Am. J. Hum. Genet. 60:174–187, 1997

Evidence of a Non-MHC Susceptibility Locus in Type I Diabetes Linked to HLA on Chromosome 6

Marc Delépine,[1] Flemming Pociot,[2] Cellia Habita,[1] Lara Hashimoto,[1] Philippe Froguel,[3] Jerome Rotter,[4] Anne Cambon-Thomsen,[5] Inge Deschamps,[6] Sami Djoulah,[7] Jean Weissenbach,[8] Jørn Nerup,[2] Mark Lathrop,[1] and Cécile Julier[1]

[1]Wellcome Trust Centre for Human Genetics, Oxford; [2]Steno Diabetes Centre, Gentofte, Denmark; [3]Institut Pasteur et CHU de Lille, Lille; [4]Division of Medical Genetics, Cedars-Sinai Medical Center and University of California, Los Angeles, School of Medicine, Los Angeles; [5]CNRS UPR8291, Centre d'immunopathologie et de génétique humaine, Hôpital Purpan, Toulouse; [6]Hôpital des Enfants Malades and [7]INSERM U93, Hôpital Saint Louis, Paris; and [8]Généthon, Evry

Summary

Linkage studies have led to the identification of several chromosome regions that may contain susceptibility loci to type I diabetes (IDDM), in addition to the HLA and *INS* loci. These include two on chromosome 6q, denoted *IDDM5* and *IDDM8*, that are not linked to HLA. In a previous study, we noticed that the evidence for linkage to IDDM susceptibility around the HLA locus extended over a total distance of 100 cM, which suggested to us that another susceptibility locus could reside near HLA. We developed a statistical method to test this hypothesis in a panel of 523 multiplex families from France, the United States, and Denmark (a total of 667 affected sib pairs, 536 with both parents genotyped), and here present evidence ($P = .00003$) of a susceptibility locus for IDDM located 32 cM from HLA in males but not linked to HLA in females and distinct from *IDDM5* and *IDDM8*. A new statistical method to test for the presence of a second susceptibility locus linked to a known first susceptibility locus (here HLA) is presented. In addition, we analyzed our current family panel with markers for *IDDM5* and *IDDM8* on chromosome 6 and found suggestions of linkage for both of these loci ($P = .002$ and .004, respectively, on the complete family panel). When cumulated with previously published results, with overlapping families removed, the affected-sib-pair tests had a significance of $P = .0001$ for *IDDM5* and $P = .00004$ for *IDDM8*.

Introduction

Type I diabetes (IDDM) is a multifactorial disease that results from immune-mediated destruction of the insulin-producing β pancreatic islet cells. The major histocompatibility complex (MHC) on chromosome 6 has been shown to contain one or more major genetic determinant(s) of disease susceptibility and accounts for >40% of the sibling recurrence risk (Rotter and Landaw 1984; Risch 1987). The insulin gene (*INS*) on chromosome 11p15.5 also contributes to IDDM susceptibility, as demonstrated by association and linkage studies (Bell et al. 1984; Thomson et al. 1989; Julier et al. 1991; Bain et al. 1992; Lucassen et al. 1993; She et al. 1994; McGinnis and Spielman 1995; Undlien et al. 1995; van der Auwera et al. 1993). The VNTR located 5′ of the *INS* gene is the most likely susceptibility variant; it could act through an effect on the transcription level of the gene (Bennett et al. 1995; Kennedy et al. 1995; Lucassen et al. 1995). Together, HLA and *INS* probably account for <50% of the familial aggregation, and thus other genes are also likely to contribute to disease susceptibility.

Results from two genome-wide linkage studies (Davies et al. 1994; Hashimoto et al. 1994) and other investigations (Field et al. 1994, 1996; Luo et al. 1995; Owerbach and Gabbay 1995) have provided evidence for the presence of other susceptibility loci in several chromosome regions. Support for several of these linkages have been found in more than one study. These are *IDDM3* on chromosomes 15 (Field et al. 1994; Luo et al. 1995, 1996), *IDDM4* on chromosome 11q13 (Davies et al. 1994; Field et al. 1994; Hashimoto et al. 1994; Luo et al. 1996), *IDDM7* on chromosome 2q (Davies et al. 1994; Copeman et al. 1995; Owerbach and Gabbay 1995), and two loci on chromosome 6, separated by 27 cM, *IDDM5* (Davies et al. 1994; Luo et al. 1996) and *IDDM8* (Davies et al. 1994; Luo et al. 1995, 1996). Studies of these loci and other regions continue, and recently an additional susceptibility locus, *IDDM11*, was reported on chromosome 14 (Field et al. 1996).

In our previous study (Hashimoto et al. 1994), we noticed that evidence for linkage in the MHC region extended over a distance of 100 cM in the Généthon genetic map (Dib et al. 1996) from D6S309, 30 cM on

Received June 17, 1996; accepted for publication October 18, 1996.
Address for correspondence and reprints: Dr. Cécile Julier, Wellcome Trust Centre for Human Genetics, Windmill Road, Oxford OX3-7BN, United Kingdom. E-mail: cecile@well.ox.ac.uk
© 1997 by The American Society of Human Genetics. All rights reserved.
0002-9297/97/6001-0024$02.00

the proximal side of HLA, to D6S283, 70 cM on its distal side. The effects of susceptibility loci such as HLA may lead to broad regions of linkage in multifactorial diseases (J. Terwilliger, W. Shannon, G. Lathrop, J. Nolan, L. Goldin, G. Chase, and D. Weeks, unpublished data); alternatively, our observation could be due to the presence of a second susceptibility gene near HLA. The other chromosome 6 regions (at 6q25 and 6q27) that have been reported to contain IDDM susceptibility loci are unlinked to HLA. However, in the nonobese diabetic (NOD) mouse, evidence from congenic NOD strains supports the presence of a susceptibility locus in an 11-cM segment of mouse chromosome 17 adjacent to, but distinct from, the murine MHC locus H2 (Ikegami et al. 1995).

