A Genomewide Scan for Type 1–Diabetes Susceptibility in Scandinavian Families: Identification of New Loci with Evidence of Interactions

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Type 1 diabetes mellitus (TIDM) has a multifactorial etiology, with major genetic-susceptibility determinants located in the HLA and insulin-gene (*INS*) regions. Linkage data implicating other disease-susceptibility loci are conflicting. This is likely due to (1) the limited power for detection of contributions of additional susceptibility loci, given the limited number of informative families available for study, (2) factors such as genetic heterogeneity between populations, and (3) potential gene-gene and gene-environment interactions. To circumvent some of these problems, we have conducted a genomewide linkage analysis for T1DM-susceptibility loci in 408 multiplex families from Scandinavia, a population expected to be homogeneous for genetic and environmental factors. In addition to verifying the HLA and *INS* susceptibility loci, the study provides confirmation of *IDDM15* on chromosome 6q21. Suggestive evidence of additional susceptibility loci was found on chromosomes 2p, 5q, and 16p. For some loci, the support for linkage increased substantially when families were stratified on the basis of HLA or *INS* genotypes, with statistically significant heterogeneity between the stratified subgroups. Our data support both the existence of non-HLA genes of significance for T1DM and interaction between HLA and non-HLA loci in the determination of the T1DM phenotype.

Introduction

Type 1 diabetes mellitus (T1DM [MIM 222100]) develops in susceptible individuals because of a gradual autoimmune destruction of the insulin-producing β-cells of the endocrine pancreas, with a consequent dependence on daily insulin injections for survival. Increased concordance for T1DM in MZ twins (30%–50%), compared to that in DZ twins (4.8%–27%) and in siblings (4.4%–12.5%), suggests that susceptibility is determined partly by genetic factors (Degnbøl and Green 1978; Tillil and Köbberling 1987; Olmos et al. 1988; Kaprio et al. 1992; Kumar et al. 1993; Lorenzen et al. 1994; Kyvik et al. 1995; Wagner et al. 1995; Davis 1996). This is also supported by familial clustering, as illustrated by an estimated recurrence risk in siblings (λ_S) of 15 (Risch 1987).

Monogenic or oligogenic forms of T1DM have been described, and genetic studies in families affected with it have allowed mapping of the corresponding genes to chromosomes 10q25 and 2p12 (Verge et al. 1998; Delépine et al. 2000). In the chromosome 2 region, disease-causing mutations in the gene for eukaryotic translation-initiation factor–2 alpha kinase–3 gene (*EIF2AK3*) have been identified in patients with Wolcott-Rallison syndrome (neonatal insulin-dependent diabetes and epiphyseal dysplasia) (Delépine et al. 2000).

The major histocompatibility complex (MHC) on chromosome 6p21.3 is a major susceptibility locus (referred to as "*IDDM1*") for the common multifactorial from of T1DM (Nerup et al. 1974; Pociot 1996), estimated to account for 40%–50% of λ_S (Risch 1987). The insulin-gene locus (*IDDM2* [MIM 125852]) has also been shown to contribute to disease susceptibility (Bell et al. 1984; Julier et al. 1991). In addition, environmental factors are also important in T1DM, as illustrated by <100% concordance in MZ twins and by

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the increase in incidence, over time, in certain age groups, particularly in the Scandinavian population (Diabetes Epidemiology Research International Group 1990; Tuomiletho et al. 1999; Dahlquist et al. 2000).

During the past decade, a large number of genetic studies have been devoted to the search for additional T1DM-susceptibility loci, either by linkage or by association with candidate genes. The major findings of these studies are summarized in table 1.

Both the relatively minor risk contribution of each of the non-HLA loci and the possible genetic and/or environmental heterogeneity within and between studies are likely to contribute to difficulties in replication of some of these linkage results. Many studies have had low power because of small sample sizes. In particular, data stratification based on HLA genotype or other criteria, which may be necessary for detection of the effects of some of the susceptibility loci, further increases the need for large sample sizes. In this context, it is probable that identification of T1DM-susceptibility loci will require the combination of data from several large linkage studies of multiple populations. Many of the previous investigations have been performed in partially overlapping groups of patients (Davies et al. 1994; Hashimoto et al. 1994; Concannon et al. 1998; Mein et al. 1998). Consequently, the aim of this investigation was to perform a genomewide search for T1DM-susceptibility genes in an independent collection of patients, consisting of 424 families (table 2) from Denmark, Norway, and Sweden—a relatively homogeneous population, in terms of its ethnic background and environmental exposures.

Subjects and Methods

Patients and Families

DNA from families with at least two affected individuals other than parent-child pairs was collected in Denmark, Norway, and Sweden (table 2). Individuals were considered affected (1) if they had been diagnosed with diabetes at age <15 years, required insulin treatment at the time of onset, and had remained on insulin treatment subsequently or (2) if diabetes with either ketoacidosis or requirement of insulin therapy from time of onset had been diagnosed at age >15 years. The median age at onset was 13 years (range 0–71 years). It previously had been shown that these criteria are consistent with the diagnosis of T1DM (Mølbak et al. 1994). To exclude families with maturity-onset diabetes of the young (MODY), the Danish families with diabetes reported in three consecutive generations were screened for MODY mutations. The Swedish families were identified through the two nationwide incidence registries: the Swedish Childhood Registry (Dahlquist et al. 1982, 1985; Nyström et al. 1990; Dahlquist and Mustonen 1994) and the Diabetes Incidence Study in Sweden (Östman et al. 1986; Blohmé et al. 1992). The Danish families were identified through the Danish Study Group of Diabetes in Childhood (Pociot et al. 1993), and the Danish IDDM Epidemiology and Genetics Group (Lorenzen et al. 1998). The Norwegian families were recruited during 1993–95, through advertisements, in the journal of the Norwegian Diabetes Association, for families with both (*a*) two sibs with T1DM and (*b*) parents without the disease, and the recruitment was followed by phone calls to include all family members. In most cases, blood samples were obtained, by mail, from local physicians. The diabetic siblings were asked to complete a form to confirm T1DM; in cases in which these sibs were uncertain of their T1DM status, their physicians were contacted. During 1996–97, recruitment was extended by letters to diabetes nurses ($n = 480$) at all pediatric and internal medicine departments in Norway. In all, 408 multiplex families from Scandinavia, comprising 464 affected full-sib pairs, were studied (table 2); when available, nonaffected siblings (216 nonaffected sib pairs) were also included in the scan.

Complementary Families: French and U.S. Families

The French- and U.S.-family panels are identical to those described elsewhere (Delépine et al. 1997). In all, 126 families with 158 affected sib pairs were available from France, and 255 families with 310 affected sib pairs were available from the United States. The latter were obtained principally from the HBDI family collection.

DNA Extraction

DNA was extracted by standard procedures, by either phenol/chloroform extraction or a salting-out method (Miller et al. 1988).

Microsatellite Genotyping

The genomewide linkage study was undertaken by use of the Medical Research Council (MRC) microsatellite panel (Reed et al. 1997). Markers that did not pass our quality-control procedures (see below) were replaced by new markers selected from the Généthon map. Additional markers were added both to regions that are sparsely covered by the MRC panel and in three regions of specific interest: HLA, on 6p21; *IDDM15* (MIM 601666), on 6q21; and the *EIF2AK3* locus, on 2p12.

