The Predisposition to Type 1 Diabetes Linked to the Human Leukocyte Antigen Complex Includes at Least One Non–Class II Gene

Benedicte A. Lie,[1] John A. Todd,[5,*] Flemming Pociot,[4] Jørn Nerup,[4] Hanne E. Akselsen,[1] Geir Joner,[2] Knut Dahl-Jørgensen,[2] Kjersti S. Rønningen,[3] Erik Thorsby,[1] and Dag E. Undlien[1]

[1]Institute of Transplantation Immunology, The National Hospital, [2]Aker Diabetes Research Centre, Aker University Hospital, and [3]Department of Population Health Sciences, National Institute of Public Health, Oslo; [4]Steno Diabetes Center, Gentofte, Denmark; and [5]The Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford

Summary

The human leukocyte antigen (HLA) complex, encompassing 3.5 Mb of DNA from the centromeric *HLA-DPB2* locus to the telomeric *HLA-F* locus on chromosome 6p21, encodes a major part of the genetic predisposition to develop type 1 diabetes, designated *"IDDM1."* A primary role for allelic variation of the class II *HLA-DRB1, HLA-DQA1,* and *HLA-DQB1* loci has been established. However, studies of animals and humans have indicated that other, unmapped, major histocompatibility complex (MHC)–linked genes are participating in *IDDM1*. The strong linkage disequilibrium between genes in this complex makes mapping a difficult task. In the present paper, we report on the approach we have devised to circumvent the confounding effects of disequilibrium between class II alleles and alleles at other MHC loci. We have scanned 12 Mb of the MHC and flanking chromosome regions with microsatellite polymorphisms and analyzed the transmission of these marker alleles to diabetic probands from parents who were homozygous for the alleles of the *HLA-DRB1, HLA-DQA1,* and *HLA-DQB1* genes. Our analysis, using three independent family sets, suggests the presence of an additional type I diabetes gene (or genes). This approach is useful for the analysis of other loci linked to common diseases, to verify if a candidate polymorphism can explain all of the association of a region or if the association is due to two or more loci in linkage disequilibrium with each other.

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Address for correspondence and reprints: Dr. Benedicte A. Lie, Institute of Transplantation Immunology, The National Hospital, 0027 Oslo, Norway. E-mail: b.a.lie@labmed.uio.no

* Present affiliation: Cambridge Institute for Medical Research, Cambridge, United Kingdom.

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Introduction

Type 1 diabetes is a multifactorial disease, in which the insulin-producing β cells of the pancreas are destroyed by the immune system, a process determined by the activity of major histocompatibility complex (MHC)–restricted T lymphocytes. In humans and in animal models, it has been shown that the disease is genetically determined, most profoundly by the MHC on chromosome 6p21 but also by a number of other loci throughout the genome. The penetrance of this genetic program is influenced by as yet unidentified environmental factors (Todd 1991). By association and ancestral haplotype mapping, DNA sequencing, and functional studies, MHC-linked susceptibility and resistance to type 1 diabetes (*IDDM1;* MIM 142857) has been fine mapped to the MHC class II loci *HLA-DQB1*, *HLA-DQA1*, and *HLA-DRB1* (Todd et al. 1987). In parallel, the mouse homologue has been mapped genetically and biologically to the homologues of *HLA-DQ* and *HLA-DR*, the *IA* and *IE* loci in the murine MHC (Ikegami et al. 1995; Wicker et al. 1995). It is likely that the T lymphocyte–mediated destruction of β cells is governed by the highly polymorphic peptide-binding properties of HLA class II alleles. The vast majority of these polymorphisms are encoded by a single exon, exon 2 of the homologous class II genes. However, it is clear from studies in mice (Ikegami et al. 1995), and it has also been suggested by studies in humans (Thomsen et al. 1988; Risch 1989; Caplen et al. 1990; Nakanishi et al. 1993; Robinson et al. 1993; Fennessy et al. 1994; Fujisawa et al. 1995; Tait et al. 1995; Erlich et al. 1996) that sequence variation within exon 2 of these genes cannot explain all of the association of the MHC region with type 1 diabetes. Certain MHC haplotypes are differentially associated with disease even though the class II exon sequences are the same (Risch 1989; Langholz et al. 1995). Owing to linkage disequilibrium among loci, particularly on certain haplotypes such as the A1-B8-DR3 haplotype, it has been difficult to detect and even more difficult to map the additional effects outside the HLA class II exon 2 sequences.