Linked susceptibility loci for a single trait have been described in rodent models of multifactorial diseases (e.g., Ghosh et al. 1993; Kreutz et al. 1995; Gu et al. 1996) and may be expected occasionally in humans. Two closely linked susceptibility loci are likely to be indistinguishable in most linkage studies, but it may be possible to separately discern their effects if they are distantly linked, although the standard nonparametric methods of linkage analysis are not applicable for this. In principal, multilocus parametric approaches, such as implemented by Lathrop and Ott (1990), or techniques, such as the two-locus maximum-LOD-score approach of Cordell et al. (1995), could be applied to test for the effects of linked susceptibility loci, but they require explicit assumptions regarding the inheritance of the disease trait, and the likelihood calculations are often computationally intense with the need of substantial computer time.

Here we describe a simple and easy to implement extension of identity-by-descent (IBD) methods to two linked susceptibility loci. The method, which is based on weighted logistic analysis, provides a test for a linked susceptibility locus, given the presence of a first susceptibility locus; sex-specific recombination distances and tests of epistasis can be incorporated. We apply the method to data from French, U.S., and Danish multiplex IDDM families and show evidence for a non-HLA susceptibility gene mapping ~40 cM distal to HLA in the sex-averaged genetic map. We also provide new data to evaluate regions on chromosome 6 in which other non-MHC susceptibility loci have been detected (*IDDM5* and *IDDM8*).

Subjects, Material, and Methods

Families

A total of 523 multiplex IDDM families of Caucasian origin containing 667 affected sib pairs from France (128 families containing 159 affected sib pairs), North Africa (23 families containing 25 affected sib pairs), the United States (225 families containing 310 sib pairs), and Denmark (147 families containing 173 affected sib pairs) were studied. DNA samples from both parents were available for 536 of the affected sib pairs. A subset of the French panel has been described elsewhere (Hashimoto et al. 1994). In the French families, diabetics were either positive for islet cell autoantibodies or had onset prior to 45 years of age, or both. All were ketone positive at the time of diagnosis and required daily treatment with insulin. The 23 families of North African origin were resident in France or in Algeria and were ascertained under the same criteria as the French panel. U.S. families were obtained from the Human Biological Data Interchange (HBDI) repository as described by Lernmark et al. (1990) or from the collection held by one of us (J.R.) in Los Angeles. Age at onset was ≤24 years in Los Angeles families. IDDM status in HBDI families was as provided by HBDI. A subset of the Danish panel has been described by Pociot et al. (1993); age at onset of Danish patients was <20 years, and all required insulin from diagnosis. The CEPH panel of 59 reference families (Dausset et al. 1990) was used to estimate genetic distances.

Marker Selection

Three microsatellite markers were selected to characterize at the HLA locus on the basis of mapping data from the study by Martin et al. (1995): a microsatellite at DQB1 (primers DQB1CAR1/DQB1CAR2 (Macaubas et al. 1995)); the anonymous marker D6S273 (AFM142xh6); and TNFB (primers TNFa-IR2/IR4) (Nedospasov et al. 1991). Two markers proximal to HLA and on 6p (D6S309 and D6S260) and 10 markers distal to HLA on 6q (D6S271, D6S286, D6S300, D6S468, D6S283, D6S434, D6S1580, D6S301, D6S447, and D6S287) were selected from the Généthon map (Dib et al. 1996) to cover the region of chromosome 6 linkage detected in our original study (Hashimoto et al. 1994). In addition, we selected four markers on chromosome 6q to test for linkage in the regions of *IDDM5* and *IDDM8*. These were ESR in the *IDDM5* region (Davies et al. 1994) and D6S264, D6S446, and D6S281, the three markers that showed the strongest evidence of linkage in the *IDDM8* region from the studies by Luo et al. (1995, 1996).

Characterization of Microsatellite Markers

Genotype characterization was performed as described by Gyapay et al. (1994), with some modifications. In brief, PCR products from six to eight systems were pooled and loaded on an acrylamide gel and then transferred to a Nylon membrane that was hybridized with a succession of radioactively labeled primers to reveal sequentially genotypes at each locus. Exact allele size classes could be determined for most markers by

comparison with a reference individual that was included in each half of acrylamide gel. This was not possible for three markers where only the allelic differences within families could be reliably assigned. The analysis of these markers was restricted afterwards to families in which parental genotypes were known.

Statistical Analysis

The marker loci were evaluated individually for disease linkage through a χ^2 test comparing observed IBD counts from informative meioses with expectations calculated from the theoretical .50 IBD probability for the absence of linkage, and with the SIBPAIR program (J. Terwilliger, W. Shannon, G. Lathrop, J. Nolan, L. Goldin, G. Chase, and D. Weeks, unpublished data), which also takes into account information when parents are not genotyped. Mutilocus analysis was performed with the ASPEX program package (version 1.45) from D. Hinds and N. Risch.

In the SIBPAIR program, linkage is evaluated with likelihood-based test statistic that is equivalent to the LOD score calculated under the assumption of a simple recessive disease model with phase-unknown matings. In brief, the likelihood contribution for meioses from a heterozygous parent with n affected offspring, of which m inherited one marker allele and $n - m$ the other, is $\{\rho^m(1 - \rho)^{n-m} + \rho^{n-m}(1 - \rho)^m\}$. Here ρ is equivalent to the recombination fraction under a recessive model, on the assumption that the parent is a obligate heterozygote for the disease locus. For the whole family, the contribution is the product of the two parental contributions if the mating is not a intercross. If parental genotypes are missing, the likelihood is a sum of terms corresponding to each of the possible parental genotype combinations, weighted by the genotype frequencies calculated under the assumption of Hardy-Weinberg equilibrium and incorporating information on all offspring (on the assumption of a Mendelian segregation ratio for unaffected or unknown offspring). Similarly, intercross matings are treated by considering likelihood terms for different possible parental origins of alleles. To test for linkage, the product of the likelihoods over all families is maximized as a function of ρ in the interval 0.0–0.5, and the likelihood ratio test statistic is calculated against the null hypothesis of $\rho = 0.5$. This choice of test statistic is based, on the one hand, on its increased power and better small sample behavior compared to alternatives in simulation experiments in which the number of affected siblings is >2 in some sibships (J. Terwilliger and G. M. Lathrop, unpublished data) and on the other, its equivalence to the IBD test when all families contain two affected offspring and parental genotypes are known (Hyer et al. 1991; Knapp et al. 1994). The estimated IBD sharing from SIBPAIR is a weighted estimate where the weights are proportional to the number of independent affected offspring pairs in a family.