Microsatellite markers were characterized by PCR amplification with fluorescence-labeled primers on PTC-225 thermocyclers (MJ Research). PCR was performed in a final volume of 15 μl (Buffer 1 × [Perkin-Elmer], 0.2 mM dNTP, 1.5–3.0 mM $MgCl_2$, 0.4 U *Taq* Gold, 0.2 mM of each primer, and 5–15 ng DNA). The samples were amplified for 35 cycles, each consisting of 30 s at 94 C, 30 s at the primer-specific annealing temperature

Table 1

Summary of Previous Linkage Results and Exclusion Data from Present Study

Locus or Region	Chromosome	Marker(s)	Data from Present Study			LOD Scores(s) from Previous Studies (Reference[s])		
			IBD[a] (%)	LOD Score[b]	λ_s Excluded	Significance	Replication	
							Independent	Overlapping
	1	D1S1644-AGT	50.0	0	1.5	⩾2.0 (Concannon et al. 1998)	…	…
IDDM7–IDDM12	2	HOXD8-D2S72-CTLA4	53.4	.79	2	⩾3.6 (Owerbach and Gabbay 1995)	…	⩾1.0 (Cordell et al. 1995; Luo et al. 1995; Concannon et al. 1998; Mein et al. 1998)
IDDM13	2	D2S137-D2S301	51.0	.04	1.5	⩾2.0 (Morahan et al. 1996)	…	⩾1.0 (Concannon et al. 1998)
IDDM9	3	D3S1279	53.0	.53	2	⩾2.0, female sib pairs (Paterson and Petronis 1999)	…	⩾1.0 (Cucca et al. 1998; Mein et al. 1998)
IDDM15	6	D6S283	(see table 4)		…	⩾3.6 (Delépine et al. 1997)	…	⩾2.0, not corrected for MHC (Concannon et al. 1998)
IDDM5	6	D6S476-D6S473	50.0	0	1.5	⩾3.6 (Luo et al. 1996)	⩾2.0 (Davies et al. 1994; Cucca et al. 1998), ⩾1.0 (Mein et al. 1998)	⩾1.0 (Davies et al. 1996; Concannon et al. 1998)
IDDM8	6	D6S446-D6S281	51.5	.12	1.5	⩾3.6 (Luo et al. 1995)	…	⩾2.0 (Luo et al. 1995; Davies et al. 1996), ⩾1.0 (Delépine et al. 1997; Concannon et al. 1998; Cucca et al. 1998; Mein et al. 1998)
IDDM10	10	D10S191-D10S220	53.4	.47	2.0	⩾3.6 (Mein et al. 1998)	⩾2.0 (Hashimoto et al. 1994)	⩾1.0 (Cucca et al. 1998)
IDDM17	10	D10S1750-D10S1773	52.0	.18	2.0	⩾3.6 (Verge et al. 1998)	⩾1.0 (Concannon et al. 1998)	
IDDM2	11	*INS*	51.3	.07	1.5	⩾2.0 (Mein et al. 1998)	…	⩾2.0, male sib pairs (Paterson and Petronis 1999)
IDDM4	11	D11S1296-FGF3	53.1	.49	2.0	⩾3.6 (Luo et al. 1996)	…	⩾2.0 (Davies et al. 1994; Hashimoto et al. 1994)
	14	D14S70-D14S276	50.0	0	1.5	⩾2.0 (Mein et al. 1998)	…	…
IDDM11	14	D14S67	50.0	0	1.5	⩾3.6 (Field et al. 1996)	…	…
	16	D16S515-D16S520	52.3	.38	1.5	⩾2.0 (Mein et al. 1998)	…	…

[a] Maximum value observed within the given interval; values <50% are shown as 50%.

[b] Maximum value observed within the given interval.

Table 2

Families of Patients Included in Genomewide Linkage Analysis

Category	Danish	Norwegian	Swedish	Total
Sib pairs:				
Affected	175	89	200	464
Unaffected	135	23	53	211
Discordant	287	127	277	691
Half-sib pairs:				
Affected	0	1	13	14
Unaffected	0	0	4	4
Discordant	0	3	20	23
Affected father-child pairs	41	3	20	64
Affected mother-child pairs	12	4	8	24
Avuncular pairs	0	8	1	9
First-cousin pairs	0	0	2	2
Families:				
Multiplex families	147	77	184	408
Simplex[a]	5	2	9	16

[a] These families were in the TDT analysis.

(45°C–61°C), and 30 s at 72°C. Initial denaturation was performed at 94°C for 10 min, and a final elongation step was performed at 72°C for 1 min. PCR products from 10–21 different primer pairs were pooled prior to separation on either an ABI373 or an ABI377 (Applied Biosystems), with either 12-cm or 36-cm well-to-read, denaturing 6% polyacrylamide gels, followed by analysis using either version 1.2 or version 3.1 of GENESCAN (Applied Biosystems). Single-strand size markers GS-350 or GS-500 (Applied Biosystems) were used. The allele assignments were made by version 2.0 of GENOTYPER (Applied Biosystems). Each gel contained an internal DNA control (individual 2 in CEPH family 1347; Coriell Institute), which was used for calibration of the allele sizes. In all, 324 markers were genotyped and included in the scan before quality control was performed.

Family structures were reassessed and corrected on the basis of the genomewide-scan data, by the SIBERROR program, with markers separated by >30 cM (Ehm and Wagner 1998). In particular, half-sibs and MZ twins were identified, and one member in each MZ pair was excluded from further analysis.

The following quality-control tests were routinely performed for each marker (and genotypes were recharacterized or removed as necessary): heterogeneity, in allele frequencies, between gels (possibly due to differences in allele sizing); Mendelian consistency, by the PEDCHECK program (O'Connell and Weeks 1998); and excess of homozygosity (generally reflecting either missed amplification of some alleles or, in heterozygotes, preferential amplification of one allele). The observed number of homozygotes was compared with the number expected on the basis of the observed allele frequencies, by a χ^2 test. A cutoff of $P < .001$ was used. Special care was also given to ensure consistency in allele sizing across all the families. These tests led to the exclusion of six microsatellite markers, which displayed excess of homozygosity that could not be corrected by reinterpretation of the gel data. Finally, we obtained consistent and corrected genotypes for a total of 318 microsatellite markers, which were used for analysis. The average success rate for genotypes after the quality-control tests were performed was 84.9%. The average heterozygosity of the markers was .79 (range .56–.91).

HLA and INS *Genotyping*

The *HLA-DRB1* (MIM 142857) genotype was determined by allele-specific PCR for the DR3 and DR4 alleles (Olerup and Zetterquist 1992, 1993). To determine the susceptibility status at the *INS* (*IDDM2*) locus, the *INS* polymorphism INS-23/*Hph*I was genotyped (Lucassen et al. 1993).

Genetic Map

The genetic map was assessed by both the CRIMAP program and the ASPEX program (see the The ASPEX Package: Affected Sib Pair Exclusion Mapping web site) (Lander and Green 1987; Hauser et al. 1996), which gave results that, in terms of marker order and distances, were similar to those for the publicly available linkage maps (e.g., that of the Center for Medical Genetics, Marshfield Medical Research Foundation). To avoid possible distortions of the map in families with diabetes due to adjacent susceptibility loci, we used the Marshfield map distances in the multilocus linkage analyses, with complementary estimates from other publicly available linkage maps, extrapolations from physical maps, and estimates from the present T1DM-Scandinavian map, where necessary. The average spacing between the 318 markers was 11.3 cM. In all, 23 intermarker distances were >20 cM.