Robinson et al. (1993) analyzed extended MHC haplotypes by using sib pairs affected with type 1 diabetes with parents homozygous for class II alleles. Their analysis suggested the presence of one or more additional disease loci by analyzing the presence of linkage in the region when the contribution from *HLA-DRB1* was fixed. We have now extended the homozygous-parent test by using the transmission/disequilibrium test (TDT) to evaluate the disease association of marker alleles of microsatellite polymorphisms across 12 Mb of chromosome 6p21, including the MHC and the hemochromatosis (*HFE*) gene region. Such a study has only become feasible recently, owing to the availability of DNA samples from very large numbers of families with type 1 diabetic children that have been HLA class II typed and to the characterization of several polymorphic microsatellites in the MHC and *HFE* regions. We present evidence for a second disease locus linked to, but distinct from, the classic HLA class II loci. Allele 3 of marker *D6S2223*, 5.5 Mb telomeric of the class II region, was strongly associated with type 1 diabetes ($P_{\text{corrected}}$ [P_c] = .0001). We cannot locate the etiological variant with any precision, but it is possible that the non–HLA class II locus is closer to the *HFE* locus, 8.5 Mb distal to the HLA class II loci, than to the classically defined MHC.

Subjects and Methods

Subjects

The regional committee for ethics in medical research in Norway approved this study. Families were selected from large data sets from Norway (526 families), Denmark (147 families), and the United Kingdom (333 families) that were used in previous studies (Undlien et al. 1995; Delepine et al. 1997; Merriman et al. 1997). To exclude associations secondary to linkage disequilibrium with already established high-risk *HLA-DRB1, HLA-DQA1,* and *HLA-DQB1* alleles during TDT analysis, all families selected to enter this study had at least one parent who was homozygous for *HLA-DRB1, HLA-DQA1,* and *HLA-DQB1* alleles. It is important to note that homozygosity is also required for specific HLA-DRB1*04 subtypes because this amino acid variation (encoded by exon 2) has an important role in type 1 diabetes (Sheehy et al. 1989; Cucca et al. 1995; Undlien et al. 1997*a*). These inclusion criteria resulted in a group of 100 Norwegian families, 51 Danish families, and 74 U.K. families for study. Of these 225 families, there were 116 DRB1*03-DQA1*0501-DQB1*0201 and 51 DRB1*0401-DQA1*03-DQB1*0302 homozygous parents; for other *DRB1-DQA1-DQB1* haplotypes, the number of homozygous parents was small ($n < 11$). This demonstrates a limitation of the approach, in that only alleles that are common can be evaluated, because only a few families have parents homozygous for haplotypes other than DR3 and DR4. Only one diabetic offspring from each family was included in the TDT analysis to make it a valid test for association. In multiplex families, the first child diagnosed was chosen. The Norwegian data set contained families with one child with type 1 diabetes diagnosed at age <15 years. In the families from Denmark, all affected children were diagnosed at age <20 years (Delepine et al. 1997). The U.K. families were restricted to whites with grandparents born in the United Kingdom, and the proband was given a diagnosis at age <17 years (Bain et al. 1990). The Norwegian and Danish family studies also consisted of DNA samples from siblings healthy (i.e., nondiabetic) at the time of sample collection.