A two-locus IBD method was developed to test for the presence of linked susceptibility loci. Let n_{ij} be the number of sib pairs, with the IBD status i for the first locus (here, HLA) and j for the second locus ($i, j = 0, 1$), ignoring pairs for which IBD status is unknown at either locus. Then $n_{i.} = n_{i1} + n_{i0}$ is the total number of sib-pair meioses with IBD status of i at the first locus. Linkage of susceptibility to the second marker will be evaluated in light of the observed IBD counts for the first marker, and thus conditional on $n_{i.}$. In the following, $q_{j|i}$ represents the IBD probability at the second marker locus conditional on IBD status at the first marker locus. We will be principally interested in the logistic transformation of these probabilities, defined as $r_i = \ln(q_{1|i}/q_{0|i}) = \ln[q_{1|i}/(1 - q_{1|i})]$.

First, consider the case where the two marker loci are not linked and IBD status at one susceptibility locus is independent of IBD status at the other susceptibility locus. Then, $r_i = r = \ln[q/(1 - q)]$, where $q = q_{1|i}$ represents the single IBD probability for the second marker, which here does not depend on IBD status at the first locus. When the second marker is unrelated to disease susceptibility, its IBD probability has the value $q = 0.5$, and thus we have $r = 0$. Segregation of the second marker with disease is evaluated by a test of $q \geqslant 0.5$ (equivalent to $r \geqslant 0$) versus $q = 0.5$ (equivalent to $r = 0$). The test can be based on the value of the statistic $T_1 = \hat{r}/\sqrt{\hat{v}}$, where $\hat{r}$ and $\hat{v}$ are the weighted least-square estimate and its estimated variance

$$\hat{r} = (\Sigma\, \hat{r}_i/\hat{v}_i)/(\Sigma\, 1/\hat{v}_i)$$
$$\hat{v} = (\Sigma\, 1/\hat{v}_i)^{-1}\,, \quad (1)$$

with $\hat{r}_i$ and $\hat{v}_i$ for the ith IBD group given by

$$\hat{r}_i = \ln(n_{i1}/n_{i0})$$
$$\hat{v}_i = n_{i.}/n_{i1}n_{i0}\,. \quad (2)$$

Under the null hypothesis, T_1 is asymptotically distributed as $N(0,1)$, and a one-sided significance value is calculated. The inverse logistic transformation applied to $\hat{r}$ leads to an estimate, $\hat{q}$, of the IBD probability for the second marker locus.

Now suppose that the two markers are linked with recombination fraction θ, and the chromosome contains a single susceptibility locus that coincides with the location of the first marker locus (this is the null hypothesis below). The probability that a sibling pair has the same IBD status at both markers, independent of disease status, is $1 - \phi$, where $\phi = 2\theta(1 - \theta)$. In an affected sibling

pair, the IBD probability at the second marker locus, which we will denote p_i (for IBD status i), is a function of the IBD probabilities at the first marker locus, denoted p_i^1

$$p_1 = (1 - \phi)p_1^1 + \phi p_0^1$$

$$p_0 = \phi p_1^1 + (1 - \phi)p_0^1 \,.$$

Conditioning on the IBD status at the first marker locus leads to

$$p_{j|i} = \begin{matrix} (1 - \phi) & \text{for} \quad i = j \,, \\ \phi & \text{otherwise} \,, \end{matrix}$$

where $p_{j|i}$ is used to symbolize the conditional IBD probability at the second marker (to distinguish it from $q_{j|i}$ when lack of linkage is assumed). In analogy with two unlinked marker loci as described above, we are interested in a test based on the logistic transformation

$$s_i = \ln(p_{1|i}/p_{0|i}) = \ln[p_{1|i}/(1 - p_{1|i})] \,.$$

Under the null hypothesis as defined above:

$$s_i = \begin{matrix} \ln(1 - \phi) - \ln(\phi) & \text{for } i = 1 \\ \ln(\phi) - \ln(1 - \phi) & \text{for } i = 0 \,, \end{matrix}$$

whereas, in the absence of linkage between the markers, the logistic transformation is zero under the null hypothesis.

More generally, we can assume that for linked marker loci

$$s_i = \begin{matrix} r_i + \ln(1 - \phi) - \ln(\phi) & \text{for } i = 1 \\ r_i - \ln(1 - \phi) + \ln(\phi) & \text{for } i = 0 \,, \end{matrix}$$

where r_i will be nonzero if the second marker is closely linked to another susceptibility locus at a distance from the first marker. Thus, when ϕ is known, r_i is estimated as

$$\hat{r}_i = \begin{matrix} \hat{s}_i - \ln(1 - \phi) + \ln(\phi) & \text{for } i = 1 \\ \hat{s}_i + \ln(1 - \phi) - \ln(\phi) & \text{for } i = 0 \,, \end{matrix} \quad (3)$$

where $\hat{s}_i = \ln(n_{i1}/n_{i0})$. If we wish to test the null hypothesis of $r_i = 0$, it would be reasonable to proceed by considering the alternative hypothesis where $r_i = r \geqslant 0$, as in the case of marker loci that are not linked. The estimated variance of $\hat{r}_i$ has the value given in (2), and the test statistic T_1 is calculated from the equations in (1) but with estimates of $\hat{r}_i$ obtained from (3). If the inverse logistic transformation is applied to $\hat{r}$ from (1), we obtain $\hat{p}$ where p can be interpreted as the IBD probability at the second locus that would be observed if $\theta = .5$ (e.g., for a locus with equivalent susceptibility effect but on a different chromosome). We refer to this as the IBD estimate at the second locus adjusted for the effects of the first locus.

