, and their functions are now being

investigated. We will not discuss in detail the genetic regions linked with AITD in which no candidate gene has yet been identified.

Genetic epidemiology of AITD

The familial occurrence of AITD has been reported by investigators for many years. Early studies showing familial aggregation of AITD were mostly observational, based on careful family histories from patients (Bartels 1941, Martin 1945). Later, in the 1960s, Hall and Stanbury (1967) showed that 33% of siblings of patients with GD or HT developed AITD themselves. Additionally, they found that 56% of siblings of AITD patients had thyroid antibodies (TAb) (Hall and Stanbury 1967). A recent survey by our own group revealed that 41/114 (36%) of GD patients with ophthalmopathy reported a family history of AITD and 26/114 (23%) had a first degree relative with AITD (Villanueva et al. 2000).

The sibling risk ratio (λ_s), which is the ratio of the prevalence of the disease in siblings of affected individuals compared to the prevalence of the disease

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in the general population (Risch, 1990), serves as a good estimate of disease heritability, with $\lambda_s > 5$ considered significant. The λ_s in AITD has been estimated to be between 5.9 (Brix et al. 1998a) and >10 in AITD (Vyse and Todd 1996, Villanueva et al. 2000), supporting a strong genetic influence on the development of AITD.

Several large twin studies have been reported from Denmark showing a higher concordance of GD in monozygotic (MZ) twins when compared to dizygotic (DZ) twins (Brix et al. 1998b, Brix et al. 2001). A recent GD twin study from California confirmed the Danish twin study results (Ringold et al. 2002). Twin studies in HT have also shown a higher concordance rate in MZ compared to DZ twins (Brix et al. 2000). The concordance rates for TABs were also reported to be higher in MZ twins compared to DZ twins (Phillips et al. 2002). Thus, the twin data confirm the presence of a substantial inherited susceptibility to AITD.

Susceptibility genes in AITD

Immune related genes

The human leukocyte antigen (HLA) gene (Table I). The major histocompatibility complex (MHC) region, encoding the HLA glycoproteins, consists of a complex of genes located on chromosome 6p21 (Todd et al. 1988). GD was initially found to be associated with HLA-B8 in Caucasians (Bech et al. 1977, Farid et al. 1980). Subsequently, it was found that GD was more strongly associated with HLA-DR3, which is now known to be in linkage disequilibrium with HLA-B8 (reviewed in Farid (1981)). The frequency of DR3 in GD patients was

generally 40–55% and in the general population ~15–30% giving a RR for people with HLA-DR3 of up to 4.0 (Farid et al. 1979, Farid et al. 1980, Volpe 1990, Manglabruks et al. 1991). A recent family-based study from UK using the transmission disequilibrium test (TDT) confirmed the results of the case control studies (Heward et al. 1998a). Among Caucasians, HLA-DQA1*0501 was also shown to be associated with GD (RR = 3.8) (Yanagawa et al. 1993, Barlow et al. 1996, Marga et al. 2001), but recent studies have suggested that the primary susceptibility allele in GD is indeed HLA-DR3 (HLA-DRB1*03) (Zamani et al. 2000). However, the exact amino-acid sequence in the DR β 1 chain conferring susceptibility to GD is unknown. In other autoimmune diseases including Type 1 diabetes (T1D) (Todd et al. 1987), there is persuasive evidence that the disease is associated with specific amino-acid sequences of the DRB1 and DQ genes. We recently sequenced the HLA-DRB1 locus in a population of GD patients and controls (Ban et al. 2004a). Sequence analysis showed an increased frequency of arginine at position 74 of the HLA-DR β 1 chain (DR β -Arg-74) in GD patients compared to controls (Ban et al. 2004a). Moreover, subset analyses showed that DR β -Arg-74 was also significantly more frequent in the HLA-DR3 negative GD patients than in controls, suggesting that the association with DR β -Arg-74 is independent of the association with HLA-DR3. The pattern of transmission of HLA alleles from parents to offspring was also studied. A recent study suggested a preferential transmission of HLA susceptibility alleles from fathers to affected offspring, whereas maternal susceptibility alleles were not transmitted

Table I. Some HLA association studies in GD performed in Caucasians.

