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Combined Segregation and Linkage Analysis of Graves Disease with a Thyroid Autoantibody Diathesis

Denis C. Shields,[1,2] Suvina Ratanachaiyavong,[3,4] Alan M. McGregor,[3] Andrew Collins,[1] and Newton E. Morton[1]

[1]CRC Genetic Epidemiology Research Group, Department of Child Health, University of Southampton, The Princess Anne Hospital, Southampton; [2]Department of Genetics, Trinity College, Dublin; [3]Department of Medicine, King's College School of Medicine, London; and [4]Department of Medicine and Biomedical Sciences, Prince of Songkla University, Songkla, Thailand

Summary

Combined segregation and linkage analysis is a powerful technique for modeling linkage to diseases whose etiology is more complex than the effect of a well-described single genetic locus and for investigating the influence of single genes on various aspects of the disease phenotype. Graves disease is familial and is associated with human leukocyte antigen (*HLA*) allele *DR3*. Probands with Graves disease, as well as close relatives, have raised levels of thyroid autoantibodies. This phenotypic information additional to affection status may be considered by the computer program COMDS for combined segregation and linkage analysis, when normals are classified into diathesis classes of increasing thyroid autoantibody titer. The ordinal model considers the cumulative odds of lying in successive classes, and a single additional parameter is introduced for each gene modeled. Distributional assumptions are avoided by providing estimates of the population frequencies of each class. Evidence for linkage was increased by considering the thyroid autoantibody diathesis and by testing two-locus models. The analysis revealed evidence for linkage to *HLA-DR* when the strong coupling of the linked locus to allele *DR3* was considered (lod score of 6.6). Linkage analysis of the residual variation revealed no evidence of linkage to *Gm*, but a suggestion of linkage to *Km*.

Introduction

Diseases with a genetic etiology are frequently complex in two ways: there may be more than one gene involved, and more than one aspect of the phenotype may be influenced. There is a need for methods of segregation and linkage analysis that address these complexities.

Traits may be quantitative or categorical, and to compress them into two groupings neglects useful phenotypic information that may distinguish between individuals of different genotype. Various approaches have been made to handle such complex phenotypes. Bonney et al. (1989) extended the theory for regressive models to consider polychotomous (or categorical) traits. They employed coefficients specific for each phenotypic category, but they recognized the difficulty that "the number of parameters increases rapidly with the number of polychotomies" (Bonney et al. 1989, p. 212). As there is often limited power in real data sets to distinguish alternative genetic models, a model should be parsimonious, with only as many parameters as are necessary. Models applied directly to quantitative data (Lalouel et al. 1985) achieve this by assuming a normal distribution of the trait; the difficulties with this approach are that departures from normality can simulate a major gene (Morton et al. 1991) and that transformations of the distribution to eliminate such departures can mask a true genetic effect (Demenais et al. 1986).

Morton et al. (1991) have presented a theory for an approach to complex phenotypes that can be applied equally well to quantitative and categorical information. This approach has three key elements. First, all the information additional to affection status is placed in ordered categories, whether the original data are categorical or quantitative. Second, the population frequency of each phenotypic class is determined prior to analysis, so that the method is not prone to misinterpretations of departures from distributional assumptions as evidence for genetic differences. Third, the ordered phenotypic categories are treated as being of two distinct types, "severity" and "diathesis" classes. Severity classes are a subclassification of affected individuals and help distinguish between affected individuals with "genetic" and "environmental" disease etiology. Diathesis classes are a subclassification of unaffected individuals that may indicate differences in genetic predisposition. (A definition of diathesis is: "a permanent condition of the body which renders it liable to certain special diseases.") While the increase in power of a polychotomy over a well-chosen dichotomy is not always very great (Armstrong and Sloan 1989), the method removes the problem of choosing a good cutoff point to form a dichotomy and is superior to an analysis of a poorly chosen dichotomy.

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Address for correspondence and reprints: Dr. Denis Shields, Department of Genetics, Trinity College, Dublin 2, Ireland.

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Linkage analysis requires knowledge of genetic parameters, usually estimated by segregation analysis. If these parameters are incorrectly specified, the evidence for linkage is weakened and the recombination rate is overestimated (Clerget-Darpoux et al. 1986). Combined segregation and linkage analysis (MacLean et al. 1984; Bonney et al. 1988) not only allows tests for linkage under a valid model, but, in addition, the linked marker can provide extra information about the segregation parameters, allowing more accurate prediction of risks. A useful model would consider a major disease locus that is linked to the marker locus, an environmental component, and a residual familial component. The mixed model (Morton and MacLean 1974) fits the latter component as a multifactorial (polygenic) background, but as this calculation requires approximate integration, it is simpler to represent this component by a second locus (MacLean et al. 1984). Here, we discuss the implementation of this theory in the program COMDS (*com*bined segregation and linkage analysis with *d*iathesis and severity) and its application to Graves disease and the diathesis of autoimmune thyroid antibody.

Graves disease is an autoimmune disease characterized by hyperthyroidism associated with other clinical features. It is partly familial, and both probands and close relatives exhibit raised levels of antibodies against thyroid antigens. Gene products of the highly polymorphic human leukocyte antigen (*HLA*) locus are involved in the immune response, and an association of *HLA-DR3* with Graves disease has been demonstrated in a number of Caucasian studies (Farid et al. 1979; Allanic et al. 1980). A linkage study of Graves disease failed to detect linkage (Roman et al. 1992), but this was not entirely surprising, as linkage equilibrium was assumed, while in fact there is a strong association with *HLA-DR3*. Here, we have carried out a segregation analysis of Graves disease and thyroid autoantibody in combination with a linkage analysis of *HLA-DR* and *Gm*. This has served (*a*) to indicate what proportion of the familial component of Graves may be accounted for by *HLA-DR3*, (*b*) to model the residual variation, and (*c*) to show how genetic factors influence both Graves and the thyroid autoantibody diathesis.

Subjects and Methods

Theory and Program

The theory implemented in the COMDS program has been documented in detail (Morton et al. 1991). Pedigree data are decomposed into nuclear families with pointers, who are relatives through whom the nuclear family was ascertained (Lalouel and Morton 1981). The phenotype includes not only affection status, but also diathesis class for normals of known antibody titer. Two autosomal disease loci are considered, each with a high-risk and a low-risk allele, whose effects are additive on a scale of liability. The loci are termed *major* and *modifier*, although the major locus need not necessarily have the greater influence.

As in the usual formulation of the liability model (Morton and MacLean 1974), COMDS uses liability classes to correct for covariates with affection, such as age and sex, which are assumed to be independent of a genetic cause. Diathesis classes are corrected for situational risk before analysis, such that they are independent of liability class (Morton et al. 1991), and the frequency of each diathesis class among normals in the population is specified.

