Common and Unique Susceptibility Loci in Graves and Hashimoto Diseases: Results of Whole-Genome Screening in a Data Set of 102 Multiplex Families

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The autoimmune thyroid diseases (AITDs), comprising Graves disease (GD) and Hashimoto thyroiditis (HT), develop as a result of a complex interaction between predisposing genes and environmental triggers. Previously, we identified six loci that showed evidence for linkage with AITD in a data set of 56 multiplex families. The goals of the present study were to replicate/reject the previously identified loci before fine mapping and sequencing the candidate genes in these regions. We performed a whole-genome linkage study in an expanded data set of 102 multiplex families with AITD (540 individuals), through use of 400 microsatellite markers. Seven loci showed evidence for linkage to AITD. Three loci, on chromosomes 6p, 8q, and 10q, showed evidence for linkage with both GD and HT (maximum multipoint heterogeneity LOD scores [HLOD] 2.0, 3.5, and 4.1, respectively). Three loci showed evidence for linkage with GD: on 7q (HLOD 2.3), 14q (HLOD 2.1), and 20q (LOD 3.3, in a subset of the families). One locus on 12q showed evidence of linkage with HT, giving an HLOD of 3.4. Comparison with the results obtained in the original data set showed that the 20q (GD-2) and 12q (HT-2) loci continued to show evidence for linkage in the expanded data set; the 6p and 14q loci were located within the same region as the previously identified 6p and 14q loci (AITD-1 and GD-1, respectively), but the Xq (GD-3) and 13q (HT-1) loci were not replicated in the expanded data set. These results demonstrated that multiple genes may predispose to GD and HT and that some may be common to both diseases and some are unique. The loci that continue to show evidence for linkage in the expanded data set represent serious candidate regions for gene identification.

Introduction

Two distinct but related autoimmune disorders affect the thyroid gland: Graves disease (GD [MIM #275000]) and Hashimoto thyroiditis (HT [MIM #140300]). In both diseases, thyroid-reactive T cells are formed and infiltrate the thyroid gland. In GD, the majority of the T cells undergo a Th2 differentiation and activate B cells to produce TSH receptor (TSHR) antibodies, which stimulate the thyroid and cause clinical hyperthyroidism (reviewed by Davies 2000). In contrast, HT is characterized by Th1 switching of the thyroid-infiltrating T cells, which induces apoptosis of thyroid follicular cells and clinical hypothyroidism (reviewed by Weetman 1996). Both disorders are common, with a prevalence in the

United States of ~1% (Jacobson et al. 1997; Hollowell et al. 2002).

The etiology of the autoimmune thyroid diseases (AITDs; i.e., GD and HT), and the mechanisms leading to the dichotomy of GD and HT are unknown. However, the AITDs are considered complex diseases, caused by an interaction between susceptibility genes (Tomer et al. 2002a) and nongenetic factors, such as infection (Tomer and Davies 1993). This paradigm is based on epidemiologic evidence demonstrating a genetic predisposition to AITD, including (1) clustering in families (Mather et al. 1980), giving a sibling risk ratio (λ_s) of >10 (Vyse and Todd 1996; Villanueva et al. 2000); (2) a high concordance rate in MZ twins when compared with DZ twins (Brix et al. 2000, 2001); (3) the presence of thyroid autoantibodies, which are markers of subclinical AITD, in as many as 50% of siblings of patients with AITD (Hall et al. 1972; Burek et al. 1982); and (4) consistent associations with HLA (Stenszky et al. 1985; Tomer et al. 1997).

In the past few years, several groups, including our own, have been trying to identify the AITD susceptibility genes by studies of candidate genes and by whole-genome screening. The candidate-gene studies have

identified HLA-DR3 (Farid et al. 1980; Zamani et al. 2000) and CTLA-4 (Yanagawa et al. 1995; Tomer et al. 2001) as putative AITD susceptibility genes, giving relative risks of 2–4. Only two whole-genome screens have been published for AITD, one in 56 multiplex white families (354 individuals) (Tomer et al. 1999) and another in 123 Japanese sib-pair families (236 individuals) (Sakai et al. 2001). In addition, several limited studies of chromosomal regions have also been reported. These studies identified several putative AITD loci (table 1). However, the previous studies have included modest data sets and need to be replicated and confirmed to identify regions likely to harbor AITD susceptibility genes. We have now almost doubled our white data set to include 102 multiplex families and have performed a genome scan on this expanded data set.

Subjects and Methods

Ascertainment of the Study Families

The project was approved by the Mount Sinai School of Medicine institutional review board. One hundred and two families (540 individuals) were analyzed in the current study (54 from North America, 29 from Italy, 10 from Israel, and 9 from the United Kingdom). All families enrolled in the study were multiplex for AITD (i.e., they had more than one affected individual) and/or multigenerational. Families were ascertained through a patient with AITD who confirmed having at least one other first-degree relative with AITD. Although as many relatives as possible were recruited from each family, the minimum requirement for participation in the study was a family consisting of four first-degree relatives (including the proband) from two generations. On the average, our families had 5.3 members.