Country	No. of patients	HLA allele	Relative risk/p-value	Reference
Belgium	194	DRB1*0301	2.53	Zamani et al. (2000)
Canada	175	B8	3.1	Farid et al. (1980)
		DR3	5.7	
Denmark	86	B8	2.80	Bech et al. (1977)
		Dw3	3.94	
Germany	253	DR3	2.52	Schleusener et al. (1989)
Hungary	256	B8	3.48	Stenszky et al. (1985)
		DR3	4.8	
Sweden	78	B8	4.4	Dahlberg et al. (1981)
		DR3	3.9	
UK	127	B8	2.77	Kendall-Taylor et al. (1988)
		DR3	2.13	
UK	101	DR3	1.10	Weetman et al. (1988)
USA	65	DR3	3.38	Manglabruks et al. (1991)
USA	92	DRB1*03	2.6	Chen et al. (1999)
		DRB1*08	3.2	
UK	120	DQA1*0501	3.8	Barlow et al. (1996)
UK	228	DRB1*0304	2.7	Heward et al. (1998a)
		DQB1*0301	1.9	
		DQA1*0501	3.2	
USA	94	DQA1*0501	3.71	Yanagawa et al. (1993)

more frequently than expected (Segni et al. 2002). This may suggest parental imprinting in the transmission of HLA susceptibility alleles to affected offspring.

Data on HLA haplotypes in HT have been less definitive than in GD. Initial studies failed to demonstrate an association between goitrous HT and HLA A- B- or C- antigens (Irvine et al. 1978). Later studies showed an association of goitrous HT with HLA- DR5 (RR = 3.1) (Farid et al. 1981) and of atrophic HT with DR3 (RR = 5.1) (Moens et al. 1978). The association of HT with HLA-DR4 in Caucasians has been confirmed in subsequent studies (Tandon et al. 1991, Ban et al. 2002a), and further supported by studies of transgenic mice (Kong et al. 1996). An association between HT and HLA-DQw7 (DQB1*0301) has also been reported in Caucasians (Badenhoop et al. 1990, Wu et al. 1994).

Linkage studies of HLA in AITD have been largely negative (Bode et al. 1973, Roman et al. 1992, Barbesino et al. 1998). Only one recent study from UK showed weak evidence for linkage between GD and the HLA region (Vaidya et al. 1999a), and an additional study reported linkage only when conditioning on DR3 (Shields et al. 1994). It is difficult to explain why the HLA genes show consistent association with GD but no evidence for linkage. The most likely explanation is that HLA is a modulating gene for AITD but not a primary susceptibility gene.

The cytotoxic T lymphocyte antigen-4 (CTLA-4) immune regulatory cluster on chromosome 2q33 (Table II). Co-stimulatory molecules are critical to the activation of T cells by antigen presenting cells (APCs). APCs activate T cells by presenting to the T cell receptor an antigenic peptide bound to an HLA

class II protein on the cell surface. However, a second signal is also required for T cell activation and these co-stimulatory signals may be provided by the APCs themselves or other local cells (Reiser and Stadecker, 1996). The co-stimulatory signals are provided by a variety of proteins which are expressed on APCs (e.g. B7-1, B7-2, B7h, CD-40) and interact with receptors (CD28, CTLA-4, and CD-40L) on the surface of CD4+ T-lymphocytes during antigen presentation (Reiser and Stadecker, 1996). Whereas, the binding of B7 to CD28 on T cells co-stimulates T cell activation, the presence of CTLA-4, which has a higher affinity for B7, down regulates T-cell activation by competing for the binding of B7 to CD28. A new member of this family of co-stimulatory molecules, inducible co-stimulator (ICOS) was identified by Hutloff et al. (1999). Unlike the constitutively expressed CD28, ICOS is induced on the T-cell surface and does not upregulate the production of interleukin (IL)-2, but induces the synthesis of IL-4 (Coyle et al. 2000). Interestingly, CD28, CTLA-4 and ICOS form a gene cluster in a 300 kb region on chromosome 2q33. Thus, associations of autoimmune diseases with this region may represent the effects of any of these three genes alone or in combination due to linkage disequilibrium.

Recently, there have been several reports demonstrating an association between CTLA-4 gene polymorphisms and AITDs, both GD and HT (Yanagawa et al. 1995, Nistico et al. 1996, Donner et al. 1997a,b, Kotsa et al. 1997a, Marron et al. 1997, Yanagawa et al. 1997, Braun et al. 1998, Heward et al. 1998b, 1999a, Petrone et al. 2001, Tomer 2001, Kouki et al. 2002, Nithiyananthan et al. 2002).