Linkage Analysis

Linkage was assessed by nonparametric linkage methods. First, the data were scanned by programs including only nuclear families. Single-locus linkage analysis was performed by the ANALYZE package. Multilocus linkage analysis and exclusion mapping were performed by the SIBIBD program from ASPEX (see the The ASPEX Package: Affected Sib Pair Exclusion Mapping web site) (provided by D. Hinds and N. Risch), under a multiplicative model, as described elsewhere (Risch et al. 1999). Allele frequencies in all family members were estimated by the DOWNFREQ program. Additional single-locus and multilocus linkage analyses were performed by ALLEGRO (Gudbjartsson et al. 2000), with similar results. For chromosome X, MAPMAKER/SIBS (Kruglyak and Lander 1995) was also used for linkage calculations, as suggested by Nyholt (2000), resulting in similar results. Loci reaching a LOD score ≥ 2.0 in either the single-locus analysis or the multilocus analysis were further investigated by single-locus and multilocus linkage analyses by ALLE-

GRO, which allowed the inclusion of all affected family members (Gudbjartsson et al. 2000). A linear model with equal weighting of affected relative pairs was used. No correction was made for number of affected individuals in each family. Locus-specific λ_S was estimated as described by Risch (1987).

Transmission/Disequilibrium Test (TDT) Analysis

TDT analysis was performed by the ANALYZE program, with all affected siblings in each family (Terwilliger 1995).

Linkage Analysis for IDDM15

For *IDDM15* on chromosome 6q, analysis was performed, as described elsewhere (Delépine et al. 1997), to correct for linkage to the HLA region. The analysis was restricted to families in which the parental origin of HLA and *IDDM15* haplotypes could be determined. HLA haplotypes were determined on the basis of *HLA-DRB1* and the nearby microsatellite markers D6S276, D6S273, and TNFA. Marker D6S283 was chosen as the marker locus for the *IDDM15* locus (Delépine et al. 1997).

Stratification Based on Population of Origin, HLA, and INS

Secondary statistical analyses were performed, with the data subdivided by either population of origin, HLA type, or *INS* genotype. For stratification based on HLA type, families were divided into four independent groups: (1) DR3/4, comprising families in which all the affected siblings were HLA DR3/4 (132 families and 146 affected sib pairs); (2) DR4, comprising families in which all affected siblings carried HLA-DR4, excluding families in category 1 (164 families and 206 affected sib pairs); (3) DR3, comprising families in which all affected siblings carried HLA-DR3, excluding those in category 1 (61 families and 69 affected sib pairs); and (4) other, comprising families in which all affected siblings are neither DR3 nor DR4 (36 families and 53 affected sib pairs).

For stratification based on *INS*, positive susceptibility ("+") was defined in terms of homozygosity (+/+) and nonsusceptibility ("−") was defined in terms of other genotypes (+/− and −/−), where "+" denotes the T1DM-associated allele at the INS-23/*Hph*I site, which is in strong linkage disequilibrium with the small-size class at the INS-5′VNTR polymorphism (Julier et al. 1994). Families were subdivided into three independent groups: (1) INS+, comprising families in which all affected sibs carried the +/+ genotype (239 families and 279 affected sib pairs); (2) INS−, comprising families in which all affected siblings carried the −/− genotype (56 families and 60 affected sib pairs); and (3) INS+/INS−, comprising families in which affected siblings were discordant, or +/− (98 families and 135 affected sib pairs). Results from the secondary statistical analyses are reported only when the results of tests for differences in linkage are significant at $P < .05$ (heterogeneity test).

Results

Primary Linkage Analysis

Genotypes for 318 marker loci were obtained in 408 multiplex families (464 affected sib pairs). Multilocus-LOD-score curves, plotted for the entire genome, together with the exclusion data (for effects of $\lambda_S \geq 1.5$), are shown in figure 1. In addition to HLA, 14 regions were detected with a LOD score of 1.0 in multilocus analysis, 8 of which also were detected at this significance level in single-locus analyses (table 3).

In multilocus analysis, a LOD score of 4.8 was observed in the *IDDM15* region (markers D6S300–D6S283), corresponding to a secondary peak in the broad HLA-linked region. LOD scores >2 were also observed on chromosome 2, at D2S113 near *EIF2AK3* (LOD score 2.1), on chromosome 5, near marker D5S407 (LOD score 2.2), and on chromosome 16p, in a wide region from D16S407 to D16S287 (LOD score 2.8). LOD scores of 1.0 in single- or multilocus analyses were also observed on chromosomes 4, 9, 10, 12, 13 (two regions), 19 (two regions), 21, and X.

Susceptibility loci with $\lambda_S = 1.5$ were excluded from 61% of the genome (LOD score <-2.0). For $\lambda_S = 2.0$, 89% of the genome was excluded. Apart from *IDDM15*, none of the 17 previously reported non-HLA putative susceptibility loci achieved a LOD score >0.8. Effects of $\lambda_S \geq 1.5$ could be excluded at eight of the loci, effects of $\lambda_S \geq 2.0$ at the other five (table 1). However, linkage in the presence of association was confirmed at the *IDDM2* locus (*INS*) by TDT, with an excess of transmission of *INS*+ from heterozygous parents to diabetic children (261 vs. 164; $P = 10^{-6}$); there was no distortion in the transmission of *INS* alleles to nonaffected children (84 vs. 89).

The *IDDM15* region on chromosome 6q is linked to HLA, with an estimated recombination rate (θ) of .32 in male meioses and .47 in female meioses (Delépine et al. 1997). Therefore, statistical analysis was performed to evaluate the significance of cosegregation of *IDDM15* with the disease, with the contribution from HLA being taken into account. This yielded suggestive evidence of linkage in the Scandinavian families, with $P = .002$ at marker D6S283, the marker that, in a previous analysis (Delépine et al. 1997), had shown the strongest evidence of linkage (table 4). We then combined the data from the Scandinavian families with data from the French and U.S. multiplex families. There was no evidence, at this locus, of heterogeneity between the Scandinavian families and the French and U.S. families. Taking into ac-