Clinical assessment.—The AITDs include GD and HT. GD was diagnosed by (1) documented clinical and biochemical hyperthyroidism requiring treatment, (2) a diffuse goiter, (3) presence of TSHR antibodies, and/or (4) diffusely increased I-131 uptake in the thyroid gland. HT was diagnosed by (1) documented clinical and biochemical hypothyroidism requiring thyroid hormone replacement and (2) presence of autoantibodies to thyroid peroxidase (TPO). Antithyroglobulin and anti-TPO antibodies were measured by specific radioimmunoassay (Kronus). All family members without AITD, whether thyroid autoantibody–positive or thyroid autoantibody–negative, were defined for this study as "unaffected." For all subjects, phenotype was determined with the cli-

Table 1

Summary of Loci Reported to Show Evidence for Linkage with AITD

Chromosome and Locus	Phenotype	Type of Study	Type of Analysis[a]	Results	Reference
2:					
2q33 (CTLA-4)	Thyroid antibodies	Whole-genome screening	Ped-LOD	HLOD 4.2	Tomer et al. 2001
2q33 (CTLA-4)	GD	Candidate-locus analysis	ASP-NPL	NPL 3.4	Vaidya et al. 1999
5:					
5q31	AITD	Whole-genome screening	ASP-LOD	LOD 3.1	Sakai et al. 2001
6:					
6p21 (HLA)	GD	Candidate-locus analysis	ASP-NPL	NPL 1.95	Vaidya et al. 1999
6p11 (AITD-1)	AITD	Whole-genome screening	Ped-LOD	LOD 2.9	Tomer et al. 1999
6p22-6q14	HT	Whole-genome screening	One large pedigree	LOD 1.52, NPL 7.53	Alkhateeb et al. 2002
8:					
8q24 (thyroglobulin)	AITD	Whole-genome screening	ASP-LOD	LOD 2.3	Sakai et al. 2001
8q24 (thyroglobulin)	AITD	Candidate-locus analysis	Ped-LOD	HLOD 3.5	Tomer et al. 2002*c*
12:					
12q22 (HT-2)	HT	Whole-genome screening	Ped-LOD	HLOD 2.3	Tomer et al. 1999
13:					
13q32 (HT-1)	HT	Whole-genome screening	Ped-LOD	LOD 2.1	Tomer et al. 1999
14:					
14q31	GD	Whole-genome screening	Ped-LOD	LOD 2.1	Tomer et al. 1999
18:					
18q21 (IDDM-6)	GD	Candidate-locus analysis	ASP-NPL	NPL 3.1	Vaidya et al. 2000
20:					
20q11 (GD-2)	GD	Whole-genome screening	Ped-LOD	LOD 3.5	Tomer et al. 1999
20q11 (GD-2)	GD	Candidate-locus analysis	ASP-NPL	NPL 2.01	Pearce et al. 1999
X:					
Xq21 (GD-3)	GD	Whole-genome screening	Ped-LOD	LOD 2.5	Tomer et al. 1999
Xp11 (IDDMX)	GD	Candidate-locus analysis	ASP-NPL	NPL 2.01	Imrie et al. 2001

[a] Ped = multiplex multigenerational pedigrees; ASP = affected sib pairs.

nician blinded to the individual's genotype. Each participant was interviewed and examined and gave written informed consent before participating. All the pertinent clinical and laboratory data were recorded and stored in our database. At the time of interview, blood was collected for DNA purification, as well as for thyroid function tests and thyroid antibody testing.

PCR Amplification of Microsatellite Markers

DNA was extracted from whole blood through use of the Puregene kit (Gentra Systems). For the whole-genome screening, we used the Perkin Elmer microsatellite panels (version 2.0, a total of 400 markers). Fluorescently labeled primers were purchased from Applied Biosystems, and microsatellite markers were typed as reported elsewhere (Tomer et al. 1999). In brief, PCRs were performed in 15-μl reaction volumes containing 50 ng of genomic DNA; 5 pmol of each primer (one of which was fluorescently labeled); PCR buffer containing 50 mM KCl, 10 mM Tris HCl (pH 8.3), 1.5 mM MgCl$_2$, and 200 μM each of dATP, dGTP, dTTP, and dCTP; and 1 U of Ampli*Taq* DNA polymerase (Perkin Elmer, Applied Biosystems). Reaction mixtures were heated to 94°C for 7 min and then cycled 30 times as follows: 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C. The PCR products were diluted 1:20 in ddH$_2$O and pooled. Two microliters of the pooled products were mixed with 0.5 μl of the internal size standard and 10 μl of deionized formamide, denatured, and separated using an ABI 310 genetic analyzer (Applied Biosystems). Allele calling was performed using Genotyper 2.0 software. The marker data were then automatically exported to our database (Ingres Database), where they were integrated with the already existing phenotype information and prepared for linkage analysis.

Linkage Analysis

Linkage analysis was performed using maximum likelihood-based (LOD score) methods of linkage analysis.

Two-point linkage analysis.—Two-point LOD scores for the different markers studied were computed using LIPED software (Ott 1976), assuming both dominant and recessive models. Population data and our own previous analyses suggest that a penetrance of 30% is appropriate (Tomer et al. 1999; Brix et al. 2000), but the actual value of the assumed penetrance does not have a major effect on the final LOD score (Greenberg 1989). We considered a LOD score ⩾1.9 in our whole-genome screen as suggestive evidence for linkage (Lander and Kruglyak 1995) and a LOD score ⩾3.3 as evidence for significant linkage, after maximizing the LOD score with respect to dominant and recessive models (Hodge et al. 1997). If a LOD score was suggestive of linkage, we then tested at a higher penetrance (80%), since it was

possible that the penetrance of AITD in our families was higher because of our method of ascertainment (multiple affected family members) (table 2). For all loci except two (10q and 12q), this did not result in increased information. Retesting at 80% penetrance resulted in a significant increase in the LOD score only for the 10q and 12q loci (see the "Results" section). All linkage analyses were performed under the assumption of a population prevalence of 1% for both diseases (0.5% for GD and 0.5% for HT), which is based on the disease prevalence data in the literature (Tunbridge et al. 1977; Vanderpump et al. 1995; Hollowell et al. 2002). On the basis of the assumed disease prevalence, the gene frequency was adjusted according to the model used (dominant or recessive) and the penetrance used, under the assumption of Hardy-Weinberg equilibrium (table 2). The phenocopy rate was set at 0; however, previous studies have shown that the actual value of the assumed phenocopy rate has little effect on the maximum LOD score (Durner et al. 1996).