The case-control sets consisted of 228 subjects with type 1 diabetes and 362 controls, who were either DRB1*03-DQA1*0501-DQB1*0201/DRB1*04-DQA1*03-DQB1*0302 heterozygotes, DRB1*03-DQA1*0501-DQB1*0201 homozygotes, or DRB1*04-DQA1*03-DQB1*0302 homozygotes. The subjects were recruited from pediatric departments throughout Norway during the period 1992–96, and they were all aged <15 years at disease onset. The controls were selected from the Norwegian Bone Marrow Donor Registry. Both cases and controls originated from the relatively ethnically homogeneous Norwegian population. Parts of this material have been used previously in studies on the contribution of DR4 subtypes (Undlien et al. 1997*a*), HLA class II large multifactorial protease polymorphisms (Undlien et al. 1997*b*), and *HLA-DPA1* and *HLA-DPB1* alleles (Lie et al. 1997) to type 1 diabetes susceptibility. A few samples were not typed for all markers, owing to either PCR failure (excluded when PCR failed three times) or lack of DNA.

Genomic HLA Typing

Typing for *HLA-DQA1, HLA-DQB1,* and DRB1*04 subtypes was performed as described elsewhere (Undlien et al. 1997*a*). Sixteen microsatellite markers (dinucleotide repeats), spanning from ~2.5 Mb centromeric to ~8.5 Mb telomeric of the HLA class II region, were selected, mainly from the article by Feder et al. (1996). In the first microsatellite-marker scan in the families, we included the following microsatellite markers: *D6S291, DQCAR, D6S273, D6S265, D6S2222, D6S2223, D6S2239,* and *D6S2235;* in the second step, we analyzed *D6S306, D6S1001, D6S105, D6S464, D6S2225, D6S2219, D6S1260,* and *D6S1558*. Primer sequences were obtained from the Genome Database (GDB). PCR products were separated on a 4.25% urea-polyacrylamide gel and were identified according to size, on an ABI 377 DNA sequencer. The alleles at each microsatellite were given a numerical value (1, 2, 3, 4, etc.),

Table 1

TDT of the Two Marker Alleles That Showed an Association with a Significant P_c Value in the Total Family Data Set

Origin of Families and Members Included in the TDT	*D6S2223* (Allele 3)					*D6S273* (Allele 2)				
	T	NT	%T (CI)	χ^2	P_{nc}	T	NT	%T (CI)	χ^2	P_{nc}
Total:										
Probandwise	9	37	20 (8–32)	17.0	.00004	21	5	81 (66–96)	9.8	.002
Healthy siblings	16	20	44 (28–60)	.4	NS	6	6	50 (22–78)	.0	NS
Norway:										
Probandwise	5	15	25 (6–44)	5.0	.03	9	1	90 (71–100)	6.4	.01
Healthy siblings	12	15	44 (25–63)	.3	NS	5	6	45 (16–74)	.1	NS
Denmark:										
Probandwise	1	7	13 (0–36)	4.5	.03	5	1	83 (53–100)	2.7	NS
Healthy siblings	4	5	44 (12–76)	.1	NS	1	0	…	1.0	NS
United Kingdom:										
Probandwise	3	15	17 (0–34)	8.0	.005	7	3	70 (42–98)	1.6	NS

Note.—Allele 3 at *D6S2223* and allele 2 at *D6S273* from DRB1*03-DQA1*0501-DQB1*0201 homozygous parents in the individual data sets of type 1 diabetes families from Norway, Denmark, and the United Kingdom. T = no. transmitted, NT = no. not transmitted, and NS = not significant.

starting at the allele with the fewest number of base pairs. In some instances, this value was not in agreement with the GDB terminology.