The following parameters are associated with the major locus: *d*, the dominance; *t*, the displacement between homozygotes, *B*, the scaling parameter, which determines the displacement between genotypes for diathesis classes, and *q*, the gene frequency. Dominance ranges between 0 for a recessive gene and 1 for a dominant. A probit model relates these parameters to the probability of phenotypic class, given genotype or penetrance. As diathesis is not necessarily colinear with affection, the displacement for diathesis between homozygotes is not always constrained to be *t*. The scaling parameter *B* is used, such that *Bt* is the distance between homozygotes used for each cumulative odds calculation. Thus, when $B = 0$, there are no genotypic differences between diathesis classes, and the analysis is equivalent to one without diathesis information. When $B < 1$, the genotypic difference between individuals drawn from the first j classes and those drawn from the subsequent classes is less than the genotypic difference between an affected and a normal. When $B > 1$, the genotypic difference is smaller between affected and normal. When $B = 1$, there is no difference. This latter case is equivalent to a simpler model, where there is a single underlying distribution split into an ordered polychotomy with the "affected" as the highest class and with diathesis classes as lower categories.

For a two-locus model, a modifier locus is added with the iterable parameters q_m, d_m, t_m, and B_m. A marker locus with up to nine alleles may also be considered: linkage of the major locus to this marker is represented by the recombination fraction θ, and the frequency of association of any of the marker alleles with the major locus disease allele is given by its coupling frequency (parameters C_1–C_9), which ranges between 0 and 1. The sum over marker alleles of the product of the population distribution and the coupling frequency is constrained to equal the frequency of the high-risk allele q (MacLean et al. 1984).

Data and Analysis

Twenty-one multiplex pedigrees were ascertained in Wales and England (table 1). Of the 271 individuals, 184 were examined, and the thyroid function test was performed in individuals who either had a goiter or were suspected of thyroid dysfunction. In all, 55 were affected. Four of the probands were male, and of the affected relatives 5 were male and 29 were female, reflecting the known sex bias of this disease. Only two of the patients in this study required medical treatment for ophthalmopathy,

Table 1

Twenty-one Graves Disease Pedigrees

ID	FID[a]	MID[b]	A[c]	P[d]	Age (years)	Sex[e]	DR[f]	*TPO*[g]	*Tg*[h]
Pedigree 1:									
1	...	...	0	...	...	1	...	...	...
2	...	...	0	...	...	2	...	...	...
3	1	2	1	...	72	2	35	1.19	.80
4	1	2	1	...	69	1	35	.41	.08
5	1	2	0	...	67	2	35	.09	.09
6	1	2	0	...	63	2	37	1.29	.35
7	1	2	1	...	61	2	35	.10	.01
8	1	2	0	...	76	2	...	...	...
9	1	2	0	...	74	1	...	...	...
10	1	2	0	...	59	1	...	...	...
11	1	2	1	...	78	1	...	...	...
12	...	...	0	...	74	1	11	.01	.00
13	12	3	1	...	52	1	13	.35	.03
14	12	3	0	...	47	1	13	.16	.00
15	12	3	1	...	46	2	15	1.23	1.01
16	12	3	0	...	44	1	15	.02	.00
17	12	3	0	...	41	1	13	.29	.00
18	12	3	0	...	39	1	15	.02	.00
19	12	3	0	...	37	1	13	.44	.03
20	12	3	1	1	32	2	13	.14	.00
21	12	3	0	...	50	2	...	...	...
22	...	...	0	...	55	2	55	.02	.00
23	4	22	0	...	28	1	35	1.27	.52
24	4	22	1	...	27	2	35	1.05	.10
25	4	22	0	...	25	2	35	.03	.02
26	...	...	9	...	50	1	...	...	...
27	26	5	1	...	43	2	25	1.29	.16
28	...	...	0	...	65	1	35	.05	.00
29	28	7	0	...	36	2	35	.08	.03
30	28	7	0	...	35	1	35	.04	.01
31	28	7	0	...	33	1	33	.02	.00
32	...	...	0	...	47	2	45	.04	.00
33	13	32	0	...	25	1	34	.01	.00
34	13	32	0	...	22	1	34	.00	.00
Pedigree 2:									
1	...	...	0	...	...	1	...	...	...
2	...	...	0	...	...	2	...	...	...
3	1	2	0	...	67	2	34	.15	.04
4	1	2	1	...	60	2	34	1.55	2.54
5	1	2	0	...	55	2	34	.10	.07
6	1	2	0	...	52	1	36	.22	.16
7	1	2	1	...	50	2	48	1.19	1.21
8	1	2	0	...	50	2	68	.25	.11
9	1	2	0	...	45	2	34	.08	.02
10	1	2	0	...	69	1	...	...	...
11	1	2	0	...	65	1	...	...	...
12	1	2	0	...	58	1	...	...	...
13	1	2	0	...	54	2	...	...	...
14	1	2	0	...	43	2	...	...	...
15	...	...	0	...	...	2	...	...	...
16	11	15	1	1	36	2	48	1.22	.67
17	11	15	0	...	30	1	44	.01	.01
18	11	15	0	...	28	1	38	.02	.01
19	11	15	0	...	34	1	...	...	...
20	...	...	0	...	67	1	37	.02	.05
21	20	4	1	...	35	2	33	1.04	.92

(*continued*)

Table 1 (continued)