Comparison of the LOD scores obtained in the expanded data set with those obtained in the original data set.—We previously performed a whole-genome scan in 56 families ("the first data set") (Tomer et al. 1999), and the current linkage analysis was performed on an expanded data set that included an additional 46 families ("the second data set"). To examine what statistical information has been gained from the addition of the second data set, we computed the statistic $Z_{total} - Z_{orig}$ for all of the markers used in the genome screen. This statistic was computed by calculating the difference between the maximized maximum LOD score (Greenberg et al. 1998) obtained for the expanded data set and the same statistic for the first data set. It should be noted that the $Z_{total} - Z_{orig}$ statistic is not identical to the LOD score obtained for the second data set alone, since the maximum LOD scores for the expanded data set and the original data set may not occur under the same inheritance parameters or at exactly the same location.

Heterogeneity testing and multipoint linkage analysis.—It was possible that genetic heterogeneity existed in our data set and/or that the simple models used in the analyses (dominant and recessive) were an incomplete description of the complex inheritance of AITD.

Table 2

Models Used in Our Analyses

Mode of Inheritance	AITD Disease Frequency	Gene Frequency	Penetrance[a]	Phenocopy Rate
Dominant	.01	.017 and .006	.3 and .8	0
Recessive	.01	.333 and .125	.3 and .8	0

[a] We computed the LOD score at a .8 penetrance only if a marker gave a LOD score suggestive of linkage.

Thus, we did a multipoint linkage analysis, under the assumption of heterogeneity, for all 23 chromosomes. The marker positions were according to the Genome Database and Généthon chromosomal genetic maps. The order of the markers and recombination fractions in the Genome Database and Généthon maps were verified in our data set. Multipoint LOD scores and multipoint heterogeneity LOD scores (HLODs) were computed by the Genehunter program (Kruglyak et al. 1996), using all the markers on each chromosome. Multipoint linkage analysis yields the maximum marker information for the area of interest. Using Genehunter, we set the inheritance parameters to the values that gave the maximum LOD scores in the two-point analyses. As we did in the two-point linkage analysis, we assumed a population prevalence of 1% for AITD and adjusted the gene frequency accordingly.

Predivided sample test.—To try to resolve the heterogeneity in our data set, we applied the predivided sample test (Morton 1956; Ott 1999). In this test, the data set is divided into different groups on the basis of clinical or demographic criteria, and the LOD scores obtained in each subset are compared. If the difference in the LOD scores for the two subsets of families is statistically significant, then it suggests that the two groups are genetically different from one another at the tested locus (Morton 1956; Ott 1999). Since our data set consisted of a large Italian subgroup that could have been different from the rest of our families owing to genetic and/or subtle ascertainment differences, we subdivided our data set into Italian and non-Italian families and applied the predivided sample test.

Affectedness and disease models.—The AITDs include GD and HT. It was not clear whether the susceptibility genes for these two disorders were unique or common to both diseases. Indeed, both disorders can occur in the same family. In our data set, 37% of the families included first-degree relatives with GD and HT. Therefore, we analyzed the data using three models:

1. Affectedness status AITD: all patients with AITD were considered as affected (loci identified using this model would confer susceptibility for both GD and HT).
2. Affectedness status GD: only patients with GD were considered as affected (under this model, patients with HT were considered as unaffected even if they had relatives with GD).
3. Affectedness status HT: only patients with HT were considered as affected (under this model, patients with GD were considered as unaffected even if they had relatives with HT).

Analysis for linkage with GD in which HT was considered unknown and analysis for linkage with HT in which GD was considered unknown were also performed, but, in all cases, the changes in the LOD scores were insig-

nificant. Family members with thyroid autoantibodies alone were classified as "unaffected." In addition, we computed the LOD scores, under the assumption of heterogeneity (HLOD), using Genehunter (Kruglyak et al. 1996).

Results

Characteristics of the Study Sample

Table 3 shows the clinical characteristics of the 102 families studied. Thirty-four (33%) had only GD-affected members, 30 (30%) had only HT-affected members, and 38 (37%) had both GD- and HT-affected first-degree relatives. Of the 250 affected individuals, 208 (83.2%) were female, and the affected female:male (F:M) ratio, 5:1, was in accordance with that reported in the literature (Volpe 1985). Thirty-five percent of the clinically and biochemically unaffected family members had thyroid antibodies, similar to the incidence reported in previous studies (Burek et al. 1982; Aho et al. 1983).

Whole-Genome Screening for the AITD Genes

Two-point LOD scores.—For mapping the AITD genes (i.e., genes causing both GD and HT), individuals with either GD or HT were considered affected. Figure 1*A* shows the results of whole-genome screening for AITD loci. Three loci, on chromosomes 6p, 8q, and 10q, gave LOD scores >2.0. At the 6p locus, the maximum two-point LOD score was 2.1 for marker D6S422 (34 cM), obtained for the recessive model, at a penetrance of 30% and a recombination fraction (θ) of 0.2 (fig. 1*A*; table 4). At the 8q locus, the maximum two-point LOD score was 2.9 for marker D8S284 (136.4 cM), obtained for the recessive model, at a penetrance of 30% and a θ of 0.1 (fig. 1*A*; table 4). The maximum two-point LOD

Table 3

Characteristics of the Study Data Set

Group	No.	%
Families:	102	100
Families with GD	34	33
Families with GD with GO[a] proband	11	11
Families with HT	30	30
Mixed families (GD+HT)	38	37
Individuals:	540	100
Affected individuals:	250	46[b]
Female	208	83[c]
Male	42	17[c]
Unaffected individuals:	290	54[b]
Female	138	48[d]
Male	152	52[d]