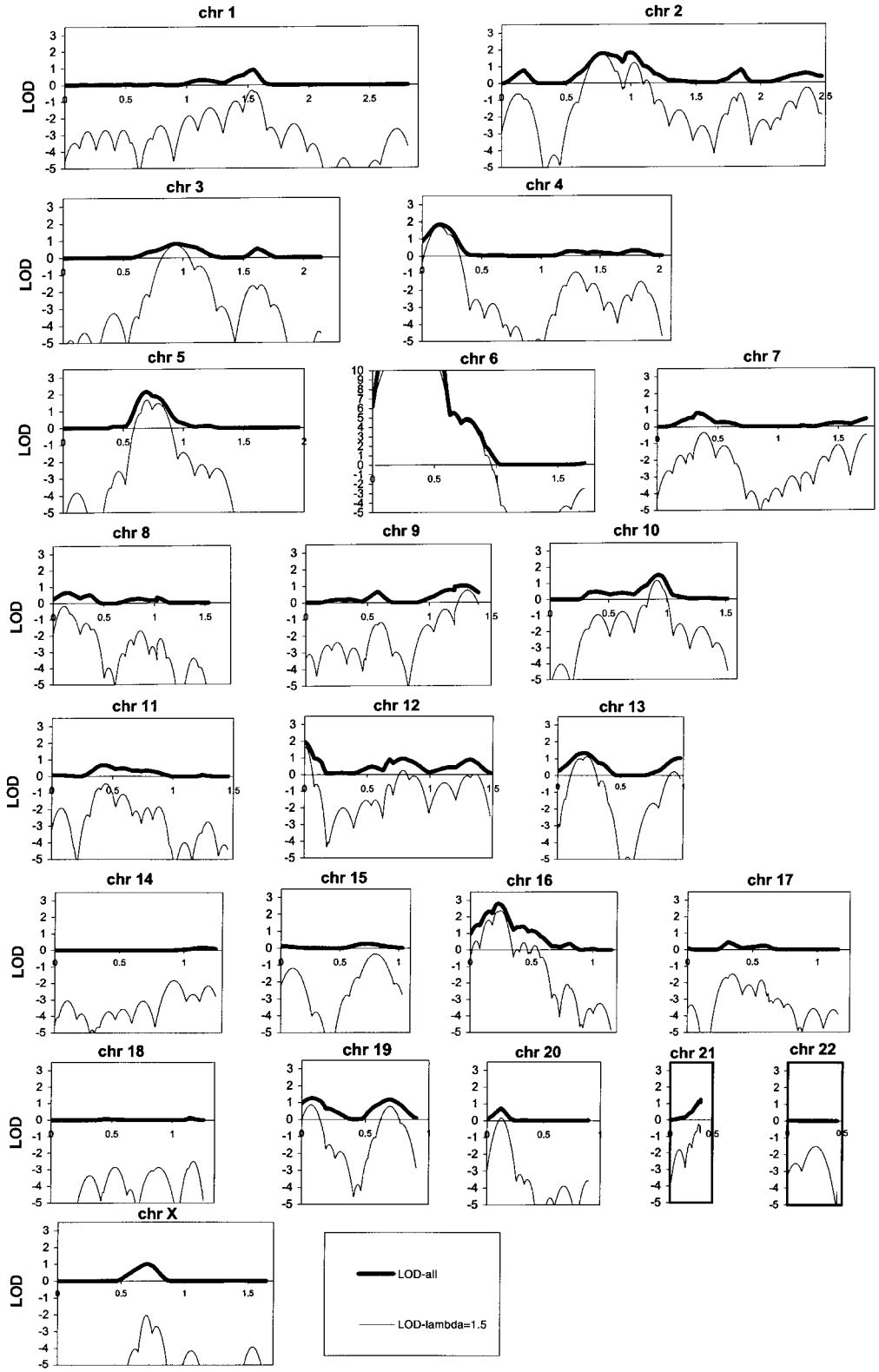

Figure 1 Multipoint linkage analysis using ASPEX multipoint linkage analysis (*solid line*) and exclusion mapping (*dashed line*) using ASPEX in all affected sib pairs. Genetic distances (in Kosambi Morgans) are given along the *X*-axis.

Table 3

Regions with LOD Score 1.0: All Families

Chromosome	Closest Marker(s)[a]	Results of Single-Locus Linkage Analysis[b]			Results of Multilocus Linkage Analysis[c]		
		LOD Score	*P*	IBD (%)	LOD Score	*P*	IBD (%)
2	D2S113	2.10	9×10^{-4}	55.8	1.82	.002	55.6
4	D4S403	1.30	.007	55.1	1.84	.002	57.4
5	D5S407	1.93	.001	55.8	2.16	8×10^{-4}	56.1
6	TNFA (HLA region)	36.97	3×10^{-39}	75.9	42.62	7×10^{-45}	74.2
6	D6S300[d]	4.27	5×10^{-6}	58.8	4.85	1×10^{-6}	59.6
9	D9S118-D9S158	.63	.044	53.4	1.02	.015	55.6
10	D10S583	.90	.021	54.5	1.49	.004	55.2
12	D12S99	1.69	.003	55.1	1.97	.001	55.4
13	D13S218	.91	.02	55.1	1.32	.007	55.2
13	D13S285	1.14	.011	54.5	1.02	.015	54.6
16	D16S405-D16S287	2.36	5×10^{-4}	57.2	2.80	2×10^{-4}	56.7
19	D19S247-D19S901	.84	.024	53.4	1.26	.008	55.3
19	D19S217-D19S180	.87	.022	54.5	1.18	.01	56.0
21	D21S65-D21S167	.95	.018	53.9	1.23	.009	54.2
X	MAOB	1.07	.013	56.5	1.00	.016	56.2

[a] Markers were considered to reflect the same susceptibility region when they were <10 cM apart and were covered by a continuous LOD-score peak.

[b] Calculated by ANALYZE programs.

[c] Calculated by ASPEX programs.

[d] Linkage around D6S300 is within the HLA-linked regions but produces a separate peak in the linkage curve. The LOD-score and *P* values for D6S300 are not corrected for linkage to HLA.

count the 95% confidence interval for the estimate of the distance between HLA and D6S283, an estimate obtained from data on the 59 large reference families from the Centre d'Étude du Polymorphisme Humain (Delépine et al. 1997), we estimated that the *P* value associated with this statistic in the combined family set would be 3×10^{-5}–5×10^{-8} (7×10^{-7} for the recombination rates given above).

Secondary Linkage Analyses

Multilocus linkage analysis was also undertaken with the data subdivided according to HLA subtype, *INS* susceptibility status, or population of origin (see the "Stratification Based on Population of Origin, HLA, and *INS*" subsection of Subjects and Methods), in regions with a LOD score >1 in the primary analysis. This allowed for testing of either heterogeneity of particular genetic effects due to interaction with specific HLA- or *INS*-risk haplotypes or population-of-origin heterogeneity for T1DM.

Table 5 shows the results when the test of heterogeneity between categories (HLA, *INS*, or population of origin) was significant at $P < .05$. A chromosome region near D16S405 on chromosome 16p displayed weak but significant evidence of heterogeneity in the stratification based on HLA ($P = .012$), with increased evidence of linkage in the DR3/4 group (LOD score 3.6; 65% IBD) (table 5). In addition, a region near marker D16S3113 on chromosome 16p demonstrates significant evidence of heterogeneity in the stratification based on *INS* ($P = .0005$), with increased evidence of linkage in the INS− group (LOD score 3.4; 71% IBD) (table 5). Weak evidence of heterogeneity in the stratification based on population of origin was found in the extended chromosome 16p region near marker D16S3131 ($P = .037$), with increased evidence of linkage in the Danish population (LOD score 2.8; 61% IBD) (table 5). Interestingly, the three peaks observed in the HLA-DR3/4, INS−, and Danish groups have distinct positions within the extended chromosome 16p region and cover a 30-cM region (fig. 2). In addition, the region on chromosome 2p near marker D2S113 showed increased evidence of linkage in the DR3/4 group (LOD score 2.7; 65% IBD), although the test for heterogeneity in the stratification based on HLA did not reach statistical significance ($P > .05$; data not shown). Evidence of linkage under stratification based on either HLA, *INS*, or population of origin was not increased in the chromosome 5q region compared with the complete data set (data not shown). There was also some evidence of heterogeneity in linkage to HLA when the data were stratified on the basis of *INS* ($P = .012$), with increased evidence of linkage in the INS− group (85% IBD) compared both with the INS+ group (71% IBD) and with the intermediate IBD value in the INS+/INS− group (76%) (table 5).

