

## Report

# Evidence for a New Graves Disease Susceptibility Locus at Chromosome 18q21

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Graves disease (GD) is a common autoimmune thyroid disorder that is inherited as a complex multigenic trait. By using a single microsatellite marker at each locus, we screened the type 1 diabetes loci *IDDM4*, *IDDM5*, *IDDM6*, *IDDM8*, and *IDDM10* and the fucosyltransferase-2 locus for linkage in sib pairs with GD. This showed a two-point nonparametric linkage (NPL) score of 1.57 ( $P = .06$ ) at the *IDDM6* marker *D18S41*, but NPL scores were  $<1.0$  at the other five loci. Thus, the investigation of the *IDDM6* locus was extended by genotyping 11 microsatellite markers spanning 48 cM across chromosome 18q12–q22 in 81 sib pairs affected with autoimmune thyroid disease (AITD). Multipoint analysis, designating all AITD sib pairs as affected, showed a peak NPL score of 3.46 ( $P = .0003$ ), at the marker *D18S487*. Designation of only GD cases as affected (74 sib pairs) showed a peak NPL score of 3.09 ( $P = .001$ ). Linkage to this region has been demonstrated in type 1 diabetes (*IDDM6*), rheumatoid arthritis, and systemic lupus erythematosus, which suggests that this locus may have a role in several forms of autoimmunity.

Graves disease (GD) (MIM 275000) is a common organ-specific autoimmune disorder, in which hyperthyroidism occurs because of the stimulation of the thyrotropin receptor by a thyroid-stimulating autoantibody. There is a strong genetic component in the pathogenesis of GD, with an estimated  $\lambda_s$  of 10–15 (Brix et al. 1998). Recent linkage and family-based association studies of GD have identified several susceptibility loci at different chromosomal regions—including 6p21 (major histocompatibility complex [*MHC*]) (Heward et al. 1998; Vaidya et al. 1999), 2q33 (cytotoxic T lymphocyte antigen-4 [*CTLA-4*]) (Heward et al. 1999; Vaidya et al. 1999), 14q31 (*GD-1*) (Tomer et al. 1998a), 20q13 (*GD-2*) (Tomer et al. 1998b; Pearce et al. 1999), and Xq21 (*GD-3*) (Barbesino et al. 1998)—providing evidence that GD,

like most autoimmune diseases, is inherited as a complex multigenic trait.

An excess concurrence of GD and type 1 diabetes (IDDM) in the same patients or their relatives suggests that IDDM and GD could share some susceptibility alleles (Payami et al. 1989). Indeed, recent studies have demonstrated that the *MHC* and *CTLA-4* loci are linked to both IDDM and GD (Davies et al. 1994; Nisticò et al. 1996; Marron et al. 1997; Heward et al. 1998, 1999; Vaidya et al. 1999). Genomewide linkage scans for IDDM have revealed many different chromosomal regions of linkage, and several of these loci have been replicated in further data sets (Davies et al. 1994; Hashimoto et al. 1994; Concannon et al. 1998; Mein et al. 1998). Replication of such a result in a different but pathogenically allied disorder would provide independent confirmation of the linkage. Thus, we screened the following putative IDDM loci in multiplex GD families for linkage: *IDDM4* (11q13), *IDDM5* (6q25), *IDDM6* (18q21), *IDDM8* (6q27), and *IDDM10* (10p11–q11) (Davies et al. 1994, 1996; Hashimoto et al. 1994; Luo et al. 1996; Delepine et al. 1997; Merriman et al. 1997, 1998; Reed et al. 1997; Concannon et al. 1998; Mein

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**Table 1**

**Phenotypes of Sib Pairs with AITD**

	Sib Pairs with GD Only (GD-GD)	Sib Pairs with GD and AH (GD-AH) <sup>a</sup>	All Sib Pairs with AITD
Full sib pairs	69	7	76
Half sib pairs	5	0	5
Total	74 <sup>b</sup>	7	81

<sup>a</sup> Families were selected only if they had at least two sibs with GD. Seven of these families had additional sibs with AH (GD-AH sib pairs).

<sup>b</sup> Seventy-one sib pairs with GD were available at the time of preliminary two-point linkage analysis.

et al. 1998; Nakagawa et al. 1998). Studies have shown that inability to secrete the soluble glycoprotein forms of the ABH blood group antigens (nonsecretor phenotype) is also associated with IDDM and GD (Blackwell et al. 1987; Collier et al. 1988). In whites this trait is due to a common nonsense mutation of the fucosyl-transferase-2 (*FUT-2*) gene (Kelly et al. 1995), located on chromosome 19q13, which suggests that markers close to this locus may also be linked to these disorders. We have, therefore, examined linkage of GD to the marker *D19S888*, which is within 1 cM of the *FUT-2* locus.