[a] GO = Graves ophthalmopathy.
[b] Percentage of total individuals.
[c] Percentage of affected individuals.
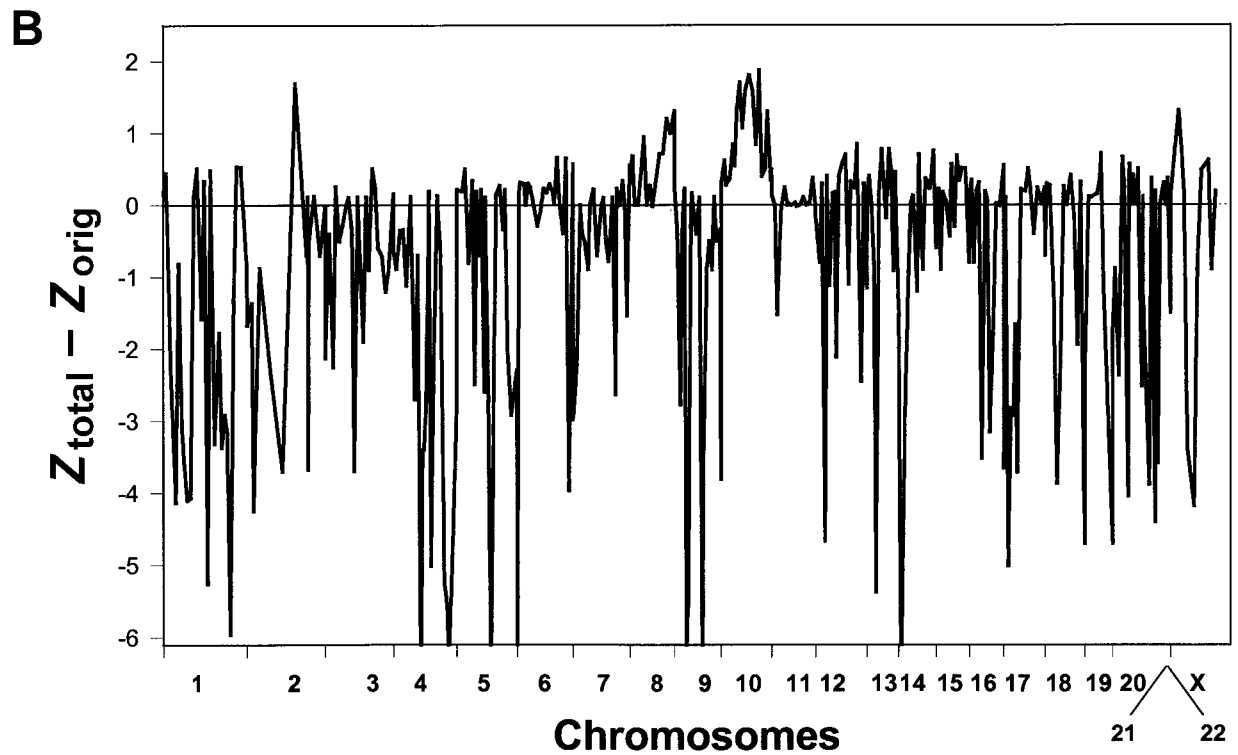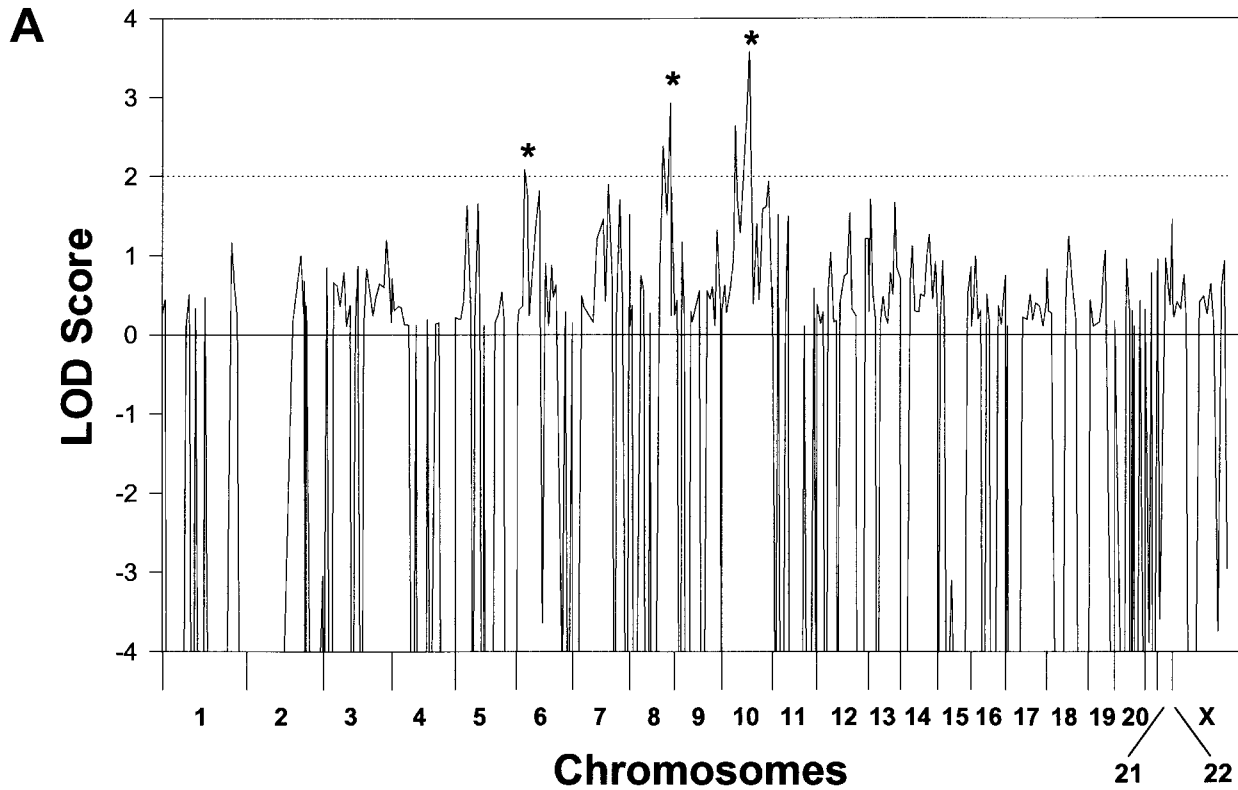[d] Percentage of unaffected individuals.