Two additional markers, D4S412 and D13S153, lo-

Table 4

Results of *IDDM15* Linkage Analysis, Adjusted for HLA Linkage, for Marker D6S283

	No. of Individuals[b]						Overall Statistic			
	All		Males		Females		Weighted χ^2			
Origin and HLA Split[a]	IBD1	IBD0	IBD1	IBD0	IBD1	IBD0	Male	Female	All	*P*
Scandinavia:										
HLA 1	217	148	122	63	95	85	7.52	1.93	8.56	.002
HLA 0	74	61	33	32	41	29				
France:										
HLA 1	84	43	48	17	36	26	4.29	1.61	5.45	.01
HLA 0	13	17	5	10	8	7				
United States:										
HLA 1	205	130	105	64	100	66	1.32	10.10	9.39	.001
HLA 0	62	57	24	33	38	24				
All:										
HLA 1	506	321	275	144	231	177	11.65	11.62	23.27	7×10^{-7}
HLA 0	149	135	62	75	87	60				

[a] HLA 1 = sharing of one HLA allele IBD; HLA 0 = sharing of no HLA allele IBD.
[b] IBD1 = inheritance of the same allele; IBD0 = inheritance of a different allele.

cated near markers detected with a LOD score >1.0 in the complete data set (21 cM from D4S403 and 12.5 cM from D13S153) showed evidence of heterogeneity when stratified on the basis of either HLA ($P = .031$ and $P = .018$, respectively). The LOD was 2.6 in the other-DR group, for D4S412, and was 3.2 in the DR4 group, for D13S153.

Linkage Analysis Using All Family Members

To include all affected individuals, linkage was calculated, by the ALLEGRO program, for regions reaching a LOD score of 2 in the primary linkage analysis. These data are displayed in figure 2. On chromosome 2, the LOD score was 2.6 among patients who also carried DR3/4. On chromosome 5, the LOD score was 2.2 in the complete data set, whereas it was 3.7 on chromosome 16 among patients who also carried DR3/4 and was 3.5 in the INS− group (these peaks were 29 cM apart).

Discussion

In the present genomewide linkage analysis in a large collection of Scandinavian families, we confirmed linkage to the disease-susceptibility loci *IDDM1* (HLA region), *IDDM2* (*INS*), and *IDDM15*. We also found suggestive evidence of linkage (LOD score >2) in three additional regions, on chromosomes 2p, 5p11-q13, and 16p. In the first and third of these three regions, the evidence of linkage was increased in secondary analyses when the data were stratified on the basis of either HLA or *INS*. HLA was confirmed as a major susceptibility locus for T1DM (multilocus LOD score 42.6), with an observed locus-specific λ_s of ~3.6. TDT analysis performed with an *INS* variant associated with T1DM susceptibility provided confirmation of linkage to *IDDM2* ($P = 10^{-6}$). The *IDDM1* and *IDDM2* loci showed marginally significant evidence of interaction ($P = .012$). Linkage to the *INS* region was not significant, as expected, because of the limited power of this approach, given the low λ_s attributed to the locus because of the high carrier frequency of the susceptibility allele in the population (Julier et al. 1991; Spielman et al. 1993).

The pattern of linkage on 6q was similar to that observed in French and U.S. families (Delépine et al. 1997), with the LOD-score curve's maxima occurring at *IDDM1* (HLA) and *IDDM15* and being separated by >30 cM. After adjusting for linkage to *IDDM1*, we obtained $P = .002$ in the test for linkage to *IDDM15* in the Scandinavian families. However, 147 of the Danish families were included in both the present study and the original report of linkage to *IDDM15* (Delépine et al. 1997). When the new data were combined with those previously published on Danish, French, and U.S. families, the significance of the HLA-adjusted test statistic was $P = 7 \times 10^{-7}$. Linkage at this locus has also been reported, in combined U.S. and U.K. families, by Concannon et al. (1998), although the U.S. families in the latter study overlap partially with both those in the report by Delépine et al. (1997) and those in the present study. Thus, the combined evidence in favor of *IDDM15* is the most compelling that has been reported thus far for any of the putative T1DM-susceptibility loci, except for *IDDM1* and *IDDM2*.

We selected additional markers on chromosome 2p12, to cover the region of *EIF2AK3*, which is mutated in patients with Wolcott-Rallison syndrome (Delépine et al. 2000). Although this syndrome is not known to involve an autoimmune process, patients develop permanent insulin-dependent diabetes shortly after birth,

Table 5

Analysis of Heterogeneity in IBD Sharing and Stratification

Stratification Type and Closest Marker	IBD (%)						
	DR3/4	DR4	DR3	Other	P[a]	HLA Group[b]	LOD Score[b]
HLA:							
D16S405	64.4	52.5	52.6	53.7	.012	DR3/4	3.60
	INS+	INS+/INS−	INS−			*INS*	
INS:							
*INS*D6S276 (HLA)	70.9	76.4	84.4		.012	INS−	
*INS*D16S3113	50.0	59.1	70.9		.0005	INS−	3.42
	Danish	Swedish	Norwegian			Population	
Population of origin:							
D16S3131	60.8	51.3	50.8		.037	Danish	2.80

[a] For test of heterogeneity in IBD sharing between groups listed.
[b] In the most linked group from the multilocus analysis.

which leads to the proposition that this gene is a candidate for T1DM (Delépine et al. 2000). The evidence of linkage at this locus was increased in the HLA DR3/4+ sib pairs (multilocus LOD score 2.6), compared with that observed in the complete data set. This suggests that *EIF2AK3*—or another gene located in this region—may contribute to T1DM susceptibility. The *EIF2AK3* region is distinct from the previously reported putative susceptibility loci—*IDDM7* (MIM 600321), *IDDM12* (MIM 601388), and *IDDM13* (MIM 601318)—on chromosome 2q (Cordell et al. 1995; Luo et al. 1995; Owerbach and Gabbay 1995; Concannon et al. 1998; Mein et al. 1998). Significant linkage to the *IDDM7–IDDM12* region has been reported, with replication, in a partly independent data set (table 1). Suggestive linkage to *IDDM13* has been reported (table 1). *CTLA4*, which maps to the *IDDM12* region, has been shown to be associated with T1DM (Nisticò et al. 1996; Marron et al. 1997; Larsen et al. 1999). Since we have excluded linkage to *IDDM7–IDDM12* for $\lambda_S \geq 2$, we cannot exclude the possibility that this region contains a gene with a relatively small effect on the risk for development of T1DM. The *IDDM13* region was excluded at $\lambda_S \geq 1.5$, suggesting that this region either does not contain a susceptibility gene in this population or contains one with a very small effect on the risk for T1DM.

The region of linkage on chromosome 16p extends over ~50 cM between D16S405 and D16S207 (fig. 2). Furthermore, increased evidence of linkage with heterogeneity was obtained in groups defined on the basis of HLA, *INS*, and population of origin, with peaks in the three groups extending over a 30-cM region. The 1-LOD support interval for the HLA-DR3/4 and INS− groups did not overlap (being located at 0–22 and 26–45 cM respectively; see fig. 2). In addition, single-point data in the INS− group reached its maximum for D16S3113 and D16S3131 (LOD score 2.9 and 3.0, respectively) whereas in the DR3/4 group the LOD score for these markers was 0.2 and 0.1, respectively. These observations suggest that this extended region contains more than one T1DM-susceptibility locus. However, simulation studies have shown that linkage analysis of multifactorial diseases may lead to linkage peaks some distance from the causative genetic effect (Roberts et al. 1999). The region partly overlaps that reported at D16S420–D16S261 (Mein et al. 1998), for which a LOD score of 1.2 was found in the UK263-family subset. In the U.K. study, the LOD score was also greater in HLA-DR3/4 sib pairs (LOD score 2.2). However, this region is distinct from a second region, on 16q22-q24, that has been reported to be linked to T1DM in U.K. families (Mein et al. 1998).