Another hypothesis of interest is $r_1 = r_0$, which is equivalent to the usual test for lack of interaction in 2×2 contingency table. Rejection of this hypothesis implies lack of independence of the IBD status for the two marker loci, after adjustment for their linkage. The statistical test is based on the difference $\hat{s}_1 - \hat{s}_0$, which has a large sample expectation of $2[\ln(1 - \phi) - \ln(\phi)]$ under the null hypothesis and estimated variance $\hat{v}_1 + \hat{v}_0$. The test statistic $T_2 = \hat{s}_1 - \hat{s}_0 - 2\ln(1 - \phi + 2\ln(\phi)/(\hat{v}_1 + \hat{v}_0)$ is then asymptotically distributed as $N(0,1)$, and its significance (two-sided) is judged accordingly.

It is usual to modify the logistic estimates to reduce small sample bias. For weighted least-squares analysis, Cox and Snell (1989, p. 32) propose the following modifications:

$$\hat{S}_i = \ln[(n_{i1} - 0.5)/(n_{i0} - 0.5)]$$
$$\hat{V}_i = (n_{i.} - 1)/(n_{i1}n_{i0}) \,, \quad (4)$$

which are used in the application below.

When male and female recombination fractions are different, the counts for paternal and maternal meioses are separated. The sums in equation (1) will then include four terms, two for each sex, with sex-specific estimates of the r_i as in (3) but as functions of $\phi_m = 2\theta_m(1 - \theta_m)$ in male meioses and $\phi_f = 2\theta_f(1 - \theta_f)$ in female meioses.

Results

Linkage Analysis of 15 Markers of Chromosome 6 in IDDM Families

A set of 15 microsatellite markers that span a 115-cM region of chromosome 6 from D6S309, 30 cM proximal to HLA, to D6S287 at 6q22 were characterized in multiplex IDDM families from France, North Africa, and the United States (a total of 494 affected sib pairs, 426 of which with both parents typed). Three of the markers were chosen to obtain a maximum of IBD information at the HLA locus. These were DQB1CAR, TNFB, and DS6273 (located between DQB1 and TNFB [Martin et al. 1995]), each of which has a heterozygosity >.78. We observed no recombinants between D6S273 and TNFB, 0.5% recombination between DQB1 and D6S273 in IDDM families, and 1.1% between DQB1 and D6S273 in the 59 CEPH families. These results are consistent with previously reported physical and genetic distances (Martin et al. 1995). IBD at the HLA locus was evaluated on the basis of the combination of the

three loci, which led to an overall heterozygosity of 95%. (The DQB1 genotype was used to determine the HLA IBD in sib pairs for which a recombinant event in the HLA region was observed.)

The linkage of disease to the markers spanning the region was initially evaluated by two methods: (1) sib-pair analysis in the families where both parents were genotyped and IBD counts could be obtained for informative meioses; and (2) IBS analysis with the SIBPAIR program, taking into account instances where parental genotype data were unknown or incomplete. The latter method could be applied to all but three of the loci for which the allele differences within families, rather than exact allele sizes, were determined. For these loci, the analyses were restricted to families in which parental genotypes were available. A complete description of the marker loci and the results are given in table 1*A* and figure 1*a*.

Significant evidence of linkage ($P = .00003$) was observed with a group of marker loci, D6S283, D6S434, and D6S1580, that span 2 cM on 6q21. These markers reside between the HLA and *IDDM5* regions and exhibit a recombination rate of ~40% with HLA (for the precise estimation of this recombination frequency, see Genetic Distances from CEPH Families) and 30% with the *IDDM5* marker ESR. It is worth noting that the pattern of linkage observed in our data is dissymetrical with respect to HLA, with significant linkage being detected over a greater distance proximal to HLA and on 6q compared to the distance distal to HLA on 6p. In addition, there are two maxima in the graphs of the estimated IBD frequencies plotted against location on the chromosome, one in the HLA region and the other at 6q21, and others in the *IDDM5* and *IDDM8* regions (fig. 1*a*). A very similar pattern was observed in multilocus affected-sib-pair analysis, which gave a maximum LOD score of 6.2 near the markers D6S283, D6S434, and D6S1580 in the 6q21 region (fig. 1*d*).

Although these observations could suggest that a second susceptibility locus could reside near D6S283, D6S434, and D6S1580, they do not provide a statistical evaluation of the evidence in favor of this hypothesis. Moreover, the region also shows a striking sex difference in recombination fractions, with female genetic distances more than three-fold greater than male genetic distances in the Généthon map of the region (Dib et al. 1996). It might be hypothesized that the linkage is a consequence of the proximity of the marker loci to HLA in male meioses, even though the estimated male recombination distance to HLA is still large ($\theta = .31$). In female meioses, D6S283, D6S434, and D6S1580 are unlinked to HLA.

Therefore, we also performed sib-pair analysis independently, with meioses separated by sex. The results, given in table 1*B* and 1*C* and figure 1*b* and 1*c*, show that both male and female meioses exhibit a second maximum for linkage near the 6q21 markers. In female meioses, none of the marker loci located between HLA and D6S283, D6S434, or D6S1580 showed strong evidence of linkage to IDDM susceptibility. Although the evidence of linkage was nonsignificant at one nearby marker (D6S468), which was also the least informative marker in the region, other flanking markers gave $P = .001$ (D6S300) and $P = .008$ (D6S301) in the affected-sib-pair test. These results clearly define a second region unlinked to HLA in female meioses that is likely to contain a susceptibility locus for IDDM ($P = .0004$ in female meioses at D6S283).

When male and female meioses are combined, the test statistic should be adjusted to take into account linkage with HLA in males as described in Subjects, Material, and Methods. Recombination distances between HLA and each markers were obtained from the Généthon data and were applied in the sex-specific form of equation (3). The results were combined with (4) to obtain the estimates and variances for calculation of the sex-specific form of test statistic T_1. As shown in table 1*D*, the D6S283-D6S434-D6S1580-D6S301 cluster exhibited the strongest evidence of linkage of all the non-HLA markers tested with this method. The most significant result in combined male and female meioses was obtained at D6S283, with $P = .00015$. In the following, the 6q21 region of linkage is denoted as *IDDM15*.