ID	FID[a]	MID[b]	A[c]	P[d]	Age (years)	Sex[e]	DR[f]	*TPO*[g]	*Tg*[h]
Pedigree 2: (*continued*)									
22	20	4	0	...	34	2	47	.02	.03
23	20	4	0	...	31	2	37	.14	.01
24	20	4	0	...	28	2	34	.90	2.13
25	20	4	0	...	26	2	37	.03	.00
26	...	...	0	...	50	1	37	.04	.02
27	26	5	0	...	26	2	38	1.18	.32
28	26	5	0	...	24	1	38	.64	.27
29	26	5	0	...	23	1	...	...	...
30	...	...	0	...	38	1	37	.05	.07
31	30	16	0	...	17	1	78	.10	.03
32	30	16	0	...	15	1	34	.02	.02
33	30	16	0	...	13	1	...	...	...
Pedigree 3:									
1	...	...	0	...	...	1	...	...	...
2	...	...	0	...	...	2	...	...	...
3	1	2	0	...	63	2	46	.01	.22
4	1	2	0	...	61	2	47	.90	.13
5	1	2	0	...	59	1	67	.39	.09
6	1	2	1	...	53	2	47	.15	.14
7	1	2	0	...	49	2	67	.91	.17
8	1	2	0	...	65	1	...	...	...
9	1	2	0	...	60	1	...	...	...
10	...	...	9	...	...	1	...	...	...
11	10	2	1	...	45	2	16	.63	.07
12	...	...	0	...	...	1	...	...	...
13	12	4	1	...	43	2	34	.68	.09
14	...	...	0	...	55	2	33	1.35	1.73
15	9	14	0	...	33	1	37	.02	.03
16	9	14	1	1	31	2	36	.57	.11
17	9	14	0	...	31	2	36	.29	.23
18	9	14	0	...	24	1	37	.00	.03
19	9	14	0	...	22	1	37	.32	1.04
20	...	...	0	...	57	1	27	.00	.00
21	20	6	0	...	32	1	27	.09	.02
22	...	...	0	...	49	1	36	.00	.02
23	22	11	0	...	24	1	13	.00	.00
24	22	11	0	...	22	1	13	.69	.58
25	...	...	0	...	...	1	...	...	...
26	25	13	0	...	23	2	36	.81	.28
27	25	13	0	...	25	2	...	...	...
Pedigree 4:									
1	...	...	0	...	...	1	...	...	...
2	...	...	0	...	81	2	23	.10	.09
3	1	2	1	1	58	1	13	.66	.63
4	1	2	0	...	56	1	13	.10	.11
5	1	2	1	...	51	2	38	1.11	1.94
6	1	2	0	...	40	1	13	.88	.27
7	1	2	0	...	53	1	...	...	...
8	1	2	0	...	53	2	...	...	...
9	...	...	0	...	55	2	46	.17	.80
10	4	9	0	...	36	1	34	.00	.00
11	4	9	1	...	34	2	36	1.17	.18
12	4	9	0	...	32	2	34	.33	.11
13	4	9	0	...	19	2	34	.02	.00
14	4	9	0	...	38	2	...	...	...

(*continued*)

Table 1 (continued)

ID	FID[a]	MID[b]	A[c]	P[d]	Age (years)	Sex[e]	DR[f]	*TPO*[g]	*Tg*[h]
Pedigree 4: (*continued*)									
15	4	9	0	...	28	1	...	...	...
16	...	...	0	...	...	1	...	...	...
17	16	5	0	...	31	2	28	.03	.00
18	16	5	0	...	29	1	18	.03	.00
19	...	...	0	...	57	2	...	...	...
20	3	19	0	...	38	1	...	...	...
Pedigree 5:									
1	...	...	0	...	...	1	...	...	...
2	17	18	0	...	84	2	24	.94	2.30
3	1	2	0	...	56	2	12	.02	.00
4	1	2	0	...	53	2	34	.01	.01
5	1	2	1	...	49	2	14	.18	.17
6	1	2	0	...	41	1	12	.00	.00
7	1	2	0	...	58	2	...	...	...
8	...	...	0	...	...	1	...	...	...
9	8	5	0	...	25	2	14	.00	.00
10	8	5	0	...	23	2	14	.00	.02
11	8	5	0	...	21	1	...	...	...
12	...	...	0	...	...	1	...	...	...
13	17	18	0	...	...	2	...	...	...
14	12	13	1	1	49	2	35	1.05	.18
15	...	...	0	...	50	1	...	...	...
16	15	14	0	...	25	1	...	...	...
17	...	...	0	...	...	1	...	...	...
18	...	...	0	...	...	2	...	...	...
Pedigree 6:									
1	...	...	0	...	...	1	...	...	...
2	...	...	0	...	...	2	...	...	...
3	1	2	1	...	62	2	47	.00	.01
4	1	2	1	...	55	2	17	.04	.07
5	1	2	0	...	64	1	...	...	...
6	...	...	0	...	59	1	34	.78	.26
7	6	4	0	...	26	2	14	.04	.00
8	6	4	1	1	25	2	13	.58	.43
Pedigree 7:									
1	...	...	0	...	...	1	...	...	...
2	...	...	1	...	...	2	...	...	...
3	1	2	1	1	68	2	56	.06	.03
4	1	2	1	...	71	2	67	.82	.10
5	...	...	0	...	...	1	...	...	...
6	...	...	0	...	...	2	...	...	...
7	5	6	0	...	75	1	67	.02	.00
8	5	6	0	...	72	1	36	.02	.00
9	5	6	1	...	65	2	36	.20	.36
10	5	6	0	...	69	2	...	...	...
11	7	3	0	...	38	1	57	.22	.69
12	7	3	0	...	36	1	66	.02	.02
13	8	4	0	...	44	1	67	.02	.00
14	8	4	0	...	40	2	37	.10	.00
Pedigree 8:									
1	...	...	0	...	...	1	...	...	...
2	...	...	0	...	...	2	...	...	...
3	1	2	0	...	74	2	37	.02	.04
4	1	2	0	...	61	2	17	.33	.34
5	1	2	0	...	70	1	37	.00	.02

(*continued*)

Table 1 (continued)

ID	FID[a]	MID[b]	A[c]	P[d]	Age (years)	Sex[e]	DR[f]	*TPO*[g]	*Tg*[h]
Pedigree 8: (*continued*)									
6	1	2	0	...	67	1	17	.00	.00
7	1	2	1	...	59	2	17	.36	.04
8	...	...	0	...	62	2	33	.00	.00
9	6	8	0	...	42	2	13	.56	.06
10	6	8	0	...	40	2	13	.00	.00
11	6	8	0	...	37	1	13	.00	.00
12	6	8	0	...	35	2	13	.19	.12
13	6	8	1	1	27	2	13	.37	.05
14	...	...	0	...	59	1	24	.00	.02
15	14	7	0	...	30	2	12	.00	.05
16	14	7	0	...	25	2	47	.00	.02
Pedigree 9:									
1	...	...	0	...	...	1	...	...	...
2	...	...	0	...	70	2	38	.20	.06
3	1	2	0	...	44	1	36	.03	.03
4	1	2	0	...	42	2	37	1.46	1.05
5	1	2	1	...	42	2	78	.90	.11
6	3	6	0	...	42	2	44	.87	.09
7	3	6	1	1	20	1	34	1.48	1.36
8	3	6	0	...	16	2	34	.02	.00
Pedigree 10:									
1	...	...	0	...	...	1	...	...	...
2	...	...	0	...	88	2	26	.13	.04
4	1	2	0	...	63	2	36	.04	.00
5	1	2	1	...	61	2	23	.03	.02
6	1	2	1	1	47	2	23	1.16	.24
7	1	2	0	...	65	1	...	...	...
8	1	2	0	...	50	1	...	...	...
9	1	2	1	...	55	2	...	...	...
10	...	...	0	...	...	1	...	...	...
11	10	6	0	...	36	1	12	.17	.60
12	...	...	0	...	...	1	...	...	...
13	12	5	0	...	20	2	27	.04	.01
14	12	5	0	...	17	1	23	.02	.00
Pedigree 11:									
1	...	...	0	...	...	1	...	...	...
2	6	7	0	...	...	2	...	...	...
3	1	2	1	...	63	2	13	.67	.32
4	1	2	1	1	55	2	17	.85	.06
5	1	2	0	...	61	2	67	.03	.00
6	...	...	0	...	...	1	...	...	...
7	...	...	1	...	...	2	...	...	...
8	6	7	0	...	...	2	...	...	...
9	...	...	0	...	...	1	...	...	...
10	9	8	1	...	...	2	...	...	...
11	...	...	0	...	60	1	...	...	...
12	11	4	0	...	35	2	...	...	...
Pedigree 12:									
1	...	...	0	...	50	1	12	.04	.00
2	...	...	1	1	50	2	35	.83	.13
3	1	2	1	...	30	1	23	.83	.10
4	1	2	0	...	26	1	13	.02	.00
5	1	2	0	...	25	1	23	.00	.00
6	...	...	9	...	50	1	...	...	...
7	6	2	0	...	20	1	56	.02	.00