Suggestive evidence of linkage on chromosome 5p11-q13 also was found. This region had not previously been linked to T1DM; however, in two independent studies of multiple sclerosis, evidence of linkage had been found in this region. In one study, a LOD >3 was reached with a dominant model of inheritance (Kuokkanen et al. 1996; Sawcer et al. 1996; Oturai et al. 1999). This region is different both from the recently reported *IDDM18* (MIM 605598) locus (*IL12B*) at 5q31.1-q33.1 (Morahan et al. 2001) and from other chromosome 5 T1DM loci reported in U.K. families (Mein et al. 1998).

Eleven other chromosome regions were identified that have nominal evidence of linkage ($.05 > P > .00074$; table 3). Although some of these overlap putative regions with T1DM-susceptibility loci from the literature, the data presently available are not sufficient to determine which, if any, of these regions contain disease-susceptibility loci. Effects of $\lambda_S \geq 1.5$ could be excluded at previously reported T1DM loci on chromosome 1, 6,

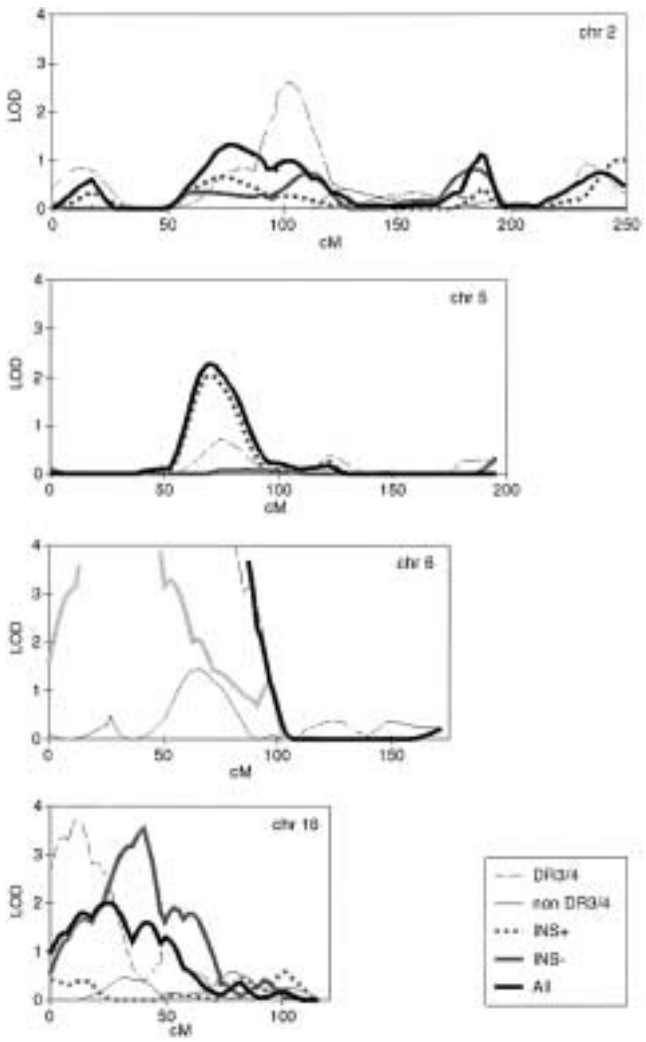

Figure 2 Multipoint linkage analysis using ALLEGRO, in HLA DR3/4 families (*thinner black hashed line*), non-DR3/4 families (*thinner black solid line*), INS+ families (*gray hashed line*), INS− families (*solid gray line*), and all families (*thicker black solid line*). A linear model and equal weighting of all families were used.

11, 14, 16, and 18 (table 1 and fig. 1), showing that these loci per se do not play a detectable role in T1DM susceptibility in the Scandinavian population.

We have observed significant heterogeneity in IBD sharing, for the chromosome 16 region, when sib pairs were grouped on the basis of shared HLA alleles (table 5). Similar results also were observed, for both the chromosome 16 region and the HLA region, when sib pairs were grouped on the basis of shared *INS* genotype (table 5). This suggests that there is both an interaction between the chromosome 16 region and both the HLA and *INS* and, similarly, an interaction between the *INS* and HLA loci. The LOD-score differences between different HLA and *INS* groups, presented in figure 2, are exploratory in nature and do not prove an interaction, for example, between the chromosome 2 region and the HLA region.

The results of this study demonstrate highly significant evidence of linkage to *IDDM15* at 6q, when data from the present study are combined with those from previous investigations. Evidence of interaction between susceptibility loci for T1DM has been obtained in a number of studies (Davies et al. 1994; Cordell et al. 1995; Mein et al. 1998). This concept is supported in the present study, in particular by the results obtained, after stratification based on either HLA or *INS*, on linkage to chromosome 16p (table 5). Gene-gene interaction is clearly an important feature of genetic predisposition, and studies addressing this are very much in demand to explain the molecular basis of disease and to develop new intervention modalities. Extensive investigations of populations, such as the Scandinavian families studied here, and the combination of data from multiple sources are required to obtain the requisite power to detect interacting loci with relatively small effects on familial recurrence risk of the disease.

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Electronic-Database Information

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

ANALYZE, ftp://ftp.ebi.ac.uk/pub/software/linkage_and_mapping/linkage_cpmc_columbia/analyze/

ASPEX Package, The: Affected Sib Pair Exclusion Mapping, ftp://lahmed.stanford.edu/pub/aspex/index.html

Center for Medical Genetics, Marshfield Medical Research Foundation, http://research.marshfieldclinic.org/genetics/

DOWNFREQ, ftp://ftp.ebi.ac.uk/pub/software/linkage_and_mapping/linkage_cpmc_columbia/analyze/

Généthon, http://www.genethon.fr/genethon_en.html

Online Mendelian Inheritance of Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for T1DM [MIM 222100], HLA-DRB1 [MIM 142857], IDDM2 [MIM 125852], IDDM7 [MIM 600321], IDDM12 [MIM 601388], IDDM13 [MIM 601318], IDDM15 [MIM 601666], and IDDM18 [MIM 605598])

References

Bell GI, Horita S, Karam JH (1984) A polymorphic locus near the human insulin gene is associated with insulin-dependent diabetes mellitus. Diabetes 33:176–183

Blohmé G, Nyström L, Arnqvist HJ, Lither F, Littorin F, Olsson PO, Scherstén B, Wibell L, Östman J (1992) Male predominance of type 1 (insulin-dependent) diabetes mellitus in young adults: results from a 5-year prospective nationwide study of the 15–34 year age group in Sweden. Diabetologia 35:56–62

Concannon P, Gogolin-Ewens KJ, Hinds DA, Wapelhorts B, Morrison VA, Stirling B, Mitra M, Farmer J, Williams SR, Cox NJ, Bell GI, Risch N, Spielman RS (1998) A second-generation screen of the human genome for susceptibility to insulin-dependent diabetes mellitus. Nat Genet 19:292–296

Cordell HJ, Todd JA, Bennett ST, Kawaguchi Y, Farrall M (1995) Two-locus maximum lod score analysis of a multifactorial trait: joint considerations of IDDM2 and IDDM4 with IDDM1 in type 1 diabetes. Am J Hum Genet 57:920–934

Cucca F, Esposito L, Goy JV, Merriman ME, Wilson AJ, Reed PW, Bain SC, Todd JA (1998) Investigation of linkage of chromosome 8 to type 1 diabetes: multipoint analysis and exclusion mapping of human chromosome 8 in 593 affected sib-pair families from the U.K. and U.S. Diabetes 47:1525–1527