Statistical Methods

Initially, we tested the microsatellites by TDT in three independent family data sets, in which at least one parent per family was homozygous at the HLA class II loci *HLA-DRB1, HLA-DQA1,* and *HLA-DQB1*. Only alleles with a frequency >5% and with ≥20 informative meiosis in the total data set were included. The TDT analyzes whether transmission of an allele, from parents heterozygous for the marker tested to the proband, deviates from the expected 50% (Spielman et al. 1993) and gives a χ^2 test statistic with 1 df. Confidence intervals (95% [CI]) for the percentage transmission (%T) of alleles were calculated by application of the formula $CI = \%T \pm 1.96\sqrt{[p(1\text{-}p)/n]}$, in which p denotes the proportion of positive transmissions and n denotes the total number of transmissions. Only transmissions from *HLA-DRB1, HLA-DQA1,* and *HLA-DQB1* homozygous parents were included in the analysis, to exclude secondary effects resulting from linkage disequilibrium with high-risk *HLA-DRB1, HLA-DQA1,* and *HLA-DQB1* alleles. We refer to this test as the "homozygous-parent TDT." The transmissions from *HLA-DRB1, HLA-DQA1,* and *HLA-DQB1* homozygous parents were evaluated separately for different HLA class II haplotypes, because an association might emerge differently on distinct haplotypes or may even be haplotype dependent. The SIMCROSS program was used to estimate haplotypes (Weeks et al. 1995), and then manual inspection of pedigrees was performed.

We compared the allele frequencies among subjects and controls using χ^2 analysis or Fisher's exact test when appropriate. Subjects were grouped according to *HLA-DRB1, HLA-DQA1,* and *HLA-DQB1* genotypes (including HLA-DRB1*04 subtypes) and were tested for association separately, to exclude secondary associations resulting from linkage disequilibrium with these high-risk alleles.

Linkage disequilibrium between markers was calculated as normalized values of either pairwise disequilibrium statistics (D') or global disequilibrium statistics (W_n) (Klitz et al. 1995). Both values show a range of from 1 to −1 (representing complete disequilibrium [1] to complete equilibrium [0] to never occurring on the same haplotype [−1]). All parental haplotypes were used in these calculations.

Results

Initially, eight microsatellite markers, spanning from ~2.5 Mb centromeric to ~5.5 Mb telomeric of the HLA complex, were tested with the homozygous-parent TDT in the selected 225 families. Allele 3 of *D6S2223* showed association with disease after correction for the total number of alleles tested ($n = 25$; $P_c = .001$ [see table 1 and fig. 1*a*]) in the 116 DRB1*03-DQA1*0501-DQB1*0201 homozygous-parent families. Marker *D6S2223* is located ~5.5 Mb telomeric of HLA class II and ~1.5 Mb centromeric of the *HFE* locus (fig. 1*a*). Linkage disequilibrium was strong between HLA class II (*DQCAR*) and *D6S2223* ($W_n = .49$, $\chi^2 = 720$, 48 df, $P \ll .001$). The reduced transmission of allele 3 to probands was consistent in all three populations studied