Genetic Distances from CEPH Families

The two-locus affected-sib-pair analysis requires accurate estimates of the sex-specific recombination fractions. Since the Généthon data is based on only eight CEPH families, we characterized the complete CEPH panel of 59 reference families with some of the microsatellite markers in the HLA (DQB1CAR and D6S273) and IDDM15 (D6S283 and D6S434) regions to obtain more precise estimates. These data confirmed the differences in recombination fractions in male and female meioses in the interval between the markers ($\chi^2_1 = 18.1$) and gave a male recombination estimate (1-LOD-unit confidence interval) of 0.32 (0.27–0.37) with a LOD score of 10.00 and a female recombination estimate of 0.48 (0.41–0.50) with a LOD score 0.32. The sex-averaged recombination rate was 0.40 (0.35–0.44). Multilocus analysis of data on eight CEPH families from the Généthon map of the markers described in table 1 predict recombination of 0.32 in males and 0.49 in females (Kosambi mapping function), very close to the estimates from the total panel. The revised recombination estimates based on the total CEPH panel are used below.

Additional Families

HLA and the three markers that exhibited the strongest evidence of linkage in the *IDDM15* region (D6S283,

Table 1

IBD for 15 Markers on Chromosome 6 Studied in the French, North African, and U.S. Families

Locus	het	A. Sex Average: All Families or Fully Typed Families[a]						B. Male Meioses: Fully Typed Families						C. Female Meioses: Fully Typed Families						D. Statistics Accounting for HLA: Fully Typed Families	
		theta	IBD1	IBD0	%IBD	z	P value	theta	IBD1	IBD0	%IBD	z	P value	theta	IBD1	IBD0	%IBD	z	P value	T_1	P value
D6S309	.83	.19	344	275	55.9	1.76	.002[a]	.14	177	134	57.4	1.41	.005	.22	167	141	54.3	.47	.07	.53	.30
D6S260	.84	.16	391	283	58.5	4.17	6×10^{-6} [a]	.10	209	128	62.7	4.62	2×10^{-6}	.20	182	155	54.4	.55	.06	1.65	.05
DQB1	.78	.01	503.9	150.0	77.1	46.38	1×10^{-48}	.01	242	67	78.3	21.52	1×10^{-23}	.01	244	76	76.3	19.15	3×10^{-21}	...	
D6S273	.81	.00	462.4	186.1	71.3	28.30	2×10^{-30}	.00	229	97	70.2	11.61	1×10^{-13}	.00	208	84	71.2	11.43	2×10^{-13}	...	
TNFB	.81		476.8	168.4	73.9	36.00	3×10^{-38}		214	84	71.8	12.31	3×10^{-14}		224	75	74.9	16.12	3×10^{-18}	...	
HLA(HAP)	.97	.22	633	228	73.5	41.37	1×10^{-43} [a]	.09	313	123	71.8	17.98	5×10^{-20}	.32	320	105	75.3	23.62	9×10^{-26}	...	
D6S271	.85	.22	407	255	61.5	7.58	2×10^{-9} [a]	.12	215	108	66.6	7.70	1×10^{-9}	.31	192	147	56.6	1.30	.007	2.19	.02
D6S286	.78	.14	363.1	266.4	57.7	3.80	.00001	.08	210	114	64.8	6.18	5×10^{-8}	.19	151	146	50.8	0.02	.39	1.98	.02
D6S300	.80	.04	352.8	252.5	58.3	4.28	5×10^{-6}	.05	166	124	57.2	1.32	.007	.04	169	118	58.9	1.97	.001	2.86	.002
D6S468	.71	.01	311.1	256.1	54.8	1.82	.002	.02	138	99	58.2	1.39	.006	.01	139	118	54.1	0.37	.10	1.86	.03
D6S283	.82	.00	386.1	288.9	57.2	3.56	.00003	.00	180	132	57.7	1.60	.003	.01	188	128	59.5	2.47	.0004	3.59	.0002
D6S434	.84	.02	395.7	312.0	55.9	2.75	.0002	.00	203	150	57.5	1.73	.002	.04	191	143	57.2	1.50	.004	3.28	.0005
D6S1580	.79	.00	378.4	291.1	56.5	3.06	.00009	.00	187	126	59.7	2.58	.0003	.01	174	136	56.1	1.01	.02	3.25	.0006
D6S301	.76	.04	325.8	255.7	56.0	2.34	.0005	.04	155	110	58.5	1.66	.003	.04	142	104	57.7	1.27	.008	3.17	.0008
D6S447	.76	.07	339.7	273.7	55.4	2.46	.0004	.03	158	107	59.6	2.13	.0009	.12	156	132	54.2	.43	.08	2.75	.003
D6S287	.77		335.0	291.2	53.5	.67	.04		155	125	55.4	.70	.04		151	136	52.6	.17	.19	1.36	.09

NOTE.—het = heterozygosity; IBD1 = estimated or observed number of pairs that have inherited the same allele; IBD0 = estimated or observed number of pairs that have inherited different alleles; %IBD = percentage of pairs that have inherited the same allele; z = affected sib-pair LOD-score statistics; HLA(HAP) = HLA haplotype defined by three marker loci as described in the text. The recombination frequencies between adjacent markers (theta) were determined from CEPH families under the assumptions of no sex difference (A) or with separate male (B) and female (C) recombination frequencies. The statistics were calculated as described in the text.

[a] For these loci, all IBD counts and statistics were obtained from families with known parental genotypes, because only allelic differences within families were ascertained. For the other marker loci, exact alleles were determined, and the IBD estimates, LOD score, and P values were calculated with the SIBPAIR program with the complete data set.