Dahlquist G, Blom L, Holmgren G, Hägglöf B, Larsson Y, Sterky G, Wall S (1985) The epidemiology of diabetes in Swedish children 0–14 years—a six-year prospective study. Diabetologia 28:802–808

Dahlquist G, Gustavsson KH, Holmgren G, Hägglöf B, Larsson Y, Nilsson KO, Samuelsson G, Sterky G, Thalme B, Wall S (1982) The incidence of diabetes mellitus in Swedish children 0–14 years of age. Acta Pædiatr Scand 71:7–14

Dahlquist G, Mustonen L (1994) Childhood onset diabetes—time trends and climatological factors. Int J Epidemiol 23: 1234–1241

Dahlquist G, Mustonen L, Swedish childhood diabetes study group (2000) Analysis of 20 years of prospective registration of childhood onset diabetes—time trends and birth cohort effects. Acta Paediatr 89:1231–1237

Davies JL, Cucca F, Goy JV, Atta ZA, Merriman ME, Wilson A, Barnett AH, Bain SC, Todd JA (1996) Saturation multipoint linkage mapping of chromosome 6q in type 1 diabetes. Hum Mol Genet 5:1071–1074

Davies JL, Kawaguchi Y, Bennett ST, Copeman JB, Cordell HJ, Pritchard LE, Reed PW, Gough SCL, Jenkins SC, Palmer SM, Balfour KM, Rowe BR, Farrall M, Barnett AH, Bain SC, Todd JA (1994) A genome-wide search for human type 1 diabetes susceptibility genes. Nature 371:130–136

Davis JA (1996) Insulin dependent diabetes mellitus in twins: differences between monozygotic and dizygotic twins may need to be taken into account. BMJ 312:313–314

Degnbøl B, Green A (1978) Diabetes mellitus among first- and second degree relatives of early diabetes. Ann Hum Genet 42:25–47

Delépine M, Nicolino M, Barrett T, Golamaully M, Lathrop GM, Julier C (2000) EIF2AK3, encoding translation initiation factor 2-α kinase 3, is mutated in patients with Wolcott-Rallison syndrome. Nat Genet 25:406–409

Delépine M, Pociot F, Habita C, Hashimoto L, Froguel P, Rotter J, Cambon-Thomsen A, Deschamps I, Djoulah S, Weissernbach J, Nerup J, Lathrop M, Julier C (1997) Evidence of a non-MHC susceptibility locus in type 1 diabetes linked to HLA on chromosome 6. Am J Hum Genet 60:174–187

Diabetes Epidemiology Research International Group (1990) Secular trends in incidence of childhood IDDM in 10 countries. Diabetes 39:858–864

Ehm MG, Wagner M (1998) A test statistic to detect errors in sib-pair relationships. Am J Hum Genet 62:181–188

Field LL, Tobias R, Thomson G, Plon S (1996) Susceptibility to insulin-dependent diabetes mellitus maps to a locus (IDDM11) on chromosome 14q24.3-q31. Genomics 33:1–8

Gudbjartsson DF, Jonasson K, Frigge ML, Kong A (2000) Allegro, a new computer program for multipoint linkage analysis. Nat Genet 25:12–13

Hashimoto L, Habita C, Beressl JP, Delepine M, Besse C, Cambon-Thomsen A, Deschamps I, Rotter JI, Djoulah S, James MR, Froguel P, Welssenbach J, Lathrop GM, Julier C (1994) Genetic mapping of a susceptibility locus for insulin-dependent diabetes mellitus on chromosome 11q. Nature 371: 161–164

Hauser ER, Boehnke M, Guo S-W, Risch N (1996) Affected-sib-pair interval mapping and exclusion for complex genetic traits: sampling conciderations. Genet Epidemiol 13:117–137

Julier C, Hyer RN, Davies J, Merlin F, Soularue P, Briant L, Cathelineu G, Deschamps I, Rotter JI, Froguel P, Boitard C, Bell JI, Lathrop GM (1991) Insulin-IGF2 region on chromosome 11p encodes a gene implicated in HLA-DR4-dependent diabetes susceptibility. Nature 354:155–159

Julier C, Lucassen A, Villedieu P, Delepine M, Levy-Marchal C, Danzé PM, Bianchi F, Coitard C, Froguel P, Bell J, Lathrop GM (1994) Multiple DNA variant association analysis: application to the insulin gene region in type 1 diabetes. Am J Hum Genet 55:1247–1254

Kaprio J, Tumiletho J, Koskenvuo M, Romanov K, Renuanen A, Erikson J, Stengård J, Kesäniemi YA (1992) Concordance

for type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes mellitus in a population-based cohort of twins in Finland. Diabetologia 35:1060–1067

Kruglyak L, Lander ES (1995) Complete multipoint sib-pair analysis of qualitative and quantitative traits. Am J Hum Genet 57:439–454

Kumar D, Gemayel NS, Deapen D, Kapadia D, Yamashita PH, Lee M, Dwyer JH, Roy-Burman P, Bray GA, Mack TM (1993) North-American twins with IDDM: genetic, etiological, and clinical significance of disease concordance according to age, zygosity, and the interval after diagnosis in first twin. Diabetes 42:1351–1363

Kuokkanen S, Sundvall M, Terwilliger JD, Tienari PJ, Wikström J, Holmdahl R, Pettersson U, Perltonen L (1996) A putative vulnerability locus to multiple sclerosis maps to 5p14-p12 in a region syntenic to the murine Eae2. Nat Genet 13:477–480

Kyvik KO, Green A, Beck-Nielsen H (1995) Concordance rates of insulin dependent diabetes mellitus: a population based study of young Danish twins. BMJ 311:913–917

Lander ES, Green P (1987) Construction of multilocus genetic linkage maps in humans. Proc Natl Acad Sci USA 84:2363–2367

Larsen ZM, Kristiansen OP, Mato E, Johannesen J, Puig-Domingo M, de Leiva A, Nerup J, Pociot F (1999) Title IDDM12 (CTLA4) on 2q33 and IDDM13 on 2q34 in genetic susceptibility to type 1 diabetes (insulin-dependent). Autoimmunity 31:35–42

Lorenzen T, Pociot F, Hougaard P, Nerup J (1994) Long-term risk for IDDM in first-degree relatives of patients with IDDM. Diabetologia 37:321–327

Lorenzen T, Pociot F, Stilgren L, Kristiansen OP, Johannesen J, Olsen PB, Walmar A, Larsen A, Albrechtsen NC, Eskildsen PC, Andersen OO, Nerup J, The Danish IDDM Epidemiology and Genetics Group (1998) Predictions of IDDM recurrence risk in offspring of Danish IDDM patients. Diabetologia 41:666–673

Lucassen AM, Julier C, Beressi J-P, Boitard C, Frougel P, Lathrop M, Bell JI (1993) Susceptibility to insulin dependent diabetes mellitus maps to a 4.1 kb segment of DNA spanning the insulin gene associated VNTR. Nat Genet 4:305–310

Luo D-F, Buzzetti R, Rotter JI, Maclaren NK, Raffel LJ, Nisticò L, Giovanni V, Pozzilli P, Thomson G, She J-X (1996) Confirmation of three susceptibility genes to insulin-dependent diabetes mellitus: IDDM4, IDDM5 and IDDM8. Hum Mol Genet 5:693–698

Luo D-F, Maclaren NK, Huang H-S, Muir A, She J-X (1995) Intrafamilial and case-control association analysis of D2S152 in insulin-dependent diabetes. Autoimmunity 21:143–147