(*continued*)

Table 1 (continued)

ID	FID[a]	MID[b]	A[c]	P[d]	Age (years)	Sex[e]	DR[f]	*TPO*[g]	*Tg*[h]
Pedigree 13:									
1	...	...	0	...	69	1	23	.03	.02
2	...	...	1	...	70	2	34	.46	.21
3	1	2	1	1	38	1	33	1.54	2.14
Pedigree 14:									
1	...	...	0	...	...	1	...	...	...
2	...	...	1	...	93	2	23	.01	.08
3	1	2	0	...	65	2	22	.00	.00
4	1	2	0	...	67	2	...	...	...
5	1	2	1	1	63	2	23	.02	.05
6	...	...	0	...	66	1	34	.06	.04
7	6	5	0	...	37	2	34	.00	.07
8	6	5	0	...	36	1	23	.00	.00
Pedigree 15:									
1	...	...	0	...	75	1	22	.04	.00
2	...	...	1	...	75	2	36	.09	.00
3	1	2	1	1	49	2	23	.02	.00
4	...	...	0	...	57	1	18	.03	.00
5	4	3	0	...	22	1	38	.00	.00
6	4	3	0	...	19	1	28	.02	.00
Pedigree 16:									
1	...	...	0	...	...	1	...	...	...
2	...	...	0	...	...	2	...	...	...
3	1	2	0	...	56	2	37	.02	.00
4	1	2	1	1	54	1	24	.09	.08
5	...	...	0	...	38	2	46	.02	.00
6	4	5	0	...	15	1	26	.00	.00
7	4	5	0	...	17	1	...	...	...
Pedigree 17:									
1	...	...	0	...	65	1	25	.02	.00
2	...	...	0	...	64	2	34	.91	.08
3	1	2	1	1	34	2	35	.82	.21
4	1	2	0	...	34	2	35	1.04	.14
Pedigree 18:									
1	...	...	0	...	...	1	...	...	...
2	...	...	0	...	60	2	35	.10	.01
3	1	2	0	...	35	2	37	1.54	2.33
4	1	2	1	1	31	2	45	.81	.21
5	1	2	0	...	30	2	45	.00	.01
Pedigree 19:									
1	...	...	0	...	...	1	...	...	...
2	...	...	0	...	...	2	...	...	...
3	1	2	0	...	63	1	34	.22	.01
4	1	2	0	...	61	2	34	.03	.00
5	1	2	0	...	60	2	34	.04	.00
6	1	2	1	1	55	2	13	.95	.09
7	1	2	1	...	53	1	...	...	...
8	...	...	0	...	55	1	45	.03	.00
9	8	6	0	...	27	2	15	.03	.00
10	8	6	0	...	29	1	...	...	...
11	8	6	0	...	25	2	...	...	...
Pedigree 20:									
1	...	...	0	...	...	1	...	...	...
2	...	...	0	...	76	2	37	.37	.16
3	1	2	0	...	48	2	36	.02	.03
4	1	2	1	1	43	2	36	.87	.34
5	...	...	0	...	40	1	14	.00	.00
6	5	4	0	...	21	1	46	.05	.00
7	5	4	0	...	19	1	16	.01	.00

(*continued*)

Table 1 (continued)

ID	FID[a]	MID[b]	A[c]	P[d]	Age (years)	Sex[e]	DR[f]	*TPO*[g]	*Tg*[h]
Pedigree 21:									
1	...	...	0	...	...	1	...	...	...
2	...	...	0	...	...	2	...	...	...
3	1	2	1	1	55	2	14	1.07	.13
4	1	2	0	...	47	2	45	.04	.00
5	1	2	0	...	46	2	16	.01	.02
6	1	2	0	...	44	1	...	...	...
7	...	...	0	...	55	1	78	.02	.00
8	7	3	0	...	31	2	47	.04	.02
9	7	3	0	...	29	2	17	.02	.07
10	7	3	0	...	27	1	47	.02	.01

[a] Father's I.D.
[b] Mother's I.D.
[c] Affection status.
[d] Proband status.
[e] 1 = male; and 2 = female.
[f] DR genotype.
[g] *TPO* titer.
[h] *Tg* titer.

which has been linked with genetic heterogeneity in other studies. Thyroid antibody information was analyzed for normal individuals only, because the antibody titer is typically lowered by treatment for Graves disease. Thyroid antibodies were measured for 135 of the 213 normal individuals. A spectrum of autoimmune thyroiditis, ranging from the subclinical thyroiditis (i.e., the presence of thyroid antibodies in euthyroid individuals with or without goiter), to postpartum thyroiditis, to the overt (hypothyroid) Hashimoto thyroiditis were also found in our data set. While Graves disease is known to cluster in families with Hashimoto thyroiditis, only two clear cases were found in this data set; for this reason, and because ascertainment through Graves disease results in underascertainment of Hashimoto thyroiditis, the analysis was restricted to Graves disease, and individuals exhibiting thyroiditis were treated in the analysis as "normal" from the perspective of disease status. The 184 were typed for *HLA, Gm,* and *Km* loci (Ratanachaiyavong et al. 1993). Preliminary statistical transformations were carried out using the SAS package (SAS Institute 1988).

Population frequencies were obtained as follows: Age-specific cumulative incidences for Graves disease were estimated from the annual incidence of TSH-receptor antibody–positive thyrotoxicosis (Phillips et al. 1985). The ratio of male to female was taken from Volpe et al. (1972) as being 0.211, and sex-specific liability classes were thus formed (table 2). While survival-analysis methods may allow a more exact correction for age at onset, we believe that assigning individuals to liability classes on the basis of age removes most of the potential bias.