Am. J. Hum. Genet. 60:174–187, 1997

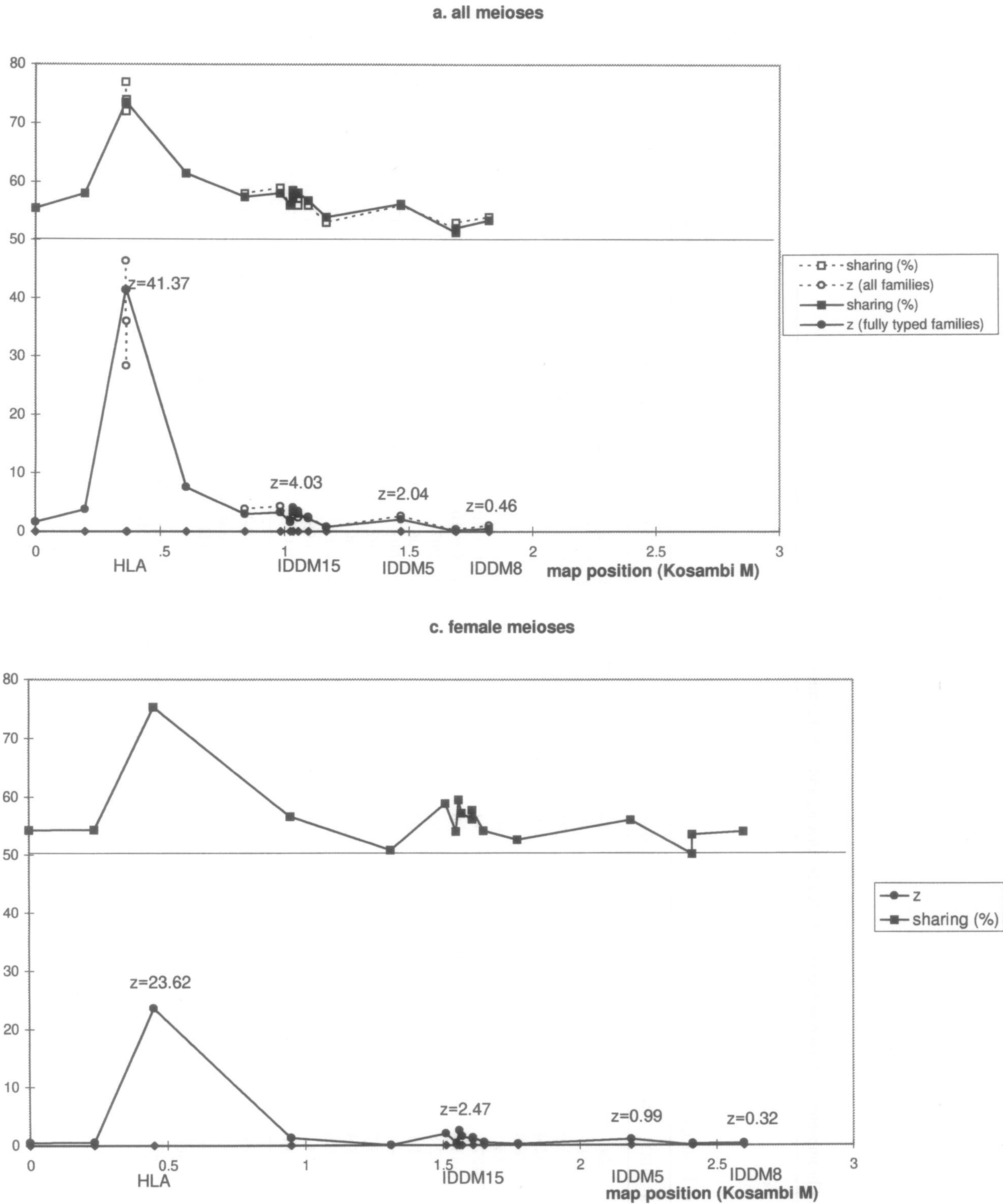
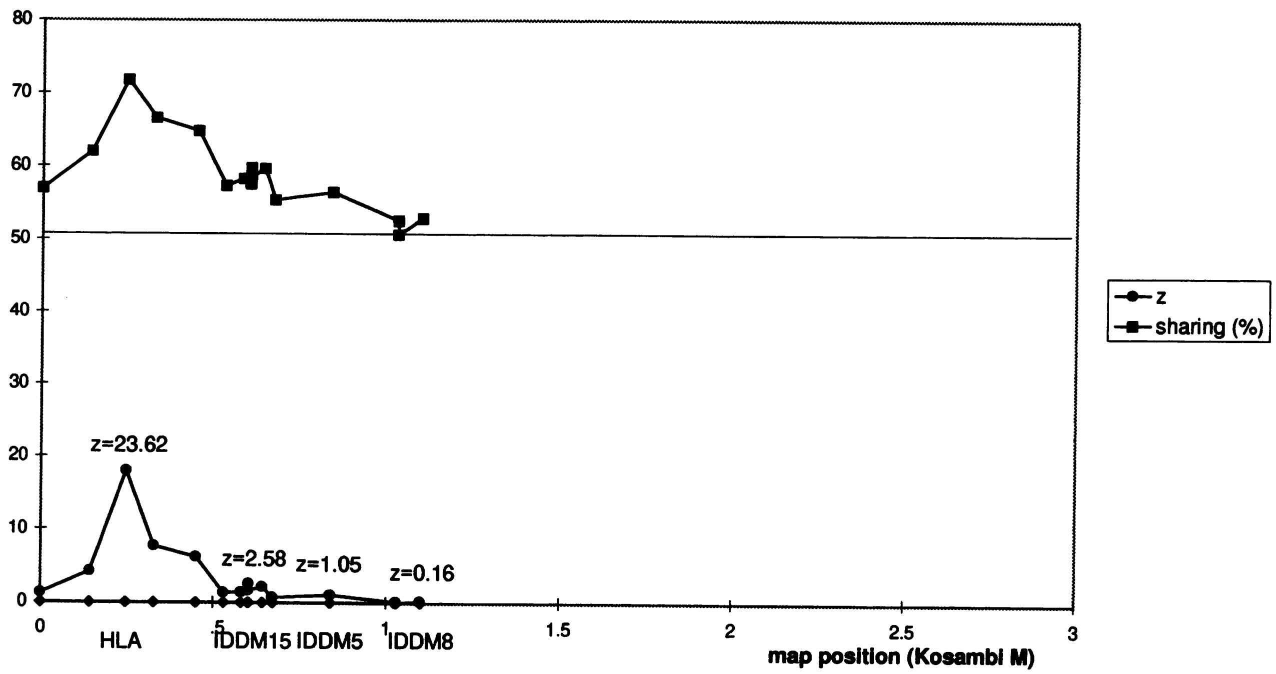
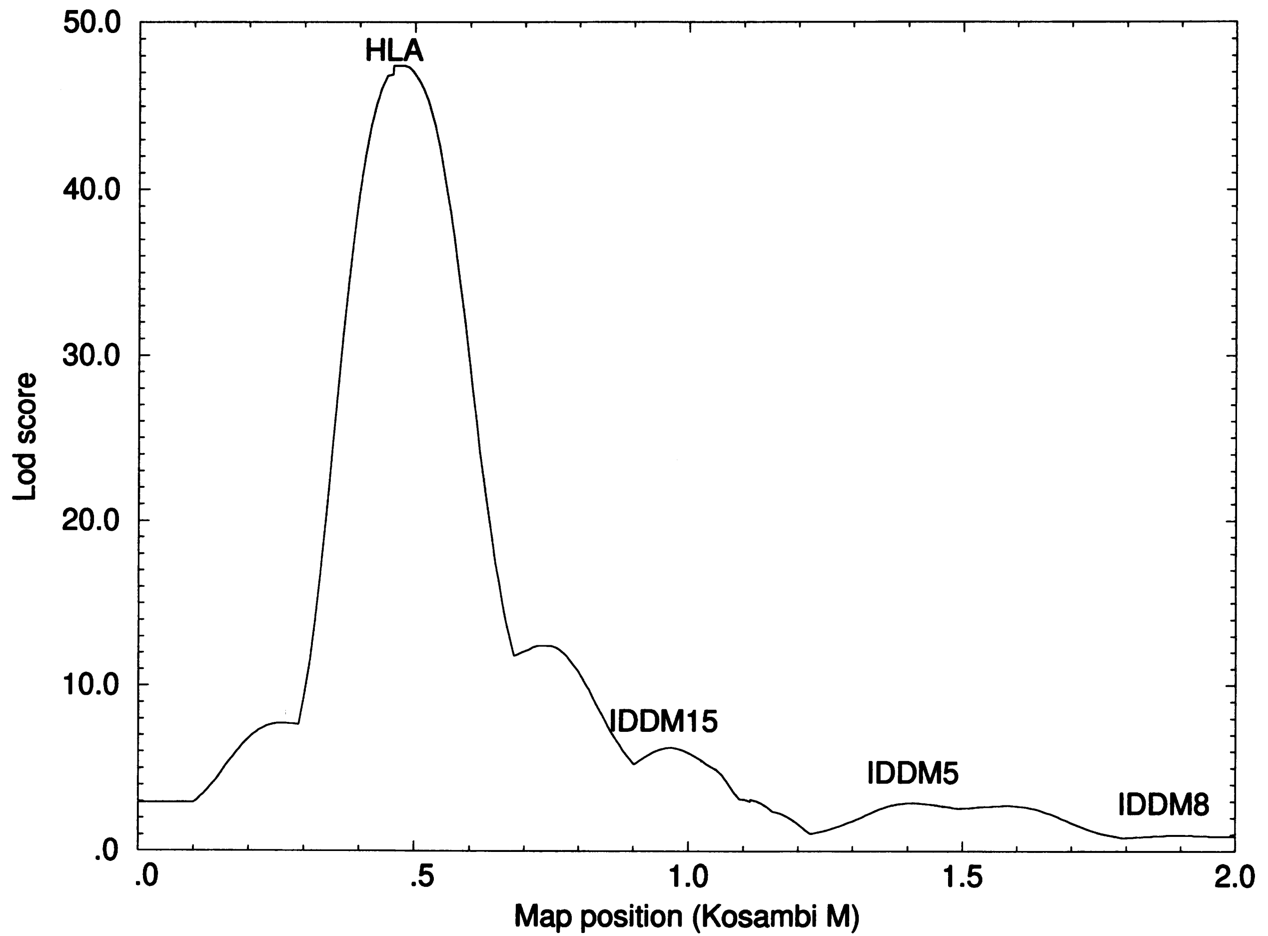