Figure 1   *A,* Whole-genome analysis for loci linked with both GD and HT (AITD). The *X*-axis shows the relative marker positions on each chromosome, and the *Y*-axis shows the two-point LOD score obtained for each marker on every chromosome. Three loci, on chromosomes 6p, 8q, and 10q (marked by asterisks [*]), gave two-point LOD scores >2. *B,* The differences between the two-point LOD scores obtained in the expanded data set and those obtained in the original data set. The *X*-axis shows the relative marker positions on each chromosome, and the *Y*-axis shows $Z_{total} - Z_{orig}$ for each marker. Two loci, on chromosomes 8q and 10q, showed a notable increase in LOD scores. One marker on 2q also showed an increase in LOD score, but the Z_{total} at this locus remained <2.

Table 4

Two-Point and Multipoint LOD Scores at Loci Showing Evidence for Linkage with AITD (LOD >2)

Phenotype and Marker Name	Chromosomal Location	Two-Point LOD	θ	Multipoint HLOD	α
AITD:					
D6S422	6p	2.1	.2	2.0	.59
D8S284	8q	2.9	.1	3.5	.6
D10S537	10q	3.6	.2	4.1	.5
GD:					
D7S502	7q	2.2	.1	2.3	.7
D14S258	14q	2.1	.2	2.1	.32
D20S195	20q	1.2[a]	.1	1.5[a]	.38
HT:					
D12S351	12q	2.6	.2	3.4	.5

[a] When we excluded the Italian subset, the two-point LOD score was 2.6 and the multipoint LOD score was 3.3, with no heterogeneity.

score at the 10q locus was 2.7 for marker D10S537 (93.3 cM), under the assumption of a recessive model, at a penetrance of 30% and a θ of 0.2. At a penetrance of 80%, the LOD score for marker D10S537 increased to 3.6 (fig. 1A; table 4). To determine the increase in information gained from the addition of the second data set, we computed the $Z_{total} - Z_{orig}$ for the 400 markers used for whole-genome screening. Figure 1B shows the results of the differences in the two-point LOD scores between the extended and the original data sets ($Z_{total} - Z_{orig}$). An increase in LOD scores was seen at the 8q and 10q loci. The multipoint HLOD scores also increased from 1.8 to 3.5 and 4.1 at 8q and 10q, respectively (table 5). One marker on 2q also showed an increase in the LOD score, but the Z_{total} at this locus remained <2.

Heterogeneity testing and multipoint analysis.—Multipoint analyses using Genehunter showed significant HLOD scores at the same three loci on chromosomes 6p, 8q, and 10q that were identified by the two-point analysis (fig. 2). The maximum multipoint LOD scores at the 6p, 8q, and 10q loci under the assumption of homogeneity were 0.2, 2.2, and −3.1, respectively. However, under the assumption of heterogeneity, the maximum multipoint HLOD scores were 2.0 for the 6p locus, 3.5 for the 8q locus, and 4.1 for the 10q locus (fig. 2; table 4). Thus, our data demonstrated strong evidence for heterogeneity at the 10q locus, whereas it is unclear whether there is genetic heterogeneity at the 6p and 8q loci. However, heterogeneity cannot be quantified by these methods, since the α statistic has been shown to be an inaccurate measure of the percentage of families with linkage (Vieland et al. 2001; Pal and Greenberg 2002).

Subset analysis by phenotype.—We examined subsets of our families (defined by clinical phenotype—i.e., GD or HT) to see whether they contributed most of the

positive LOD scores at the three identified AITD loci. We therefore tested these loci separately in our GD, HT, and mixed families (table 6). Subset analysis for the 10q locus showed that all three subsets contributed equally to the total LOD score. However, for the 6p and 8q loci, only the families with HT or GD gave positive LOD scores, whereas the mixed families gave negative LOD scores (table 6). This may imply that the mixed families form a subset of our families that is influenced by different loci than the families with GD or HT. Alternatively, the mixed families may show intrafamilial heterogeneity at the 6p and the 8q loci.

Whole-Genome Screening for GD Genes

For screening the unique GD genes (i.e., genes causing GD but not HT), we defined as "affected" only individuals with GD (therefore, the families with HT were excluded from analysis by definition, only the families with GD and mixed families were screened, and only the GD-affected family members were treated as affected). Two loci gave two-point LOD scores >2.0, on chromosomes 7q and 14q. The maximum two-point LOD score at the 7q locus was 2.1 for marker D7S502 (81.2 cM), obtained for the recessive model, at 0.3 penetrance and a θ of 0.1 (table 4). The maximum two-point LOD score at the 14q locus was 2.1 for marker D14S258 (71.6 cM), obtained for the dominant model, at 0.3 penetrance and a θ of 0.2 (table 4). The previously identified GD-2 (20q) locus (Tomer et al. 1998) showed a maximum two-point LOD score of 1.2 for marker D20S195 (47.8 cM), obtained for the recessive model, at 0.3 penetrance and a θ of 0.1 (table 4). Since our data set consisted of a large subset of Italian families, we reanalyzed this locus with the Italian families excluded ($n = 29$). The GD-2 (20q) locus gave a LOD score of 2.6 without an assumption of heterogeneity when we divided the families into subsets on the basis of the coun-

Table 5

Comparison of the Multipoint HLOD Scores Obtained in the First Data Set and in the Expanded Data Set

LOCUS	MARKER	MARKER POSITION[a] (cM)	First Data Set ($n = 56$)	Expanded Data Set ($n = 102$)
			HLOD SCORE IN	
GD-2	D20S195	47.8	3.5	3.3[b]
GD-3	DXS8020	101.2	2.5	−.5
HT-1	D13S173	103.6	2.1	−.9
HT-2	D12S346	102.0	2.3	3.4
7q	D7S502	81.2	1.4	2.3
8q	D8S284	136.4	1.8	3.5
10q	D10S537	93.3	1.8	4.1

[a] The distance of the marker from the p-terminal end of the chromosome.