A measure of microsome (thyroid peroxidase [*TPO*]) and thyroglobulin (*Tg*) thyroid antibodies in unaffecteds was used as diathesis. Antibody in normals was coded as "diathesis." No distributions of antibody titer are available for random population surveys, and the sample of pedigrees has higher titers because of the selection for Graves disease in relatives. However, it is possible to estimate the population frequencies from the sample, by correcting for the degree of relationship to an affected person. A measure of degree of relationship to the nearest affected, *R*, was coded as 0 for unrelated, 0.5 for third-degree relatives, 1 for second degree, 2 for first degree, and 4 for identical twins. A regression of various polynomials of the two antibody measures was used to investigate what was the best predictor of relationship *R*. Raising titer to the power

Table 2

Population Frequencies of Affection

Liability Class	Age Range (years)	Sex	$P(A \mid L)$[a]
1	10–19	Male	.00004
2	20–39	Male	.00072
3	40–59	Male	.00278
4	60+	Male	.00468
5	10–19	Female	.00016
6	20–39	Female	.00268
7	40–59	Female	.01042
8	60+	Female	.01752

[a] Probability of affection, given liability class.

Table 3

Diathesis-Class Population Frequencies

Class	Range[a]	$P(D)$[b]
1	<−.4	.1975
2	−.4–.0	.2469
3	.0–.2	.2284
4	.2–.5	.2284
5	>.5	.0988

[a] On a scale of combined antibody titer, estimated as $TPO + (0.130/0.358)Tg - 0.222 - 0.0040(\text{age}) - 0.104(\text{sex})$, where sex is 1 for male and 2 for female.

[b] Probability of an unaffected lying in each diathesis class.

of 0.125 gave the best prediction. A multiple regression of R versus the transformed titers obtained weights of 0.358 for TPO and 0.130 for Tg; a combined antibody measure for further analysis was then taken to be equal to $TPO^{0.125} + (Tg^{0.125})0.358/0.130$. A multiple regression of antibody against age, sex, and R was carried out. For the inferred population the residual was thus obtained corrected for these three factors; for the pedigree data the residual was obtained for the age and sex factors alone. The inferred population distribution was subdivided into five diathesis classes (table 3). The diathesis class of each individual without Graves disease was added to the data file. HLA class II specificities (table 4) were obtained by *Taq*I-RFLP using HLA-DRB, DQα, and DQB probes (Bidwell 1988; Ratanachaiyavong et al. 1990).

The families were mainly selected as having more than one affected, but as there was no requirement for the affected probands to have a specific relationship (e.g., two sibs affected), there is no exact correction available for ascertainment bias. A reasonable correction was obtained by assuming multiplex selection, either by conditioning on a multiplex sibship (Morton et al. 1991) or by conditioning on an affected relative outside the nuclear family, termed a *pointer*. Pointers were coded according to the scheme given by Morton et al. (1983). Portions of pedigrees that were extended without containing an affected member were excluded from the analysis. It was assumed that thyroid antibody status did not influence selection. An ascertainment probability of .001 was assumed. The coded data set comprised 64 nuclear families with 92 children. Some individuals were duplicated, appearing as parents, pointers, or children in different nuclear families. This does not present a problem with regard to analysis of the disease phenotype, as the likelihood is conditioned on parent and pointer phenotypes. However, the marker likelihoods are not conditioned, and therefore, to prevent a bias due to replication of the data, no marker typings were included more than once.

Single- and two-locus models were fitted using COMDS. Parameters in parentheses (tables 5–7) were held fixed at the given values, while the others were estimated. The likelihood of the set of sibships (conditional on parental phenotypes) is transformed to $-2\ln(L) + C$, which has a χ^2 distribution. (The constant C is present because certain calculations relating to the ascertainment probability and the marker typings are ignored, as they are independent of the genetic model.) A hypothesis is nested in another when only a subset of the parameters from the more general model are estimated. The difference in $-2\ln(L) + C$ between the two hypotheses is a χ^2 with the number of degress of freedom equal to the difference in the number of independently estimated parameters. A lod score represents the $\log_{10}$ of the ratio of the likelihoods of linked and unlinked models; these were obtained (table 8) from tables 5–7 by taking the difference in $-2\ln(L) + C$ between linked and unlinked hypotheses and dividing by the constant $2\ln(10)$. A pseudopolygenic model was fitted as two

Table 4

***HLA, Gm,* and *Km* Allele Population Frequencies**

Allele	Frequency
HLA-DR[a]:	
DR1/DR10	.1543
DR2	.1609
DR3	.1130
DR4	.2033
DR5	.0674
DR6	.1380
DR7/DR9	.1402
DR8	.0228
Gm[b]:	
fb	.6450
a	.2270
ax	.1180
anb	.0100
HLA-DQα[a]:	
DQa1	.4454
DQa2	.1989
DQa3	.3457
HLA-DQβ[a]:	
DQb1	.4359
DQb2	.2130
DQb3	.3511
Km[c]:	
1	.0750
0	.9250

[a] HLA class II specificities were obtained by *Taq*I/DNA-RFLP by using *HLA-DR, DQα,* and *DQβ* probes (Bidwell 1988; Ratanachaiyavong et al. 1990). Allele frequencies obtained from a U.K. sample, provided by Dr. J. L. Bidwell, U.K. Transplant Service, Southmead Hospital, Bristol (Ratanachaiyavong 1992).

[b] Gm phenotypes typed for *f, b, n, a, z, q,* and *x* allotypes were coded as four haplotypes: *fb, a, ax,* and *anb*. The population distribution of *fb, a,* and *ax* were taken from a U.K. sample (Steinberg and Cook 1981), and the frequency of *anb* was assumed to be .01.

[c] Allele frequencies from an Irish population (Steinberg and Cook 1981).