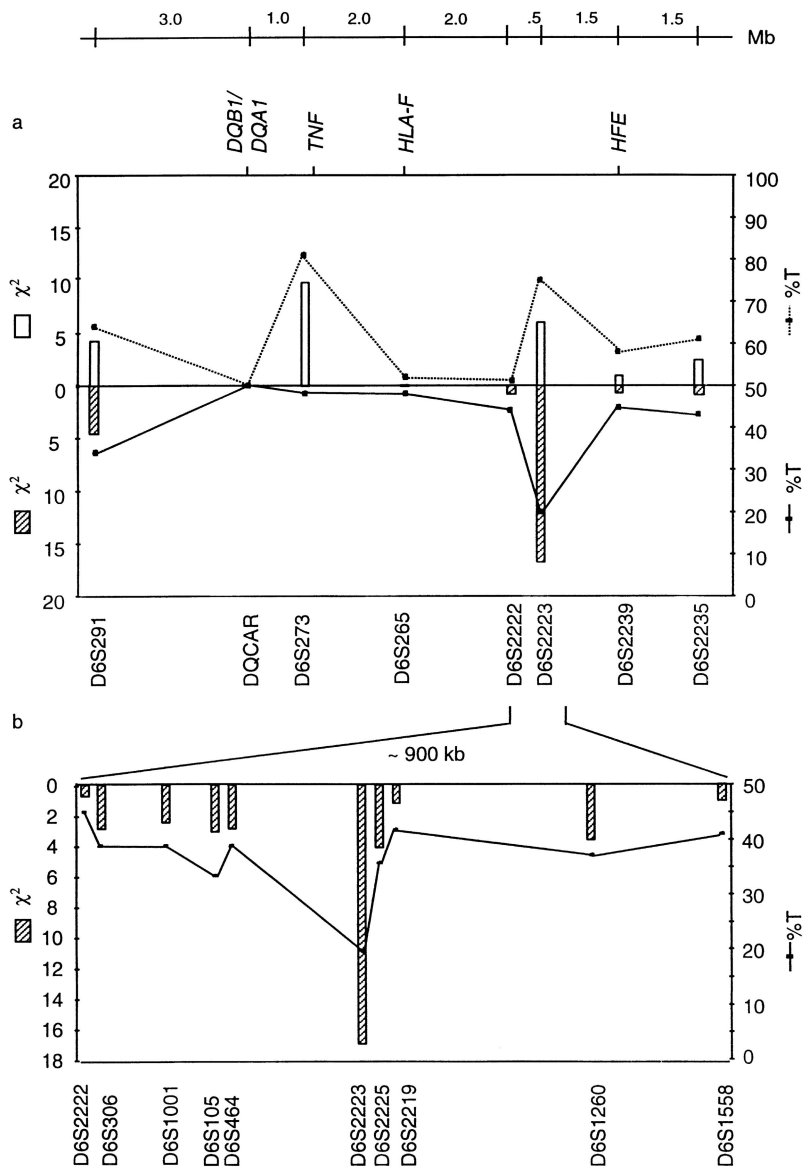

Figure 1 TDT from DRB1*03-DQA1*0501-DQB1*0201 homozygous parents, for all microsatellites analyzed in this study, presented for the total family data set (from Norway, Denmark, and the United Kingdom). Both the χ^2 test statistics and %T of positively (denoted by unhatched bars and doted lines) and negatively (denoted by hatched bars and solid lines) associated alleles are shown. The location of nearby genes is outlined, and the distances are approximate; n = no. of informative meiosis for each marker allele. *a*, Microsatellites analyzed in the initial screen covering ~12 Mb. The positively and negatively transmitted alleles presented are the following: allele 3 (n = 53) and allele 2 (n = 47) at *D6S291*, allele 2 (n = 26) and allele 7 (n = 48) at *D6S273*, allele 1 (n = 18) and allele 5 (n = 27) at *D6S265*, allele 3 (n = 55) and allele 2 (n = 50) at *D6S2222*, allele 2 (n = 24) and allele 3 (n = 45) at *D6S2223*, allele 2 (n = 39) and allele 3 (n = 57) at *D6S2239*, and allele 4 (n = 50) and allele 2 (n = 46) at *D6S2235*. Only a few *DRB1-DQA1-DQB1* homozygous parents were heterozygous for *DQCAR*, and there was no biased transmission. Only alleles with $\chi^2 > 9.1$ are still significant after correction for multiple tests (25 alleles). *b*, More densely spaced markers in 900-kb region flanking marker *D6S2223*. The most negatively transmitted alleles for the additional markers are presented. These are as follows: allele 6 (n = 61) at *D6S306*, allele 11 (n = 51) at *D6S1001*, allele 10 (n = 27) at *D6S105*, allele 10 (n = 59) at *D6S464*, allele 5 (n = 53) at *D6S2225*, allele 3 (n = 56) at *D6S2219*, allele 4 (n = 54) at *D6S1260*, and allele 1 (n = 22) at *D6S1558*.