[b] This multipoint LOD score was obtained for the white non-Italian families ($n = 73$) without assuming heterogeneity.
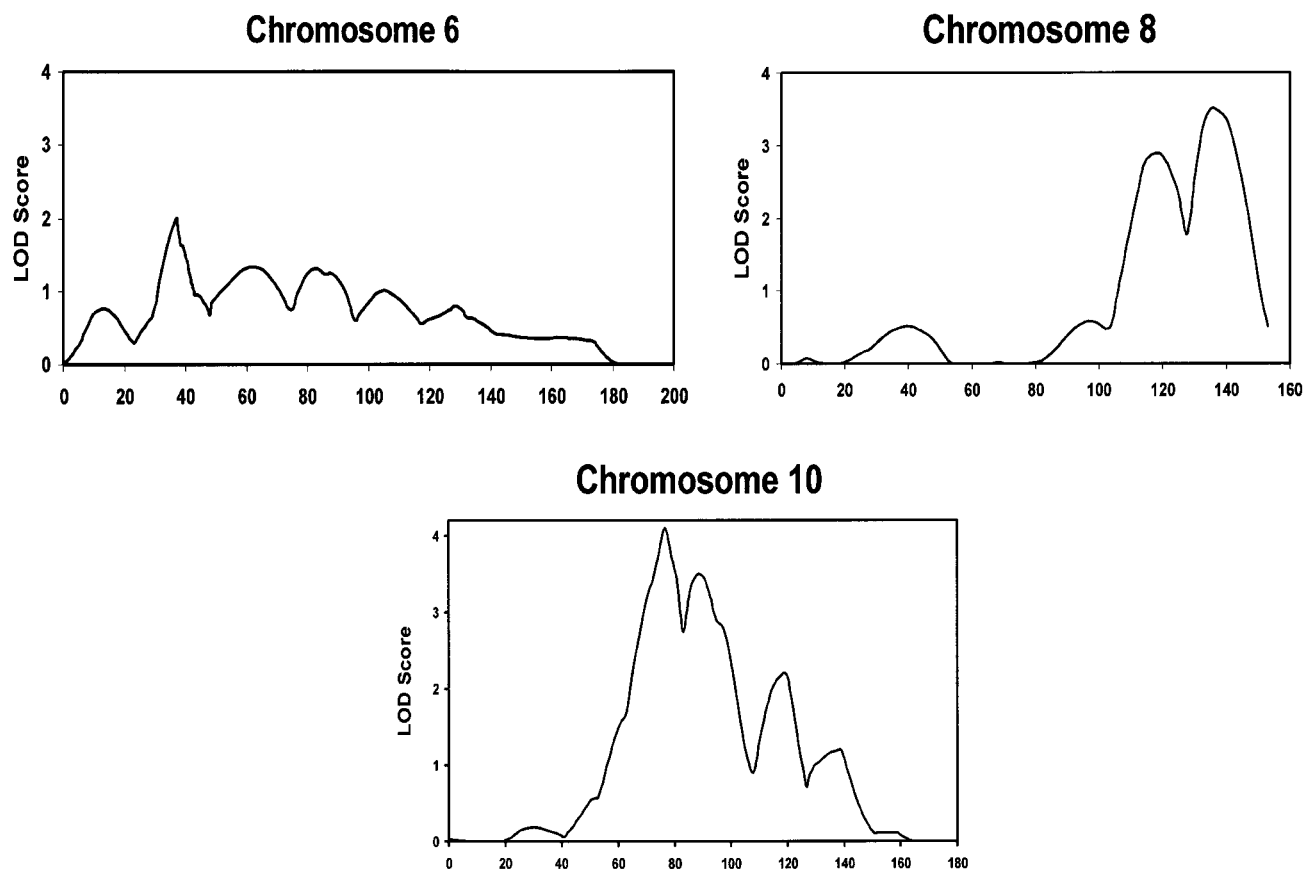
Chromosome 6

Chromosome 8

Chromosome 10

Figure 2   Multipoint analysis for chromosomes 6, 8, and 10. The *X*-axis shows the relative marker position in centimorgans, and the *Y*-axis shows the multipoint HLOD score. The maximum multipoint HLOD scores were 2.0 for the 6p locus, 3.5 for the 8q locus, and 4.1 for the 10q locus.

try of origin and excluded Italian families from the analysis (Tomer et al. 2002*b*). The multipoint LOD score at GD-2 (20q) was 3.3 when we excluded the Italian families (table 4). Application of the predivided-sample test (Morton 1956; Ott 1996) showed that there was statistically significant evidence for linkage heterogeneity at 20q in the Italian families versus the non-Italian white families, with the Italian families showing evidence against linkage at this locus ($\chi^2 = 9.2$; 1 df; $P < .01$) (Tomer et al. 2002*b*).

Whole Genome Screening for HT Genes

For screening the unique HT genes (i.e., genes causing HT but not GD), we defined as "affected" only individuals with HT (therefore, the families with GD were excluded from analysis by definition, only the families with HT and mixed families were used, and only the HT-affected family members were classified as affected). One locus showed evidence for linkage only with HT on 12q. The maximum two-point LOD score was 1.9

at marker D12S346 (102 cM), under the assumption of a recessive model, at a penetrance of 0.3 and a θ of 0.2. At a penetrance of 80%, the LOD score increased to 2.6. The maximum multipoint HLOD (recessive model, 80% penetrance) was 3.4 (table 4).