Table 5

Single-Locus Models

Hypothesis	d	t	q	B	θ	C_3	$-2 \ln L + C$
Sporadic	...	...	(0)	...	...	...	960.0
Graves disease without thyroid antibody diathesis (B=0):							
Dominant	(1)	1.97	.005	(0)	(.5)	...	921.5
Recessive	(0)	2.41	.126	(0)	(.5)	...	929.2
General	1.0	1.97	.005	(0)	(.5)	...	921.5
Graves disease and thyroid antibody diathesis colinear (B=1):							
Dominant	(1)	1.61	.010	(1)	(.5)	...	902.6
Recessive	(0)	1.75	.195	(1)	(.5)	...	909.2
General	1.0	1.61	.010	(1)	(.5)	...	902.6
Graves disease and thyroid antibody diathesis (estimated B):							
Dominant	(1)	2.08	.012	.56	(.5)	...	896.3
Recessive	(0)	2.27	.202	.59	(.5)	...	905.3
General	1.0	2.08	.012	.56	(.5)	...	896.3
Complete linkage to HLA-DR; coupling frequencies equal:							
Dominant	(1)	1.86	.013	.47	(0)	(q)	903.7
Recessive	(0)	1.97	.226	.54	(0)	(q)	910.7
General	.64	2.97	.050	.40	(0)	(q)	901.7
B = 0	.61	2.97	.009	(0)	(0)	(q)	924.0
Complete linkage to HLA-DR; coupling to DR3 estimated:							
Dominant	(1)	1.54	.013	.60	(0)	.07	878.4
Recessive	(0)	1.77	.198	.65	(0)	.54	884.9
General	1.0	1.54	.013	.60	(0)	.07	878.4
B = 0	1.0	1.72	.003	(0)	(0)	.02	900.1

Table 6

Two-Locus Models of Graves Disease, with Thyroid Antibodies in Normals

	Major-Locus Parameters				Modifier-Locus Parameters				*HLA-DR*		
Hypothesis	d	t	q	B	d_m	t_m	q_m	B_m	θ	C_3	$-2 \ln L + C$
No linkage to HLA:											
Pseudopolygenic	(.5)	1.98	(.5)	.64	(.5)	1.94	(.5)	.24	(.5)	...	905.7
Dominant/dominant	(1)	1.87	.335	.95	(1)	.76	.014	.54	(.5)	...	891.0
Dominant/recessive	(1)	1.68	.011	.44	(0)	2.48	.971	1.38	(.5)	...	892.0
Recessive/recessive	(0)	1.24	.224	.48	(0)	1.96	.489	.57	(.5)	...	899.8
General two-locus	1.0	1.87	.335	.95	1.0	.76	.014	.54	(.5)	...	891.0
Graves alone[a]	1.0	1.97	.005	(0)	.0	52.54	.0002	(0)	(.5)	...	921.5
Complete linkage of major locus to HLA-DR; coupling frequencies equal:											
General two-locus	1.0	1.96	.534	.63	1.0	1.24	.012	.80	(0)	(q)	894.3
Two-locus, Graves[a]	.61	2.97	.009	(0)	.64	.62	.000	(0)	(0)	(q)	924.0
Complete linkage of major locus to HLA-DR; coupling frequency of DR3 estimated:											
General two-locus	1.0	.72	.115	.58	1.0	1.93	.024	.47	(0)	1.0	860.4
Two-locus, Graves	1.0	1.04	.012	(0)	1.0	1.66	.119	(0)	(0)	.10	895.4

[a] Equivalent to single-locus model.

Table 7

Linkage to *HLA-DR* with an Estimated Recombination Fraction θ

Hypothesis	Major-Locus Parameters				Modifier-Locus Parameters				*HLA-DR*		
	d	t	q	B	d_m	t_m	q_m	B_m	θ	C_3	$-2 \ln L + C$
Coupling Frequencies Equal:											
One locus (B=0)	1.0	1.97	.006	(0)	...	...	(0)	...	.32	(q)	921.5
One locus ($\hat{B}$)	1.0	2.07	.012	.56	...	...	(0)	...	.28	(q)	895.8
Two-locus (B=0)	1.0	1.96	.006	(0)	.52	.55	1.000	(0)	.32	(q)	921.5
General two-locus[a]	1.0	1.93	.327	.91	1.0	.59	.014	.72	.32	(q)	890.8
Estimated DR3 Coupling Frequency:											
One-locus (B=0)	1.0	1.96	.002	(0)	...	...	(0)	...	.42	.02	(878.6)
One-locus ($\hat{B}$)	1.0	1.83	.014	.64	...	...	(0)	...	.35	.09	863.6
Two-locus (B=0)	1.0	.99	.113	(0)	1.0	1.89	.021	(0)	.36	1.0	884.9
Two-locus ($\hat{B}$)	1.0	.98	.115	.57	1.0	1.94	.023	.49	.34	1.0	854.1

[a] Equivalent to a single-locus model.

semidominant loci ($d=d_m=q=q_m=.5$). It is only an approximation of the polygenic model fitted in the POINTER program and is less parsimonious since more parameters are fitted.

To test linkage to *Gm* and *Km*, the influence of *DR3* on liability was removed. Liability classes (and diathesis classes) were recalculated to allow for not only age and sex, but also for the effect of the *DR3* allele, as modeled in the two-locus model with zero recombination (table 6). This is an approach similar to the stepwise oligogenic analysis employed by Wilson et al. (1990), except that the parameters of the major *HLA-DR* locus were obtained under a two-locus model, which should specify the linked locus penetrances more accurately.

Table 8

Evidence of Linkage Expressed as Lod Scores

Model[a]	C_3[b]	θ[c]	Lod Score[d]
Graves disease alone (B=0):			
One-locus	(q)	(0)	−.54
One-locus	(q)	.32	0.0
One-locus	.02	(0)	4.65
[Two-locus][e]	(q)	(0)	−.54
[Two-locus][e]	(q)	.32	.0
Two-locus	.10	(0)	5.67
Graves disease with thyroid antibody diathesis (B estimated):			
One-locus	(q)	(0)	−1.17
One-locus	(q)	.28	.11
One-locus	.07	(0)	3.89
Two-locus	(q)	(0)	−.72
Two-locus	(q)	.32	.04
Two-locus	1.0	(0)	6.64

[a] General models were obtained by combined segregation and linkage analysis, using the COMDS program (see text).

[b] Frequency with which *HLA* allele DR3 is coupled with the major disease allele.

[c] Recombination fraction between *HLA-DR* and the major disease locus.

[d] Log_{10} of the ratio of the likelihoods of the shown hypothesis, with the hypothesis of no linkage.

[e] Equivalent to single-locus test.

Results

Segregation Analysis without Marker Information

Single-locus models are presented in table 5. A single-locus model of Graves disease alone is more likely than the sporadic model (χ^2=960.0−921.5=38.5; 3 df). A model where the underlying single locus contributes not only to Graves, but also to thyroid antibody diathesis (when $B>0$), fits significantly better (χ^2=921.5−896.3=25.2; 1 df). The simplifying hypothesis (B=1) that Graves disease can be considered colinear with the diathesis of thyroid antibody (i.e., similar to a treatment where Graves disease was simply an additional diathesis class) was rejected in favor of a value of B = 0.56 (χ^2=902.6−896.3=6.3; 1 df). Thus, the putative gene has less effect on antibody diathesis than on affection. Recessive models are rejected with and without the thyroid autoimmune diathesis, (χ^2=6.6 and 7.7, respectively, 1 df), and dominant models fit well.