Since CTLA-4 is a non specific co-stimulatory molecule, it is expected to confer susceptibility to AITD and autoimmunity in general, and not

Table II. Some CTLA-4 association studies in autoimmune thyroid diseases in Caucasians and non-Caucasian population.

CTLA-4 polymorphism	Country	Ethnic group	Dis.	No.	RR*/P value	Reference
CTLA-4(AT)	USA	Caucasians	GD	133	2.82	Yanagawa et al. (1995)
CTLA-4(AT)	UK	Caucasians	GD	112	2.1	Kotsa et al. (1997a)
			HT	44	2.2	
CTLA-4(AT)	Hong-Kong	Chinese	GD	94	$p = 0.037$	Nistico et al. (1996)
CTLA-4(AT)	Japan	Japanese	GD + HT	349	1.8	Volpe (1990)
Thr/Ala (A/G) ₄₉	Germany	Caucasians	GD	305	2.0	Donner et al. (1997a)
Thr/Ala (A/G) ₄₉	UK	Caucasians	GD	94	$p = 0.003$	Vaidya et al. (1999b)
Thr/Ala (A/G) ₄₉	UK	Caucasians	GD	379	1.6	Heward et al. (1999a)
Thr/Ala (A/G) ₄₉	UK	Caucasians	GD	484	$p < 0.0001$	Allahabadia et al. (2001)
Thr/Ala (A/G) ₄₉	USA	Caucasians	GD	85	1.6	Villanueva et al. (2000)
Thr/Ala (A/G) ₄₉	Germany	Caucasians	HT	73	$p < 0.04$	Donner et al. (1997b)
Thr/Ala (A/G) ₄₉	Italy	Caucasians	HT	126	NS*	Petrone et al. (2001)
Thr/Ala (A/G) ₄₉	UK	Caucasians	HT	158	1.57	Nithiyananthan et al. (2002)
Thr/Ala (A/G) ₄₉	Slovenia	Caucasians	TAB's	67	$p < 0.005$	Zaletel et al. (2002)
Thr/Ala (A/G) ₄₉	Japan	Japanese	GD	153	2.64	Yanagawa et al. (1997)
Thr/Ala (A/G) ₄₉	Korea	Korean	GD	97	1.6	Park et al. (2000)
			HT	110	NS	

*RR, relative risk; NS, not significant.

specifically to GD (Tomer 2001). Indeed, CTLA-4 was reported to be associated and linked with all forms of AITD (GD, HT, and TABs, see below), and with many autoimmune diseases such as Type 1 diabetes (T1D) (Nistico et al. 1996, Donner et al. 1997a, Marron et al. 1997, Ueda et al. 2003), Addison's disease (Vaidya et al. 2000), and myasthenia gravis (Huang et al. 1998).

Two studies have now shown that CTLA-4 confers susceptibility to the production of TABs. Our group has shown strong evidence for linkage between the CTLA-4 gene region and the production of TABs with a maximum LOD score (MLS) of 4.2 (Tomer et al. 2001). Recently, another report has described an association between the G allele of the CTLA-4 A/G₄₉ SNP and thyroid autoantibody diathesis (Zaletel et al. 2002). Since the development of TABs often represents the pre-clinical stage of AITD (Vanderpump et al. 1995), it is possible that CTLA-4 predisposes non-specifically to the development of thyroid autoimmunity. Additional genetic and/or environmental factors must be necessary for the development of the specific GD/HT phenotypes (Tomer 2001).

As mentioned, the region on chromosome 2q33 containing the CTLA-4 gene harbors in addition the CD28 and ICOS genes and it was unclear whether the CTLA-4 gene itself or another immune regulatory gene in the region was involved in the genetic susceptibility to AITD. Recently, we tested additional genes and markers in the 2q33 region, and the strongest association was with the CTLA-4 markers (Tomer et al. 2001). These results were in agreement with results obtained in T1DM (Marron et al. 2000, Wood et al. 2002). More recently, Ueda et al. (2003) also showed that CTLA-4 was indeed the AITD susceptibility gene in this region. They identified a new 3' untranslated region SNP that was strongly associated with AITD.