(20% transmission overall). Importantly, transmission to unaffected siblings was not different from 50% (table 1).

One other allele, allele 2 of marker *D6S273*, located between *TNF* and *HSP70* (D'Alfonso and Richiardi 1996), showed some evidence for nonrandom transmission from DRB1*03-DQA1*0501-DQB1*0201 homozygous parents (81% T, P_c = .05; table 1). This marker

has been reported previously to be associated with type 1 diabetes in a Belgian case-control study (Moghaddam et al. 1998). In contrast to our results, Moghaddam and colleagues found allele 2 (130 bp) on the DRB1*03 haplotype to be less frequent (although not significantly) among patients. They observed a negatively associated allele at *D6S273* (140 bp), which was equally distributed among subjects and controls (both independent control subjects and family-based ones) in our data sets. This allele is not in linkage disequilibrium with allele 3 at *D6S2223* ($D' = .07$, $P = .02$).

To extend support for the disease association observed for allele 3 at *D6S2223* and for allele 2 at *D6S273,* we analyzed these two marker alleles in an independent and completely *HLA-DRB1, HLA-DQA1,* and *HLA-DQB1* matched Norwegian case-control set (table 2). Allele 3 at *D6S2223* was found with decreased frequency among DRB1*03-DQA1*0501-DQB1*0201 homozygous subjects, compared with DRB1*03-DQA1*0501-DQB1*0201 homozygous controls ($P = .03$, $P_c = .27$), which is consistent with the observations from all three family data sets. Allele 2 at *D6S273* had increased frequencies in heterozygous subjects carrying either the genotype DRB1*0401-DQA1*03-DQB1*0302/DRB1*03-DQA1*0501-DQB1*0201 ($P = .04$, $P_c = .36$) or DRB1*401-DQA1*03-DQB1*0302/DRB1*0404-DQA1*03-DQB1*0302 ($P = .01$, $P_c = .09$), compared with *DRB1-DQA1-DQB1* matched controls.

We wanted to test whether the associations with *D6S2223* and *D6S273* were independent from or a result of linkage disequilibrium between alleles of the two markers. The global disequilibrium statistic between *D6S273* and *D6S2223* was $W_n = .27$ ($\chi^2 = 222$, 32 df, $P \ll .001$). When the TDT was applied only to parents homozygous for DRB1*03-DQA1*0501-DQB1*0201 and alleles at *D6S273,* the disease association with allele 3 at *D6S2223* was even stronger (15%T, $P = .002$, $P_c = .004$). For allele 2 of *D6S273,* the disease association was slightly reduced when the parents were homozygous for both class II and *D6S2223* (71%T, P = ns). Some degree of negative linkage disequilibrium between allele 3 at *D6S2223* and allele 2 at *D6S273* exists ($D' = -.4$, $P_c = .003$).

To evaluate the extent of the associated region containing marker *D6S2223,* we analyzed a much more densely spaced set of polymorphic markers, spaced an average of every 100 kb and covering ~400 kb and ~500 kb of DNA centromeric and telomeric, respectively, of *D6S2223*. The linkage disequilibrium values between the adjacent microsatellites are as follows (calculated by W_n): *D6S2222*–(.41)–*D6S306*–(.43)–*D6S1001*–(.35)–*D6S105*–(.41)–*D6S464*–(.60)–*D6S2223*–(.30) –*D6S2225*–(.16)–*D6S2219*–(.34)–*D6S1260*–(.34)–*D6S1558,* and all $P \ll .001$. Negative transmission of a marker allele to diabetic probands from DRB1*03-DQA1*0501-DQB1*0201 homozygous parents, observed with *D6S2223,* was also observed for allele 5 of *D6S2225* (37%T, $P = .05$, $P_c = 1$), the closest marker to *D6S2223*.