When Graves disease was considered alone, two-locus models failed to improve the likelihood, with the second locus being only a minor contributor to Graves (table 6). When thyroid antibodies were included, two loci provided a better fit than one, but the improvement was not significant (χ^2=896.3−891.0=5.3; 4 df). The favored model was of two dominant loci, although a dominant/recessive model was not ruled out (χ^2=1.0; 1 df).

Combined Segregation and Linkage Analysis

HLA associations with Graves disease are well documented, and the *HLA* gene products have a role in the immune response. Therefore, models of linkage that assume zero recombination are of the most biological relevance, especially those that permit *HLA* alleles to be in disequilibrium with the disease allele. This is achieved in COMDS by holding the recombination fraction θ at 0 and iterating on the coupling frequencies.

For the single-locus model, assuming complete linkage to *HLA-DR* (θ=0) with equal coupling frequencies actually marginally worsened the fit, compared with no linkage; the lod scores were negative, regardless of whether thyroid autoantibody was considered (table 8). This simple model is not very useful, as it ignores the significant association with the *DR3* allele. This result is consistent with the finding of Roman et al. (1992), who failed to detect linkage when assuming equal coupling frequencies. Estimating the recombination fraction θ only marginally improved the lod scores (table 8). However, when the coupling frequency of *DR3* is allowed to vary, there is strong evidence for linkage (lod scores of 4.65 and 3.89, excluding and including thyroid autoantibodies, respectively). Allowing the coupling frequencies of other *DR* alleles to vary on their own did not significantly improve the model: a slight improvement with *DR8* was not significant (lod score =0.22). While the best-fitting model suggested complete coupling of *DR3* and the disease-causing effect, this is not proof that *DR3* is a direct causative agent of the disease, as there is considerable linkage disequilibrium at the *HLA* locus. Single-locus dominant models (estimating *t*, *q*, and *B*) were used to test linkage to the *DQ*α and *DQ*β serotypes (estimating *t*, *q*, *B*, and individual coupling frequencies, with θ=0). A lod score of 2.42 was obtained when the coupling frequency of *DQ*α allele 2 was estimated, and a lod score of 1.74 was obtained when the *DQ*β allele 2 was estimated. However, because the highest lod score was obtained with the *DR3* association, *DR3* is the most likely causative agent. Two-locus models are presented in table 3. Introducing an unlinked modifier locus into the model not only improves the fit (χ^2=878.4−860.4=18.0; 4 df), but also increases the evidence for linkage, raising the lod score to 6.64. The linked locus in the two-locus model is a common gene in complete coupling with *DR3*, with a low penetrance.

In tests of linkage, deviation from the correct segregation model tends to lead to an inflation of the recombination fraction. Under a general two-locus model with the DR3 coupling frequency estimated, fitting θ (table 7) gave a significant improvement of fit (χ^2=6.3; 1 df). The lod scores are also increased, but they have not been tabulated, as they have 2 df and are thus not comparable with the usual lod scores. This model is biologically implausible, as complete coupling with *DR3* and a high recombination rate would be unstable over a few generations. However, it may indicate the segregation of an additional factor, either within or outside the *HLA* locus. The heterozygote interaction of particular alleles at the *HLA* locus could form a susceptible genotype, and jointly estimating the coupling frequencies of various allele pairs should indicate which interactions are significant. Single-locus models were fitted where the coupling frequencies of other alleles were estimated along with that of *DR3* (two-locus models were not fitted because of convergence problems). The most significant association was with *DR8* (table 9; χ^2=12.3; 1 df), which is still significant after the Bonferroni adjustment is made for tests of multiple alleles. The likelihood improvement resulting from estimating the recombination fraction θ was smaller than for the case when the coupling frequency of *DR8* was not estimated (χ^2=8.3, compared with 14.8), but the effect is still significant, suggesting some residual effect not explained by the model.

To test linkage of *Gm*, a combined segregation and linkage analysis was carried out under a single-locus model, corrected for the *HLA-DR3* association. No evidence of linkage to *Gm* was found (table 10). There was evidence against complete linkage (lod score of −1.9), and the recombination fraction θ was estimated to be .5. When coupling frequencies were estimated along with the recombination fraction θ, an improvement in likelihood was obtained when the coupling to haplotype *a* was 0 at a recombination fraction θ of .28, but it was not significant (χ^2=2.2; 2 df).

Linkage to *Km* was carried out in the same manner. When the coupling frequency of allele 1 was estimated under complete linkage, the χ^2 improvement was significant (χ^2=6.5; 1 df), corresponding to a lod score of 1.4 (table 10). This indicates a possible role of the *Km* allele 1.

It is possible to check these results by carrying out a multiple regression of disease phenotype against presence of each allele. A stepwise multiple regression of affection status against age, sex, and each of the alleles of *HLA*-DR, Gm, and *km* was carried out, with .15 probability level for entry into the model. The model obtained included three variables: a positive contribution of age and sex (female) and a negative association with *HLA-DR7* (probabilities of .07, .00, and .03, respectively). The measure of thyroid autoantibody used in the linkage analysis was used in a similar stepwise regression against the presence of each allele. The model obtained included four variables: *HLA-DR3*, *Km-1*, *HLA-DR5*, and *HLA-DR8* (probabilities of .00, .01, .04, and .06, respectively). All four contributed positively to thyroid autoantibody. Thus, while the regression of Graves disease alone does not confirm any of the associations found in the linkage study, the regression of autoantibody corroborates all three. The probabilities given here overestimate the significance, as they are not corrected for multiple testing, and the observations on related individuals are not independent. However, overall

Table 9

Single-Locus Models, Estimating Coupling Frequencies of DR3 and Other Alleles

DR_3, DR_i[a]	d	t	q	B	θ	C_3	C_i[a]	$-2 \ln L + C$
DR3, DR1/DR10	.52	2.38	.016	.74	(0)	.09	.00	878.4
DR3, DR2	.58	2.46	.046	.53	(0)	.19	.00	871.5
DR3, DR4	.46	2.53	.028	.66	(0)	.14	.00	872.7
DR3, DR5	.49	2.37	.072	.57	(0)	.26	.10	871.4
DR3, DR6	.49	2.48	.045	.59	(0)	.18	.00	873.4
DR3, DR7/DR9	.43	2.40	.042	.62	(0)	.18	.00	878.1
DR3, DR8	.52	2.46	.056	.56	(0)	.22	.21	866.1
DR3, DR8, θ	1.00	1.79	.014	.65	.33	.08	.06	857.8

[a] The coupling frequency of the allele shown was estimated, as was that of DR3.

they are in agreement with the associations identified by linkage and in addition suggest a possible positive association with *DR5* and a possible negative association with *DR7*.