The CD40 gene. Two linkage studies, one by our group (Tomer et al. 1998) and one by Pearce et al. (1999) have shown evidence that a locus on 20q11 was linked with GD. This GD locus was not linked to HT, since analysis of the data for the HT families gave strongly negative LOD scores. Moreover, in families with GD- and HT-affected individuals, the locus was linked only with GD, demonstrating its high specificity for GD (Tomer et al. 1998, Wood et al. 2002). The CD40 gene, an important regulator of B cell function, is located within the linked region on chromosome 20q11 and, therefore, it was a likely positional candidate gene for GD. CD40 is a transmembrane glycoprotein that is expressed predominantly on B cells, and also on monocytes, dendritic cells, epithelial cells and other cells (reviewed in Durie et al. (1994)). It is a member of the tumor necrosis factor receptor

superfamily and it binds to a ligand (CD40L or CD154), which is expressed mainly on activated T cells. Binding of CD40L to CD40 induces B cells to proliferate and undergo immunoglobulin isotype switching (Banchereau et al. 1994). CD40 has been shown to play an important role in the regulation of humoral immunity, central and peripheral T-cell tolerance, and APC function (reviewed in Foy et al. (1996)). Moreover, *in vivo* blockade of CD40 has been shown to suppress the induction of experimental autoimmune thyroiditis (Carayanniotis et al. 1997). Therefore, we tested whether CD40 was the GD susceptibility gene on chromosome 20q11. Sequencing of the CD40 gene revealed a C/T SNP in the 5' untranslated region (5' UTR) of the gene. Analysis of the CD40 5' UTR SNP in 154 Caucasian GD patients and 118 Caucasian controls showed an association between the CC genotype and GD but with a low relative risk of 1.6 (Tomer et al. 2002a). TDT analysis also showed preferential transmission of the C allele of the CD40 5' UTR SNP to affected individuals (Tomer et al. 2002a). Other investigators who found evidence for linkage in this region have not found an association between this SNP and GD in their dataset (Pearce, personal communication) and it is possible that other polymorphisms in the CD40 gene, or another gene in linkage disequilibrium with CD40, is the GD susceptibility gene.

Other immune related genes. Other immune related genes tested for association with GD include the T cell receptor β chain (Demaine et al. 1987, Weetman et al. 1987, Manglabruks et al. 1991), the IgG heavy chain (IgH) gene (Roman et al. 1989, Fakhfakh et al. 1999), the IL-1 receptor antagonist gene (Blakemore et al. 1995, Cuddihy and Bahn 1996, Muhlberg et al. 1998, Heward et al. 1999b), tumor necrosis factor α (TNF α) gene (Barbesino et al. 1998), interferon γ gene (Siegmond et al. 1998), the transporters associated with antigen presentation (TAP) genes (Rau et al. 1997), and the IL-4 gene (Heward et al. 2001). However, none of these have produced replicable associations with GD. The vitamin D binding protein, which may have some immune modulatory functions, has also been reported to be associated with GD (Pani et al. 2002).

Thyroid associated genes

The thyroglobulin (Tg) gene

Two studies have found evidence for linkage between a locus on chromosome 8q24 and AITD. Our group has shown strong evidence for linkage at the Tg gene locus with an MLS of 3.5 between D8S514 and D8S284 (Tomer et al. 2002b). Recently, another study in Japanese sib-pairs identified a major AITD locus on 8q24 very close to the locus, which we identified

(Sakai et al. 2001). Since the Tg gene was located within this linked region we proceeded to analyze the Tg gene directly. We identified two new Tg microsatellites in intron 10 (designated Tgms1) and intron 27 (designated Tgms2). Linkage analysis using Tgms2 gave a 2-point LOD score of 2.1 and a multipoint LOD score of 2.9, confirming that it was the Tg gene linked with AITD (Tomer et al. 2002b).