Figure 1 Results from affected-sib-pair analysis (*a, b,* and *c*) and from multilocus sib-pair analysis (*d*) of chromosome 6 markers characterized in French, North African, and U.S. families. In *a, b,* and *c,* IBD sharing (*top*) and LOD-score values (*bottom*) are from the data in table 1. Map positions were estimated from the recombination values in table 1 with the Kosambi mapping function.


b. male meioses
80
70
60
50
40
30
20
10
0
z=23.62
z=2.58
z=1.05
z=0.16
0
.5
1
1.5
2
2.5
3
HLA
IDDM15
IDDM5
IDDM8
map position (Kosambi M)
z
sharing (%)


d. multilocus analysis
50.0
40.0
30.0
20.0
10.0
.0
Lod score
HLA
IDDM15
IDDM5
IDDM8
.0
.5
1.0
1.5
2.0
Map position (Kosambi M)

Am. J. Hum. Genet. 60:174–187, 1997

D6S434, and D6S1580) were characterized in an independent panel of 147 Danish multiplex IDDM families to seek confirmation of the results. Without accounting for linkage to HLA, the affected-sib-pair test for D6S1580 had a significance of $P = .01$. When the data on Danish families were combined with the other panels, the tests gave $P = .00002$, $P = .0005$, and $P = .000006$ for D6S283, D6S434, and D6S1580, respectively. A contingency table analysis was performed on the observed IBD counts to test for homogeneity of the results in the French, U.S., and Danish panels. (North African families were excluded because of the small number of affected sib pairs in this panel.) None of the homogeneity tests were significant (results not shown).