Comparison of the Whole-Genome Screen Results Obtained in the First Data Set with Those Obtained in the Expanded Data Set

Our initial whole-genome screen was performed in 56 families, and the expanded data set included an additional 46 families (a total of 102 families). Comparison of the results obtained for the expanded data set with the results obtained from the original data set showed that, at four of six sites, there is continued evidence for linkage (tables 5 and 7). Two loci (HT-2 and GD-2) continued to show strong evidence of linkage in the expanded data set, with LOD scores >3 (table 5). For two additional loci (AITD-1 and GD-1), we obtained peak LOD scores of >2 ~20–30 cM away from the original

Table 6

Two Point LOD Scores Obtained for the Families with GD Only, HT Only, and Mixed Families

	LOD SCORE IN			
LOCUS	Families with GD ($n = 34$)	Families with HT ($n = 30$)	Mixed Families ($n = 38$)	All Families ($N = 102$)
6p	1.0	1.9	−.8	2.1
8q	1.5	1.8	−.4	2.9
10q	1.0	1.5	1.1	3.6

locus (table 7), and, at present, it is unclear whether they represent the same loci or different loci. In contrast, for two additional loci that gave positive LOD scores in the first data set (GD-3 and HT-1), the LOD scores became negative in the expanded data set; thus, these loci could not be confirmed (table 5).

Discussion

The mechanisms underlying the genetic predisposition to AITD are unknown. Although a hereditary component in the pathogenesis of these diseases has long been recognized, the inheritance appears to be complex, involving multiple genes with variable penetrances. Moreover, the genetic relationship between GD and HT is not known, and, even within GD and HT, there exists significant phenotypic heterogeneity. Previous linkage studies by our own group and others have identified several loci as potential genetic regions harboring AITD susceptibility genes (table 1). However, previous studies employed only small numbers of families and/or individuals. We report here the largest whole-genome linkage study performed in AITD to date. Our data demonstrated significant LOD scores (>2) at seven loci: three loci (6p, 8q, and 10q) showed evidence for linkage with both GD and HT, three loci (7q, 14q, and 20q) showed evidence for linkage with GD, and one locus (12q) showed evidence for linkage with HT.

Comparison of the results obtained for this expanded data set with the results obtained for the original data set showed that the addition of the second data set resulted in a notable increase in statistical information (fig. 1B; table 5). Our first genome screen, performed in 56 families, showed evidence for linkage at six sites: 6p (AITD-1) showed evidence for linkage with both GD and HT; 14q (GD-1), 20q (GD-2), and Xq (GD-3) showed evidence for linkage with GD; and 13q (HT-1) and 12q (HT-2) showed evidence for linkage with HT. The results obtained in the expanded data set showed that four of six loci show continued evidence for linkage (tables 5 and 7), whereas two loci, GD-3 and HT-1, gave negative LOD scores in the expanded data set and,

thus, could not be confirmed (table 5). The other four loci continued to show evidence for linkage in the expanded data set. Two loci (HT-2 and GD-2) continued to show strong evidence for linkage in the expanded data set (table 5). For HT-2 (12q) the maximum two-point LOD score increased from 2.3 in the original data set to 3.4 in the expanded data set. For GD-2 (20q), the LOD scores remained >2.0 in the expanded data set, but only when we excluded the Italian families from the analysis. Since the original data set had few Italian families and the expanded data set consisted of ~30% Italian families, this effect was noticeable only in the expanded data set. We have now identified a putative susceptibility gene at the GD-2 locus region, the CD-40 gene (Tomer et al. 2002b). Since the second data set was "enriched" in Italian families, we did similar subset analysis (excluding the Italian families) at the other loci we identified, but in none of them except GD-2 did we resolve the heterogeneity by dividing the families into subsets on the basis of geographic origin (data not shown).

For two additional loci (AITD-1 and GD-1), we obtained peak LOD scores of ≥2 ~20–30 cM away from the original locus. Marker D6S257 (AITD-1) gave a maximum multipoint LOD score of 2.9 in the original data set and 1.5 in the expanded data set (table 7). However, in the expanded data set, there was a peak LOD score of 2.0 at marker D6S422 located ~30 cM telomeric to D6S257 (table 7). Although this is a large distance, it may represent the same locus (AITD-1), and the differences in map positions may be due to inaccuracies of the current genetic maps and/or due to genetic heterogeneity. A similar phenomenon was observed for the GD-1 locus. Marker D14S81 (GD-1) gave a maximum multipoint LOD score of 2.5 in the original data set and 1.2 in the expanded data set (table 7). However, in the expanded data set, marker D14S258, located 30 cM centromeric to D14S81, gave a maxi-

Table 7

Comparison of the Multipoint HLOD Scores Obtained in the First Data Set and in the Expanded Data Set for the AITD-1 and GD-1 Loci

LOCUS AND MARKER	MARKER POSITION[a] (cM)	HLOD SCORE IN	
		First Data Set ($n = 56$)	Expanded Data Set ($n = 102$)
AITD-1:			
D6S257	62.8	2.9	1.5
D6S422	34.0	2.0	2.0
GD-1:			
D14S81	91.7	2.5	1.2
D14S258	71.6	1.0	2.1

[a] The distance of the marker from the p-terminal end of the chromosome.

mum multipoint LOD score of 2.1 and, again, may represent the same locus. Thus, of the six loci originally identified, two showed increased evidence for linkage (GD-2 and HT-2), two showed significant LOD scores in the same chromosomal regions as the original loci (AITD-1 and GD-1), and two were not replicated (GD-3 and HT-1). In addition, for three loci that gave low positive LOD scores (1.0–2.0) in the original data set (D8S284, D10S537, and D7S502), the LOD scores increased to significance levels (>2.0) in the expanded data set (table 5). As mentioned above, our analyses were performed assuming two modes of inheritance (dominant and recessive) and two penetrances (30% and 80%). Greenberg et al. (1998) have shown that maximizing LOD scores over modes of inheritance, as we did, increases the power of the analysis significantly. However, because of the multiple testing, the recommendation is to subtract 0.3 from the obtained LOD score (Hodge et al. 1997). In our case, that would have resulted in a LOD score of 1.7 for the 6p locus, 3.2 for the 8q locus, 3.8 for the 10q locus, 2.0 for the 7q locus, 1.8 for the 14q locus, 3.0 for the 20q locus, and 3.1 for the 12q locus (see table 4). Thus, at all loci except 6p and 14q, the LOD scores remained above the cutoffs suggested by Lander and Kruglyak (1995). The significance of the 6p and 14q loci will have to be determined by studying additional data sets.