We then used the same two Tg microsatellites to test whether the Tg gene was associated as well as linked with AITD. Using an unselected group of 190 Caucasian GD patients and 134 age- and sex-matched Caucasian controls we found only a weak association between Tgms2 and AITD ($p = 0.05$, $RR = 1.4$) (Tomer et al. 2002b). However, the association was more impressive when the probands from the linked families ($n = 32$) were used ($p = 0.004$, $RR = 2.3$). TDT analysis also showed an association of Tgms2 with AITD ($p = 0.02$, Table III), but with a different allele, suggesting that Tgms2 was in linkage disequilibrium with another polymorphism of the Tg gene. These results have been replicated recently in a UK dataset (Collins et al. 2003). As in our study, the UK study also showed a significant association between Tgms2 and AITD ($p < 0.001$). Moreover, the same Tgms2 allele that we found to be associated with AITD was found to be associated by Collins et al. (2003). Thus, the Tg gene was both linked and associated with AITD and, therefore, is an important AITD susceptibility gene. Recently, sequence changes in the Tg gene which were associated with AITD were identified (Ban et al. 2003). Case control association studies for 14 Tg SNPs in AITD patients and controls showed that one SNP cluster (in exons 10–12) and an exon 33 SNP were significantly associated with AITD (Ban et al. 2003). However, it remains possible that another gene on 8q24 in linkage disequilibrium with Tg was the AITD susceptibility gene responsible for the observed linkage and association at this locus.

Table III. Transmission disequilibrium test for markers D8S284, Tgms1 and Tgms2 in 102 AITD families.

Marker	Allele/ Haplotype	Transmitted	Untransmitted	<i>p</i> -value
D8S284	3	54	34	0.03
	9	6	16	0.03
	All others	111	121	NS*
Tgms2	3	48	34	NS
	4	14	4	0.02
	7	32	52	0.02
	All others	62	66	NS
D8S284/ Tgms1	3/3	32	12	0.002
	All others	101	121	NS

* NS, not significant; Tgms1, microsatellite marker located in intron 10 of the Tg gene; Tgms2, microsatellite marker located in intron 27 of the Tg gene.

The TSH receptor (TSHR) gene

The hallmark of GD is the production of the TSHR antibodies. Therefore, the TSHR gene was thought to be a likely candidate gene for GD. Three common germline SNPs of the TSHR have been described (Tonacchera and Pinchera 2000). Two of them are located in the extracellular domain of the TSHR: an aspartic acid to histidine substitution at position 36 (D36H), and a proline to threonine substitution at position 52 (P52T). The third SNP is a substitution of glutamic acid for aspartic acid (D727E) within the intracellular domain of the receptor. Most studies on the contribution of the TSHR gene to the genetic susceptibility to GD have focused on the two SNPs in the extracellular domain of the TSHR (Cuddihy et al. 1995, Kotsa et al. 1997b, Allahabadia et al. 1998, Simanainen et al. 1999, Chistyakov et al. 2000, Kaczur et al. 2000), because this domain is the major site for TSH and TSHR antibody binding. Amino acid changes in the extracellular domain of the TSHR could theoretically change the amino acid sequence of TSHR T-cell epitopes (Rapoport et al. 1998). Initial studies suggested that the P52T SNP was associated with GD in females (Cuddihy et al. 1995). However, other authors were unable to confirm the association between the P52T SNP and GD in Caucasians (Kotsa et al. 1997b, Allahabadia et al. 1998, Simanainen et al. 1999, Chistyakov et al. 2000, Kaczur et al. 2000). The D36H SNP has also been reported not to be associated with GD (Simanainen et al. 1999). Linkage studies in GD families using three microsatellite markers within introns 2 and 7 of the TSHR gene were also negative in Caucasians (De Roux et al. 1996, Tomer et al. 1999). Recently, the D727E SNP was reported to be associated with GD in a Caucasian Russian population (Chistyakov et al. 2002), but these results were not replicated in a subsequent study (Muhlberg et al. 2000). Recently, we also tested whether the D727E SNP was associated with GD, but there was no association between the D727E SNP and GD, and no effect of the D727E SNP on the GD phenotype or disease severity was seen (Ban et al. 2002b). In addition, the frequency of the G allele was not increased in patients with more severe forms of GD (i.e. ophthalmopathy and goiter) and in patients with early disease onset. Our own study and other negative TSHR studies have not excluded a weak association between GD and the TSHR gene since very large datasets may be needed to detect associations with low RRs. We, therefore, performed a meta-analysis combining our data with the data reported in the previous two negative TSHR studies. The results showed a weak association between the D727E SNP E allele and GD ($p = 0.03$, $RR = 1.6$) (Ban et al. 2002b). Therefore, at this time it remains possible that the TSHR is a minor susceptibility gene for GD.