The models presented make certain predictions about risks to relatives. The two-locus model, with *HLA-DR3* in complete coupling with the first locus, predicts a higher risk to females than to males: the penetrance of the highest-risk genotype is .224 in males and .524 in females, in the oldest age group. This is consistent with Graves disease being primarily a female disorder, which is modeled here as a component of the environmental risk. A model in which there is a stronger genetic effect in one sex is not possible in COMDS. The risks to a sib or offspring that are predicted by this model are .046 (compared with a population risk of .005). This is similar to observed risk to a DZ twin of ~.05 (Volpe 1978). However, the risk to an MZ twin, from this model (.100), is much lower than the literature estimate of .50 (Volpe 1978). While this could reflect a risk factor, such as polygenes, gene interactions, or an environment common to MZ twins, not adequately modeled here, it must be pointed out that the moderate size of our data set makes it of use primarily for predictions concerning linkage, and only secondarily for predictions concerning population risks. In addition, the twin studies are derived from compilations of various data whose ascertainment is unclear (Verschuer 1958), a large portion of which are from a study in which thyrotoxicosis and goiter were considered together (Harvald and Hauge 1956). The model also makes predictions for affected sib pairs. The ratio for pairs with 0, 1, and 2 alleles identical by descent is predicted to be .2:.5:.3, which only slightly deviates from that for an unlinked marker. This is because such calculations fail to take into account the strong coupling with *DR3*. Only four affected sib pairs in this study were informative for identity by descent at *HLA-DR*, in a ratio of 0:4:0. This sample was too small to test the fit, but it is clear that affected-sib-pair methods would require a very

Table 10

Combined Segregation and Linkage Analysis, to *Gm* and *Km* (after Correction for *HLA-DR3* Coupling)

Hypothesis	d	t	q	B	θ	C_1	C_2	C_3	C_4	$-2 \ln L + C$[a]
Linkage to *Gm:*										
Null	(0)	(0)	(0)	(0)	(.5)	...	...	...	...	607.9
No linkage	1.00	1.92	.010	.40	(.5)	...	...	...	...	565.0
Complete linkage	1.00	1.64	.014	.43	(0)	...	...	...	...	573.8
Estimated coupling	(1)	1.92	.010	.40	.42	.01	...	...	...	565.0
	(1)	1.86	.010	.45	.28	...	.00	...	...	562.8
	(1)	1.82	.017	.42	.35	...	...	.04	...	564.9
	(1)	1.92	.011	.40	.49	...	...	...	.00	564.5
Estimated linkage	1.00	1.92	.011	.40	.50	...	...	...	...	565.0
Linkage to *Km:*										
Null	(0)	(0)	(0)	(0)	(.5)	...	...	...	...	350.5
No linkage	1.00	1.92	.011	.40	(.5)	...	...	...	...	307.6
Complete linkage	(1)	1.97	.011	.39	(0)	...	...	...	...	304.3
Estimated coupling	(1)	2.01	.010	.39	(0)	.03	...	...	...	301.1
Estimated linkage	(1)	1.97	.011	.39	.00	...	...	...	...	304.3
	(1)	2.01	.010	.39	.00	.03	...	...	...	301.1

large sample to detect a significant bias. A larger sample of sib pairs with autoimmune thyroid disease failed to detect a significant departure from that expected (Roman et al. 1992).

Discussion

This combined segregation and linkage analysis of Graves disease helps clarify the apparent conflict between association and linkage studies of *HLA-DR* (Farid 1992; Roman et al. 1992), by carrying out a linkage study in which Graves disease is in disequilibrium with the *DR3* allele. The evidence obtained for linkage is significant, and it becomes more so when residual heritability is modeled as a modifier locus. While simulations have shown that a two-locus model does not increase the power to detect linkage (Vieland et al. 1992), they assumed complete penetrance, which is not true in this case. When the complex phenotype incorporating thyroid autoantibodies is included, the lod score reaches 6.64. This illustrates how careful modeling of a complex phenotype by using an oligogenic model can increase the power to detect linkage.

The evidence for a familial component additional to *HLA-DR3* was significant when the single- and two-locus models were compared. The modifier is fitted as a dominant gene unlinked to *HLA*, with a frequency of 2% and a larger effect on phenotype than has *DR3*. However, it must be emphasized that the modifier effect could represent almost any residual cause of family resemblance, such as another factor segregating at the *HLA* locus, polygenes, family-specific environment, or an amalgam of such factors. The most important feature of the two-locus model is that it allows the segregation parameters of the linked locus to be estimated independently of those of the residual component. Tests of linkage of *Gm* and *Km* to the residual component that was corrected for *DR3* provided evidence against linkage to *Gm* but suggested an association with the *Km-1* allele. A significant coupling with *HLA-DR8* was also shown. Thus, *HLA-DR3, HLA-DR8,* and perhaps *Km-1* alleles have direct pleiotropic effects on Graves disease and thyroid autoantibody or are in tight disequilibrium with the causative agents; future tests of linkage to other loci should ideally be corrected for these identified associations.

In previous analyses of disease status and associated traits, such as colon cancer and adenomas (Cannon-Albright et al. 1988) and breast cancer and fibrocystic breast disease (Skolnick et al. 1990), the only model tested was that the disease and the ancillary trait were both under the influence of the same locus. With COMDS, if the segregation of the two aspects of the phenotype are independent, under a two-locus model it is anticipated that *B* would be large and *Bm* would be small, or vice versa. In this study, the diathesis parameter *Bm* was of a magnitude similar to that of *B*, suggesting that the modifier effect, like the *HLA-DR3* effect, influences both Graves disease and the thyroid antibody diathesis.

The COMDS program allows a treatment of severity among affecteds that is exactly analogous to the treatment of diathesis among normals. However, this study did not use the thyroid autoantibody phenotype of Graves patients to form severity classes, because treatment of the disease (such as thyroidectomy) lowers autoantibody titer. In the analysis presented here, a quantitative trait was converted to an ordered polychotomy for analysis. Clearly, categorical phenotypes may also be treated as an ordered polychotomy. In a reversal of the approach used here, Curtis and Gurling (1991) placed an ordered polychotomy onto a quantitative scale, to increase power, in linkage studies, over a dichotomy. This approach would be unsatisfactory for segregation analysis, when distributional assumptions are violated. Combined analysis allows segregation parameters to be improved by the marker data when there is linkage and increases the power to detect linkage. This is superior to selecting the highest lod score from various segregation hypotheses, which biases in favor of linkage (Weeks et al. 1990). As genetic analyses are increasingly being applied to complex diseases for which there are no good segregation model and uncertain definitions of phenotype, the approach used here should prove useful in addressing some of the problems.

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