No significant biased transmission of alleles from other *HLA-DRB1, HLA-DQA1,* and *HLA-DQB1* homozygous parents (not DRB1*03-DQA1*0501-DQB1*0201) were observed ($n = 107$; data not shown). Finally, the paternal and maternal transmissions to probands were analyzed separately, but no heterogeneity was observed (data not shown).

Discussion

The homozygous-parent TDT is a new approach for fine mapping of disease-linked chromosome regions. Our data for MHC in type 1 diabetes suggest that there is at least one additional component outside exon 2 of *HLA-DRB1, HLA-DQA1,* and *HLA-DQB1*. Allele 3 of *D6S2223* appears to be in linkage disequilibrium with an allele at a locus that affects the etiology of type 1 diabetes on DRB1*03-DQA1*0501-DQB1*0201 haplotypes. This conclusion is supported by $P_c = .001$ in families, with the same trend shown in a second independent HLA class II–matched case-control data set ($P_c = .27$). Our data are consistent with previous estimates by Robinson et al. (1993) that 40% of DR3 haplotypes confer a significantly higher risk than other DR3

Table 2

Case-Control Analysis of *D6S2223* and *D6S273*

DRB1-DQB1 Genotypes	*D6S2223* (Allele 3)			*D6S273* (Allele 2)		
	Patients (%)	Controls (%)	P_{nc}	Patients (%)	Controls (5)	P_{nc}
03-0201/03-0201	38 (66)	119 (80)	.03	5 (8.6)	5 (3.5)	NS
03-0201/0401-0302	167 (67)	163 (73)	NS	16 (6.5)	6 (2.6)	.04
03-0201/0404-0302	48 (80)	141 (71)	NS	7 (11.7)	9 (4.5)	NS
0401-0302/0401-0302	27 (56)	27 (68)	NS	2 (4.2)	0	NS
0401-0302/0404-0302	18 (90)	30 (75)	NS	4 (20)	0	.01

Note.—Only the *DRB1-DQA1-DQB1* genotype groups with more than five individuals among both cases and controls are included. NS = not significant.

haplotypes, by use of the homozygous-parent affected–sib pair method. Nevertheless, the result has to be confirmed in further data sets.

Results of a case-control study that suggested an association between marker *D6S273* and type 1 diabetes were reported recently (Moghaddam et al. 1998). We also found some evidence for an association for *D6S273* by using the homozygous-parent TDT with allele 2, conferring disease susceptibility on the DRB1*03-DQA1*0501-DQB1*0201 haplotype. Moghaddam et al. (1998) found this allele (130 bp) to provide susceptibility on the DRB*0401 haplotype, whereas on the DRB1*03 haplotype it showed a nonsignificant tendency of being protective. In our case-control study, allele 2 appeared at a higher frequency in all patient groups, subdivided by class II haplotypes, compared with controls, but only provided association at $P < .05$ in the DRB1*03 homozygous and DRB1*0401/DRB1*0404 heterozygous individuals. Therefore, our results for allele 2 at *D6S273* are partly consistent with the study by Moghaddam et al. (1998). However, the associations they detected for other alleles at *D6S273* were not replicated in our analyses.

The fact that no biased transmission to healthy siblings was observed in the Danish and Norwegian families (table 1) strongly supports the validity of the homozygous-parent TDT and of our conclusions, and it also rules out segregation distortion as an explanation for the associations observed with type 1 diabetes. The observation of linkage disequilibrium between class II and *D6S2223,* separated by 5.5 Mb of DNA, shows the necessity of controlling for *DRB1-DQA1-DQB1* by use of the homozygous-parent TDT, even for markers located far from the known etiological loci.

The DRB1*03-DQA1*0501-DQB1*0201 haplotype was the most frequent among our *DRB1-DQA1-DQB1* homozygous parents (52%) and was the only haplotype for which we detected nonrandom transmission of microsatellite-marker alleles. The lack of association for other *DRB1-DQA1-DQB1* haplotypes could be because of insufficient sample size or because the effect of the additional gene(s) may only occur on DRB1*03-DQA1*0501-DQB1*0201 haplotypes. The limitation of the homozygous-parent TDT is that only DR-DQ haplotypes with high risk to develop the disease are present in sufficiently high frequencies to make them useful for statistical analyses. Only parents homozygous for the established or suspected disease alleles are informative in this test.