Comparison of our results with those obtained in the genome screen performed by Sakai et al. (2001) demonstrated evidence for linkage, at the 8q locus in their Japanese data set, that was replicated in our data set; however, the 5q locus that gave a LOD score of >3.0 in the Japanese data set did not show evidence for linkage in our data set (two-point LOD 0.54). The AITD-1 (6p) locus is within a broad region that showed evidence for linkage with HT in a genome scan performed in a large family in which vitiligo and HT occurred in numerous individuals (Alkhateeb et al. 2002). Another locus, GD-2 (20q), showed evidence for linkage in a U.K. data set (nonparametric linkage [NPL] score 2.0) (Pearce et al. 1999). Thus, three of the seven loci we identified showed evidence for linkage in independent data sets.

The AITDs are unique; even though their clinical manifestations are different, they share common immunopathogenetic mechanisms. Therefore, two hypotheses have been proposed to explain the etiology of the AITDs. According to one hypothesis, GD and HT share common genetic susceptibility, whereas differing environmental encounters and immunological modulating factors determine the final phenotype (Phillips et al. 1990). The second hypothesis proposes that, even though GD and HT are autoimmune diseases of the same organ (thyroid), they represent separate disease entities caused by distinct genes. Our study shed light

on the genetic relationship between GD and HT. Three loci (6p, 8q, and 10q) showed evidence for linkage with both GD and HT, implying that there is significant shared genetic susceptibility to GD and HT. This is also suggested by the fact that more than a third of the families have both diseases (table 3). Moreover, subset analysis showed that these three loci showed positive LOD scores in both the families with GD and the families with HT, again supporting a strong shared genetic susceptibility for GD and HT (table 6). However, the mixed families gave positive LOD scores only for the 10q locus and not for the 6p and 8q loci (table 6). This may imply that, in the mixed families, the development of AITD is influenced by unique loci not contributing to AITD in the families with GD or HT. Alternatively, the mixed families may represent intrafamilial heterogeneity. Therefore, the shared genetic susceptibility to GD and HT may be different in the mixed families than in the families with GD or HT. Preliminary data suggest that the shared genetic susceptibility for GD and HT may involve both immune regulatory genes (e.g., CTLA-4 [Vaidya et al. 1999; Tomer et al. 2001]) and thyroid-specific genes (Tomer et al. 2002c). However, we also found loci that were unique to GD and HT, implying that genetic factors also contribute to the distinct GD and HT phenotypes. Since all loci showed evidence for linkage with heterogeneity, the relative weights of the shared susceptibility loci and the unique loci for GD and HT cannot be assessed until the heterogeneity is resolved.

Some of the loci we have identified harbor interesting positional candidate genes. These include both immune regulatory genes and thyroid-specific genes. The 6p (AITD-1) locus harbors the SOX-4 gene, an immune modulator (Schilham et al. 1997). This locus is also close to a major immune regulatory gene cluster, the human leukocyte antigen (HLA) complex. However, previous studies by our group (Barbesino et al. 1998; Ban et al. 2002) and others (Bode et al. 1973; Hawkins et al. 1985; Shields et al. 1994) have shown strong evidence against linkage of the HLA locus to AITD. These data suggest that the 6p (AITD-1) locus is distinct from the HLA gene cluster. It is interesting that an insulin-dependent diabetes locus (IDDM-15 [MIM #601666]) (Delepine et al. 1997) and a systemic lupus erythematosus (SLE) locus (Gaffney et al. 1998) were identified in the same location as AITD-1, and this may imply that a general autoimmunity susceptibility gene is located in this region. Indeed, AITD is known to be associated with insulin-dependent diabetes (Gray et al. 1983; Kordonouri et al. 2002) and with SLE (Miller et al. 1987; Weetman and Walport 1987) in the same individuals and to cluster together in families. The 8q locus harbors the thyroglobulin gene, and our preliminary data suggest that the thyroglobulin gene may be

the AITD susceptibility gene in this region (Tomer et al. 2002*c*). In addition, the 20q locus (GD-2) harbors the CD40 gene, and we have preliminary data showing that an SNP in the Kozak sequence of CD40 is associated with GD (Tomer et al. 2002*b*).

Complex diseases are likely to be caused by the interaction of several genes, and their combined effects may differ in different individuals and families (Vyse and Todd 1996). In type I diabetes mellitus, at least 12 susceptibility loci have been identified by several groups (Davies et al. 1994; Hashimoto et al. 1994; Luo et al. 1995, 1996); in AITD, we identified seven loci, and additional loci were identified by others (table 1). It is most likely that these loci interact and that their interactions may influence disease phenotype and severity (Tomer et al. 1999). Indeed, locus interactions were reported in type 2 diabetes (Cox et al. 1999), in murine lupus (Vyse et al. 1997), and in epilepsy (Durner et al. 2001). 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 that ultimately determine disease phenotype. Another unresolved question is that of which loci harbor major genes (i.e., genes that are necessary for developing the disease) and which loci harbor minor modulating genes (i.e., genes that alter the effects of the major genes but are not necessary for disease development). To better understand these effects, we need to identify the susceptibility genes for AITD and the underlying molecular mechanisms by which they allow the induction of thyroid autoimmunity.

Acknowledgments

Electronic-Database Information

The URLs for data presented herein are as follows:

Généthon, http://www.genethon.fr/php/index.php

Genome Database, http://www.gdb.org/
Online Mendelian Inheritance in Man (OMIM), http://www .ncbi.nlm.nih.gov/Omim/ (for GD, HT, and IDDM-15)

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