The thyroid peroxidase (TPO) gene

The TPO gene was tested for linkage and association with AITD in two studies using a microsatellite inside the TPO gene. However, these studies showed no evidence of linkage and/or association of the TPO gene with AITD (Pirro et al. 1995, Tomer et al. 1997b). Therefore, the TPO gene is most likely not a major susceptibility gene for AITD.

The effect of ethnicity in the development of AITD*The HLA gene (Table IV)*

As previously mentioned, HLA-DR3 is associated with GD in Caucasians. The HLA genes were also shown to be associated with GD in non-Caucasians, albeit the associated alleles were different (Table IV). Studies in the Japanese population have shown associations of GD with HLA-B35 (Kawa et al. 1977, Inoue et al. 1992). However, other class I and II HLA alleles have also been reported to be increased in Japanese GD patients (Katsuren et al. 1994, Onuma et al. 1994, Ohtsuka and Nakamura 1998). In Chinese an increased frequency of HLA-Bw46 has been reported (Chan et al. 1978, Tan et al. 1988, Yeo et al. 1989, Chan et al. 1993, Cavan et al. 1994). It is interesting that in Asians the HLA associations are with class I genes while in Caucasians they are with class II genes. This may imply that other non-HLA genes in the region in linkage disequilibrium with class I genes are the susceptibility genes in Asians. In contrast, DR3 is believed to be the causative gene in Caucasians (see below). In African-Americans an increased frequency

of HLA DRB3*0202 has been reported (Table IV) (Chen et al. 2000a). Interestingly, one study of a mixed population in Brazil showed association with HLA-DR3 implying that this allele may confer susceptibility in other ethnic groups and not just Caucasians (Maciel et al. 2001). Alternatively, this Brazilian population may have been mostly of European ancestry.

Also, HLA association studies in HT have not been consistent in non-Caucasian ethnic groups, e.g. HLA-DRw53 in Japanese (Honda et al. 1989), and HLA-DR9 in Chinese (Hawkins et al. 1987) (Table IV). In addition, linkage studies of HLA in AITD have been consistently negative in non-Caucasians, including Chinese (Hawkins et al. 1985a) and Japanese (Sakai et al. 2001). The negative linkage studies imply that HLA are also minor AITD genes in non-Caucasians.

The CTLA-4 gene

The association between GD and the CTLA-4 3' UTR microsatellite and A/G₄₉ SNP has been consistent across populations of different ethnic backgrounds such as Japanese (Yanagawa et al. 1997, Akamizu et al. 2000), and Koreans (Park et al. 2000). As was reported in Caucasians (Heward et al. 1998b), the C/T₋₃₁₈ SNP of CTLA-4 has not been associated with GD in Chinese (Heward et al. 1998b). It has also been reported that the frequency of the G allele and the GG genotype of the CTLA-4 A/G₄₉ SNP was significantly higher in GD patients who did not go into remission after five years on anti-thyroid medications in Japanese (Kinjo et al. 2002). Similarly, CTLA-4 has been reported to be associated with HT in non-Caucasians including Japanese (Akamizu et al. 2000, Sale et al. 1997).

Table IV. Some HLA association studies in AITD performed in non-Caucasian populations.

Country	Ethnic group	Dis.	No. of Patients	HLA allele(s)	RR*/p-value	Reference
Hong Kong	Chinese	GD	132	Bw46	4.8	Hawkins et al. (1985b)
Hong Kong	Chinese	GD	97	B46	2.3	Cavan et al. (1994)
				DR9	2.2	
				DQB1*0303	3.2	
Singapore	Chinese	GD	35	B46	8.2	Chan et al. (1993)
Singapore	Chinese	GD	159	Bw46	4.2	Yeo et al. (1989)
Japan	Japanese	GD	33	Bw35	$p < 0.02$	Kawa et al. (1977)
Japan	Japanese	GD	106	B46	$p < 0.000$	Onuma et al. (1994)
Japan	Japanese	GD	76	A2	2.86	Dong et al. (1992)
				DPB1*0501	5.32	
Korea	Korean	GD	128	B13	3.8	Cho et al. (1987)
				DR5	4.4	
				DRw8	2.3	
India	Asian	GD	57	B8	4.1	Tandon et al. (1990)
	Indian			DQw2	5.4	
USA	African-American	GD	73	No association		Sridama et al. (1987)
USA	African-American	GD	49	DRB3*0202	3.6	Chen et al. (2000b)
South Africa	African	GD	103	DR1	3.5	Omar et al. (1990)
				DR3	2.4	
China	Chinese	HT	53	DRw9	$p < 0.05$	Hawkins et al. (1987)
Japan	Japanese	HT	99	DRw53	3.33	Honda et al. (1989)

* RR, relative risk.