Although our results indicate the presence of another gene(s) outside the HLA class II region, we cannot map its location. The problem with linkage-disequilibrium mapping is that the association of a marker with disease does not necessarily reflect its proximity to the etiological locus, because the distribution of marker alleles on predisposing and nonpredisposing chromosomes is unpredictable. However, each of the markers used in this study have been shown to be in linkage disequilibrium with its adjacent markers. Therefore, given the fact that no microsatellites flanking *D6S2223* showed any association in the homozygous-parent TDT, the most likely location of the proposed and novel type 1 diabetes–involved gene in linkage disequilibrium with *D6S2223* would be telomeric of *HLA-F,* in the vicinity of *D6S2223*. The *HLA-A* gene, which has been proposed by others to be involved in type 1 diabetes (Nakanishi et al. 1993), is 2.8-Mb centromeric of *D6S2223,* but our results do not point to this locus. This does not mean that there is no separate association at this locus but simply that it could not be detected by use of the microsatellite markers evaluated in the present study. Moreover, we cannot formally rule out that the second locus is within the class II region itself, outside the exon 2 sequences.

How can the putative etiological determinant near *D6S2223* be mapped? *D6S2223*-defined haplotypes could be further characterized by use of a dense set of microsatellites and single-nucleotide polymorphisms (SNPs) around the associated marker. This would enable us to define ancestral chromosome segments shared identical-by-descent between nonpredisposing *D6S2223* allele 3–positive haplotypes that are distinct from those of other haplotypes showing no association ("cross-match" haplotype mapping) (Todd et al. 1987, 1989; Degli-Esposti et al. 1992; Bennett and Todd 1996). It will be possible to identify ancient recombination breakpoints that should yield fine-mapping information. We can also analyze the association of haplotype segments in different populations, such as Asian, African, and Sardinian, in the same way these populations were used to map the class II loci as etiological determinants (Todd et al. 1989; Sheehy et al. 1989; Cucca et al. 1995). Perhaps the most productive way to proceed is to search all known genes in linkage disequilibrium with *D6S2223* for SNPs, to test each as a candidate that might explain the association of *D6S2223*. Candidate polymorphisms that have allele distributions consistent with the haplotype associations described here will be studied for functional activity. With an ongoing worldwide effort to identify very large numbers of common SNPs and emerging technology for typing SNPs, use of this approach with every gene in the MHC should become feasible in the near future. A combination of genetic and functional analysis, as used for the identification of *IDDM1* and *IDDM2,* will be necessary, as well as continued analysis of the mouse and rat models of disease.

In conclusion, this study provides consistent evidence for at least one novel diabetes gene other than those defined by the exon 2 polymorphism of the *HLA-DRB1, HLA-DQA1,* and *HLA-DQB1* loci. Our data provide

consistent evidence for linkage and association to marker *D6S2223*, located 5.5 Mb telomeric of the HLA class II region. *IDDM1* appears to be a cluster of linked disease determinants. Clustering of separate disease loci in regions defined by a single peak of linkage may be characteristic of common diseases caused by low-penetrance alleles at multiple loci. In the spontaneous mouse model of type 1 diabetes, we now know that a linkage peak on chromosome 3 comprises at least four separate disease loci (Podolin et al. 1998). The possibility of multiple closely linked disease loci makes the prospect for their identification even more challenging, particularly in outbred human populations. The homozygous-parent TDT can be a useful tool for this purpose.

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Electronic-Database Information

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

Genome Database (GDB), http://gdb.gdbnet.ad.jp/gdb/docs/gdbhome.html (for primer sequences)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for *IDDM1* [MIM 142857])

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