Sixty-seven white families with two or more sibs affected with GD (including 152 individuals with GD [26 males], 22 with autoimmune hypothyroidism [AH], and 73 unaffected subjects) were recruited from the north of England and the Lothian region of Scotland (table 1). Parents ( $n = 49$ ) and unaffected sibs ( $n = 37$ ) were studied if available. The diagnostic criteria for GD and the

**Table 2**

**Two-Point Linkage Analysis at the Various Candidate Loci in Families with GD**

Locus	Chromosome	Marker	NPL score	<i>P</i>	Information Content
<i>IDDM4</i>	11q13	<i>D11S1917</i>	-.62	.73	.40
<i>IDDM5</i>	6q25	<i>ESR</i>	.91	.18	.64
<i>IDDM6</i>	18q21	<i>D18S41</i>	1.57	.06	.44
<i>IDDM8</i>	6q27	<i>D6S281</i>	.48	.31	.58
<i>IDDM10</i>	10p11-q11	<i>D10S193</i>	-1.05	.85	.61
<i>FUT-2</i>	19q13	<i>D19S888</i>	-.85	.80	.62

characteristics of these families have been described elsewhere (Vaidya et al. 1999).

We screened the cohort of sib pairs affected with GD (71 sib pairs available at the time of screening) (table 1) for linkage to the single markers at *IDDM4*, *IDDM5*, *IDDM6*, *IDDM8*, *IDDM10*, and *FUT-2*. This showed a two-point nonparametric linkage (NPL) score of 1.57 ( $P = .06$ ) at the *IDDM6* marker *D18S41*. NPL scores were all <1.0 at the other five loci (table 2). Thus, we extended linkage analysis of the *IDDM6* region by genotyping 10 additional microsatellite markers (a total of 11 markers), spanning a 48-cM region of chromosome 18q12-q22. Multipoint linkage analysis in the cohort of 81 sib pairs affected with autoimmune thyroid disease (AITD) (table 1) showed a peak NPL score of 3.46 ( $P = .0003$ ), at the marker *D18S487* (fig. 1). The designation of only subjects with GD as "affected" (74 sib pairs) showed a peak NPL score of 3.09 ( $P = .001$ ) (fig. 1). The proportion of the 76 full sibs with AITD sharing zero alleles ( $z_0$ ) reached a minimum of 0.137 close to marker *D18S487*, which suggests that the locus-specific  $\lambda_s$  for this region is 1.8. The 26 families with affected

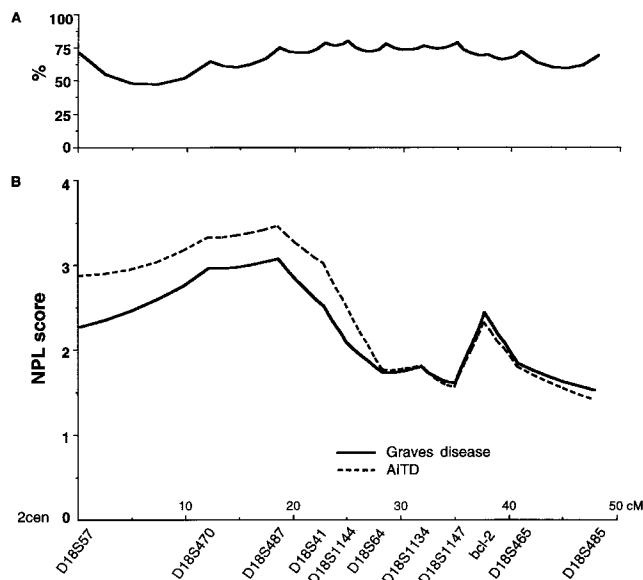
**Table 3**

**Intrafamilial Association between Proband with GD and Unaffected Sib Controls at Chromosome 18q12-q22 Markers**

Marker	Marker Map (cM)	Allele (mu)	Probands with GD <sup>a</sup>	Sibs Unaffected with GD <sup>a</sup>	<i>P</i>	Corrected <i>P</i> <sup>b</sup>
<i>D18S57</i>	.0	107	9/7	5/11	.143	.429
<i>D18S470</i>	12.0	289	4/4	0/8	.038	.114
<i>D18S487</i>	18.4	119	14/10	6/18	.020	.020
<i>D18S41</i>	22.7	203	19/11	16/14	.300	...
<i>D18S1144</i>	24.8	165	3/5	1/7	.285	...
<i>D18S64</i>	28.3	207	6/6	2/10	.096	.288
<i>D18S1134</i>	31.8	213	22/14	16/20	.118	.354
<i>D18S1147</i>	34.9	213	9/9	3/15	.038	.114
<i>bcl-2</i>	37.7	195	13/5	10/8	.244	...
<i>D18S465</i>	40.8	239	7/5	1/11	.014	.042
<i>D18S485</i>	47.8	183	13/11	7/17	.071	.213

<sup>a</sup> Number of occurrences/nonoccurrences of candidate alleles (Curtis 1997). Totals of the candidate alleles differentially occurring in the probands and in the unaffected siblings were compared.