Although no linkage study has been reported in non-Caucasians, the association of CTLA-4 across populations of different ethnic backgrounds shows that it is an important susceptibility gene for thyroid autoimmunity.

Other immune related genes

The IgH gene was found to be associated with GD in the Japanese (Nakao et al. 1980, Nagataki, 1986). However, these results have not been reproduced in Caucasians (Roman et al. 1989, Fakhfakh et al. 1999). This might imply that the IgH gene may contribute to the susceptibility to GD only in the Japanese if a founder effect exists. Alternatively, these could result from random event due to sampling small populations. Recently, the TNF α gene (Kamizono et al. 2000) and the vitamin D receptor, which may have some immune modulatory functions, has been reported to be associated with GD in Japanese (Ban et al. 2000). A C/T SNP in the promoter region of the CD40 gene has also been reported to be associated with GD in Koreans (Kim et al. 2003). These results need to be confirmed and it cannot be excluded that other genes in linkage disequilibrium with these genes are the susceptibility genes at these loci. Other immune related genes such as the interferon γ gene have not been tested yet in non-Caucasians, and warrant further studies.

The Tg gene

A Japanese whole genome screen in 123 Japanese sib-pair families identified two loci giving strong evidence for linkage [i.e. $MLS > 2.0$]. One of these loci is located on chromosome 8q24 and showed evidence for linkage with both AITD ($MLS = 2.31$) and HT ($MLS = 3.77$) (Sakai et al. 2001). This locus is identical to the one found to be linked in Caucasians (Tomer et al. 2002b) and contains the Tg gene. Since the Tg locus was linked with AITD both in Caucasians and in Japanese, this supports that it is a major gene. Indeed, Tgms2 was associated with HT in Japanese ($p = 0.04$) (Ban et al. 2004b). However, since the significance was borderline, this association could still be due to random chance event.

The TSHR gene

Associations between AITD and TSHR microsatellite markers have been reported in the Japanese (Sale et al. 1997, Akamizu et al. 2000). However, these results have not been reproduced in Caucasians (Kotsa et al. 1997b, Allahabadia et al. 1998, Simanainen et al. 1999, Chistyakov et al. 2000, Kaczur et al. 2000, Villanueva et al. 2000). These results suggest that TSHR gene may contribute to the susceptibility to GD only in Japanese especially if there is a founder effect. For example, NOD2 mutations in Crohn's

disease were shown only in Caucasians, and not in Japanese (Yamazaki et al. 2002).

Conclusion

The AITD are complex diseases believed to be caused by the combined effects of multiple susceptibility genes and environmental triggers. There are sufficient epidemiologic data to support an important genetic contribution to the development of AITD, and in the past few years several loci and genes have shown evidence for linkage and/or association with AITD. The genetic susceptibility to AITD seems to involve several genes with varying effects. With the completion of the human genome project and the establishment of large SNP databases the identification of additional AITD susceptibility genes will become more feasible.

The AITD loci identified so far show that some putative AITD susceptibility genes may be immune related genes which increase the susceptibility to autoimmunity in general (e.g. HLA, CTLA-4) while others may be specific to AITD (e.g. TSHR, Tg). The next step in investigating the role of these genes in the development of AITD is by functional studies and genotype-phenotype correlations. Preliminary functional studies have been performed for HLA (Sawai and DeGroot 2000) and CTLA-4 (Kouki et al. 2000, Xu et al. 2002). More functional studies are needed for these and other genes which have shown association with AITD.

It is most likely that the susceptibility genes for AITD interact and that their interactions may influence disease phenotype and severity (Tomer et al. 1999). The molecular basis for the interactions between susceptibility genes in complex diseases is unknown. These interactions could represent the cumulative effect of increased statistical risk, or alternatively, there may be molecular interactions between the susceptibility genes or their products which ultimately determine disease phenotype. We are slowly progressing towards identification of the AITD susceptibility genes and once they are identified, we will begin to understand the underlying molecular mechanisms by which they induce thyroid autoimmunity.

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