Marron MP, Raffel LJ, Garchon H-J, Jacob CO, Serrano-Rios M, Larrad MTM, Teng W-P, Park Y, Zhang Z-X, Goldstein DR, Tao Y-W, Beaurain G, Bach J-B, Huang H-S, Luo D-F, Zeidler A, Rotter JI, Yang MCK, Modilevsky T, Maclaren NK, She J-X (1997) Insulin-dependent diabetes mellitus (IDDM) is associated with CTLA4 polymorphisms in multiple ethnic groups. Hum Mol Genet 6:1275–1282

Mein CA, Esposito L, Dunn MG, Johnson GCL, Timms AE, Goy JV, Smith AN, Sebag-Montefiore L, Merriman ME, Wilson AJ, Pritchard LE, Cucca F, Barnett AH, Bain SC, Todd JA (1998) A search for type 1 diabetes susceptibility genes in families from the United Kingdom. Nat Genet 19: 297–300

Miller SA, Dykes DD, Poleskey HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215

Morahan G, Huang D, Tait BD, Colma PG, Harrison LC (1996) Markers on distal chromosome 2q linked to insulin-dependent diabetes mellitus. Science 272:1811–1813

Morahan G, Huang D, Ymer SI, Cancilla MR, Stephen K, Dabadghao P, Werther G, Tait BD, Harrison LC, Colman PG (2001) Linkage disequilibrium of a type 1 diabetes susceptibility locus with a regulatory IL12B allele. Nat Genet 27: 218–221

Mølbak AG, Christau B, Marner B, Borch-Johnsen K, Nerup J (1994) Incidence of insulin-dependent diabetes mellitus in age groups over 30 years in Denmark. Diabetic Med 11: 650–655

Nerup J, Platz P, Andersen OO, Christy M, Lyngsøe J, Poulsen JE, Ryder LP, Thomse M, Nielsen LS, Svejgaard A (1974) HL-A antigens and diabetes mellitus. Lancet 2:864–866

Nisticò L, Buzzetti R, Pritchard LE, Van der Auwera B, Giovanni C, Bosi E, Larrad MTM, Rios MS, Chow CC, Cockram CS, Jacobs K, Mijovic C, Bain SC, Barnett AH, Vanderwalle CL, Schuit F, Gorus FK, Belgian Diabetes Registry, Tosi R, Pozzilli P, Todd JA (1996) The CTLA-4 gene region of chromosome 2q33 is linked to, and associated with, type 1 diabetes. Hum Mol Genet 5:1075–1080

Nyholt DR (2000) All LODs are not created equal. Am J Hum Genet 67:282–288

Nyström L, Dahlquist G, Rewers M, Wall S (1990) The Swedish childhood diabetes study: an analysis of the temporal variation in diabetes incidence 1978-1987. Int J Epidemiol 19:141–146

O'Connell JR, Weeks DE (1998) PedCheck: a program for identification of genotype incompatibilities in linkage analysis. Am J Hum Genet 63:259–266

Olerup O, Zetterquist H (1992) HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. Tissue Antigens 39:225–235

——— (1993) DR "low-resolution" PCR-SSP typing—a correction and an update. Tissue Antigens 41:55-56

Olmos P, A'Hern R, Heaton DA, Millward BA, Risley D, Pyke DA, Leslie RG (1988) The significance of concordance rate for type 1 (insulin-dependent) diabetes in identical twins. Diabetologia 31:747–750

Östman J, Arnquist H, Blohmé G, Lithner F, Littorin B, Nyström L, Sandström A, Scherstén B, Wall S, Wibell L (1986) Epidemiology of diabetes mellitus in Sweden: results of the first year of a prospective study in the population age group 15–34 years. Acta Med Scand 220:437–445

Oturai A, Larsen F, Ryder LP, Madse HO, Hillert J, Fredrikson A, Sandberg-Wollheim M, Laaksonen M, Koch-Henriksen N, Sawcer S, Fugger L, Sorensen PS, Svejgaard A (1999) Linkage and association analysis of susceptibility regions on chromosome 5 and 6 in 106 Scandinavian sibling pair families with multiple sclerosis. Ann Neurol 46:612–616

Owerbach D, Gabbay KH (1995) The HOXD8 locus (2q31)

is linked to type I diabetes: interaction with chromosome 6 and 11 disease susceptibility genes. Diabetes 44:132–136

Paterson AD, Petronis A (1999) Sex of affected sibpairs and genetic linkage to type 1 diabetes. Am J Med Genet 84:15–19

Pociot F (1996) Insulin-dependent diabetes mellitus—a polygenic disorder? Dan Med Bull 43:216–248

Pociot F, Nørgaard K, Hobolth N, Andersen O, Nerup J, Danish Study Group of Diabetes in Childhood (1993) A nationwide population-based study of the familial aggregation of type 1 (insulin-dependent) diabetes mellitus in Denmark. Diabetologia 36:870–875

Reed P, Cucca F, Jenkins S, Merriman M, Wilson A, McKinney P, Bosi E, Joner G, Rønningen K, Thorsby E, Undlien D, Merriman T, Barnett A, Bain S, Todd J (1997) Evidence for a type 1 diabetes susceptibility locus (IDDM10) on human chromosome 10p11-q11. Hum Mol Genet 6:1011–1016

Risch N (1987) Assessing the role of HLA-linked and unlinked determinants of disease. Am J Hum Genet 40:1–14

Risch N, Spiker D, Lotspeich L, Nouri N, Hinds D, Hallmayer J, Kalaydjieva L, et al (1999) A genome screen of autism: evidence for a multilocus etiology. Am J Hum Genet 65: 493–507

Roberts SB, MacLean CJ, Neale MC, Eaves LJ, Kendler KS (1999) Replication of linkage studies of complex traits: an examination of variation in location estimates. Am J Hum Genet 65:876–884

Sawcer S, Jones HB, Feakes R, Gray J, Smaldon N, Chataway J, Robertson N, Clayton D, Goodfellow PN, Compston A (1996) A genome screen in multiple sclerosis reveals susceptibility loci on chromosome 6p21 and 17q22. Nat Genet 13:464–468

Spielman RS, McGinnis RE, Ewens WJ (1993) Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). Am J Hum Genet 52:506–516

Terwilliger JD (1995) A powerful likelihood method for the analysis of linkage disequilibrium between trait loci and one or more polymorphic loci. Am J Hum Genet 56:777–787

Tillil H, Köbberling J (1987) Age-corrected empirical genetic risk estimates for first-degree relatives of IDDM patients. Diabetes 36:93–99

Tuomiletho J, Karvonen M, Pitkäniemi J, Virtala E, Kohtamäki K, Toivanen L, Tuomiletho-Wolf E, The Finnish Childhood Type 1 Diabetes Registry Group (1999) Record-high incidence of type 1 (insulin-dependent) diabetes mellitus in Finnish children. Diabetologia 42:655–660

Verge CF, Vardi P, Babu S, Bao F, Erlich HA, Bugawan T, Tiosano D, Yu L, Eisenbarth GS, Fain PR (1998) Evidence for oligogenic inheritance of type 1 diabetes in a large Bedouin Arab family. J Clin Invest 102:1569–1575

Wagner A, Tibben A, Bruining GJ, Aanstoot HJ, Tiems I, Blondeau MJCE, Niermeijer MF (1995) Preliminary experience with predictive testing for insulin-dependent diabetes mellitus. Lancet 346:380–381