<sup>b</sup> Fisher's exact test, corrected for multiple allelic comparisons. The three most common alleles were tested for association, but not at marker *D18S487*, where a candidate allele was already known (Merriman 1997).



**Figure 1** Multipoint linkage analysis of 11 markers on chromosome 18q12-q23. *A*, Percent information content over the marker map. *B*, The NPL score obtained by “scoring all” affected subjects with GD (solid line) and AITD (broken line) with the use of the GENEHUNTER package (Kruglyak et al. 1996) is shown, against the marker map on the x-axis. The peak NPL score of 3.46 occurs at marker *D18S487*. The microsatellite markers were genotyped with the use of fluorescently-labeled PCR and resolved on a semiautomated 373 ABI sequencer. The primers were taken from the Genethon genetic linkage map, except for *bcl-2* (Mehrian et al. 1998). The marker map was derived from the Genetic Location Database and the Center for Medical Genetics–Marshfield Medical Research Foundation databases. The minimum proportion of full sib pairs sharing zero alleles ( $z_0$ ) was calculated with the use of MAPMAKER/SIBS (Kruglyak and Lander 1995). The population allele frequencies for each marker were derived by genotyping local white controls.

AITD males (brother-sister sib pairs) showed a peak NPL of 2.03 ( $P = .02$ ,  $z_0 = 0.172$ ,  $\lambda_s = 1.5$ ), compared with 3.20 ( $P = .0008$ ,  $z_0 = 0.058$ ,  $\lambda_s = 4.3$ ) in the 41 families with only affected females.

To assess potential interactions between the chromosome 18 locus and other putative GD loci in our population, we weighted data from the chromosome 18 markers for allele-sharing status (0 or 1 to model epistasis; 1 or 0 to model heterogeneity) at *D2S117* (*CTLA-4*) and *TNF $\alpha$*  (*MHC*), using the modified GENEHUNTER-plus version 2, as described (Kong and Cox 1997; Cox et al. 1999). Weighting the chromosome 18 data for allele sharing at *D2S117* under a heterogeneity model showed a peak NPL score of 2.92 ( $P = .002$ , 32 families), which was similar to the NPL of 3.09 obtained in the whole cohort before weighting. No other evidence of interaction between the chromosome 18 locus and *CTLA-4* or *MHC* loci was found.

Family-based association analysis, with the use of un-

affected sibs as controls (Curtis 1997), showed an excess of the 119 mobility unit (mu) allele of *D18S487* ( $P = .020$ ) in probands with GD (table 3), which is the same allele found to be associated with IDDM (Merriman et al. 1997; T. Merriman, personal communication). This association of GD with the 119 mu allele of *D18S487* was also observed in a population-based case-control study with 166 unrelated probands with GD and 165 controls (27.3% vs. 17.3% in controls,  $P = .002$ ).

The present study has identified a susceptibility locus for GD at chromosome 18q21, which is close to the *IDDM6* locus. Using a multiplicative model, we can estimate that with a  $\lambda_s$  value of 1.8, the chromosome 18 locus may confer  $\leq 25\%$  of the total genetic susceptibility to GD in our population (Risch 1987). With both intrafamilial and population-based association analyses, we also found weak evidence for an association of GD with the 119 mu allele of *D18S487*. Our data provide some evidence to support genetic heterogeneity between the chromosome 18 locus and *CTLA-4* in our cohort, which suggests that these susceptibility loci may play complementary roles in conferring susceptibility to GD in different families.

One genomewide linkage scan involving 56 AITD families has not found evidence for linkage to this region of chromosome 18 (Tomer et al. 1999). However, our study and other studies showing linkage of this chromosomal region to other autoimmune diseases, such as IDDM (*IDDM6*) (Davies et al. 1994; Merriman et al. 1997, 1998), rheumatoid arthritis (Cornelis et al. 1998), and systemic lupus erythematosus (Shai et al. 1999), provides evidence that this region is likely to harbor an important autoimmunity locus. Candidate genes close to the region of association include the immunoglobulin gene transcription factor *ITF2* and the MAP kinase *MAPK4*, with the anti-apoptotic *bcl2* gene being located  $\sim 10$ cM from the region of strongest linkage. In addition, our findings further strengthen the hypothesis that common susceptibility loci are involved in different autoimmune disorders.

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## Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

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Foundation, [http://www.marshmed.org/genetics/Map\\_Markers/maps/indexmap.html](http://www.marshmed.org/genetics/Map_Markers/maps/indexmap.html) (for marker maps)  
 Genethon, [http://www.genethon.fr/genethon\\_en.html](http://www.genethon.fr/genethon_en.html) (for microsatellite marker primers)  
 Genetic Location Database, [http://cedar.genetics.soton.ac.uk/public\\_html/gmap.html](http://cedar.genetics.soton.ac.uk/public_html/gmap.html) (for marker maps)  
 Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim> (for GD [MIM 275000])

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