Next, the test statistics were calculated for the three marker loci D6S283, D6S434, and D6S1580 in the combined family panel, taking account of linkage to HLA. Strong evidence of linkage was found, particularly at D6S283 ($P = .00003$), with sex-specific recombination fractions (table 2). When the two completely linked markers D6S283 and D6S434 were combined into a single haplotype, the test for linkage was also significant ($P = .0002$), a value intermediate between those obtained at each of these markers. When the sex-averaged rate was assumed for male and female meioses, the test statistic was $\chi_1^2 = 18.9$ ($P < .00001$) for D6S283. The data were also analyzed with other recombination fractions from the 1-LOD-unit confidence intervals reported above. At D6S283, the sex-specific values of the statistic ranged from $\chi_1^2 = 2.8$ ($\theta = .27$) to $\chi_1^2 = 8.8$ ($\theta = .37$), for male meioses, and from $\chi_1^2 = 9.6$ ($\theta = .41$) to $\chi_1^2 = 11.3$ ($\theta = .5$) for female meioses. When families were divided by origin, consistent evidence for linkage was found in the French, U.S., and Danish family sets (table 2). Although the North African panel showed no evidence of linkage (data not shown), heterogeneity could not be confirmed, because only 18 affected sib pairs were informative for HLA and D6S283. The tests of interaction between the IBD status at HLA and the other loci (T_2) were not significant ($P = .04$ for D6S283; $P > .05$ for D6S434 and D6S1580). Finally, we also performed the same tests for linkage with IBD counts for independent sib pairs (a single affected proband was chosen in families with three or more affected offspring, and only those affected sib pairs containing this proband were counted). The combined statistics adjusted for linkage to HLA gave $P = .0002$ for D6S283 and $P = .001$ for D6S434 and $P = .0001$ for D6S1580.

An estimate of the IBD probability, p, at *IDDM15* with adjustment for the HLA effect was calculated by taking the inverse logistic transformation of the weighted least-squares estimate of r. For D6S283, this was .57 (0.59–0.56, 95% confidence interval). An the assumption that IBD in the paternal and maternal meioses are independent, the (HLA-adjusted) probability that affected sibling pairs have inherited different alleles from both parents is .18 for this locus. Thus, the estimated sibling recurrence risk attributable to *IDDM15* is .25/.18 = 1.35, with correction for the HLA effect. As a comparison, if linkage to HLA is ignored, the sibling recurrence risk is 1.48.

Analysis of the IDDM5 *and* IDDM8 *Regions*

Markers in the regions of the previously designated susceptibility loci on chromosome 6, *IDDM5* and *IDDM8,* were characterized in the French, North African, U.S., and Danish families (table 3). ESR, the marker that exhibits the strongest evidence of linkage for *IDDM5* in the combined data from Davies et al. (1994) and Luo et al. (1995, 1996), gave evidence in favor of linkage ($P = .002$) in our families. D6S281, which provided the strongest evidence of linkage for the *IDDM8* region in the data compiled by Luo et al. (1996), also showed evidence of linkage ($P = .004$) in our data. As shown in table 4, the combination of data presented here with nonoverlapping information from the literature that have been compiled by Luo et al. (1996) gave overall P values of .0001 for *IDDM5* and .00004 for *IDDM8,* where our data included only independent affected sib pairs with a single proband per family. (The IBD values in tables 3 and 4 differ because the former have been estimated by the SIBPAIR program, which provides weighted estimates that take account of all sib pairs in sibships with more than two affected offspring and which also uses data from families in which one or both parents have not been genotyped.)

Discussion

We have provided evidence of a previously unreported susceptibility locus for IDDM, provisionally denoted *IDDM15,* located near chromosome 6q21, in the region of the markers D6S283, D6S434, and D6S1580. These markers are unlinked to HLA in female meioses but are distantly linked in male meioses ($\theta = .32$). We developed a simple IBD method to take account of linkage between susceptibility loci in order to evaluate the evidence for *IDDM15*. Overall, this gave strong evidence ($P = .00003$) in favor of a non-MHC susceptibility linked to markers in the region, with positive evidence in three large family panels from France ($P = .01$), USA ($P = .001$), and Denmark ($P = .02$). The estimated IBD probability for *IDDM15* was 0.57, leading to an estimated recurrence risk in siblings, λ_s, of 1.35, after adjustment for HLA. By comparison, estimates of λ_s due to specific loci in the French, North African, and U.S. family panels are 3.6 for HLA and 1.28 for *IDDM4* in the 11q13 region (calculations on the basis of equations given in the article by Risch [1987]).

Confirmation of linkage to *IDDM15* must now be

Table 2

IBD for HLA and Markers in 6q21 Region with the Test Statistic Adjusted for the HLA Effect

	All		Male		Female		Test Statistics			
Locus and HLA Split	IBD1	IBD0	IBD1	IBD0	IBD1	IBD0	Male	Female	Overall	*P* value
					A. All Families					
D6S283:										
HLA1	349	223	185	106	164	117	2.38	3.33	4.04	.00003
HLA0	110	98	47	54	63	44				
D6S434:										
HLA1	349	235	191	108	158	127	2.13	2.33	3.16	.0008
HLA0	120	120	54	70	66	50				
D6S1580:										
HLA1	324	219	179	97	145	122	2.81	2.12	3.49	.0002
HLA0	111	98	52	57	59	41				
					B. French Families					
D6S283:										
HLA1	82	43	47	17	35	26	2.11	1.16	2.28	.01
HLA0	13	16	5	9	8	7				
D6S434:										
HLA1	78	36	45	17	33	19	1.73	2.09	2.70	.003
HLA0	15	18	5	11	10	7				
D6S1580:										
HLA1	76	37	41	13	35	24	2.28	1.27	2.45	.007
HLA0	14	17	6	9	8	8				
					C. U.S. Families					
D6S283:										
HLA1	195	124	97	64	98	60	.76	3.65	3.11	.001
HLA0	62	54	24	32	38	22				
D6S434:										
HLA1	209	139	112	65	97	74	1.33	2.26	2.53	.006
HLA0	74	75	34	47	40	28				
D6S1580:										
HLA1	189	129	106	59	83	70	1.75	1.85	2.55	.005
HLA0	63	56	27	34	36	22				
					D. Danish Families					
D6S283:										
HLA1	65	47	36	23	29	24	2.08	.91	2.09	.02
HLA0	33	19	17	6	16	13				
D6S434:										
HLA1	55	51	30	24	25	27	1.09	.10	.66	.26
HLA0	28	19	14	6	14	13				
D6S1580:										
HLA1	56	48	30	25	26	23	1.19	.97	1.54	.06
HLA0	32	17	18	8	14	9				

Note.—HLA1 = alleles shared IBD at HLA; HLA0 = alleles not shared IBD at HLA; other abbreviations as in table 1. The recombination fractions used for the statistical analysis were the values estimated in the 59 CEPH families for HLA and D6S283-D6S434: .394 (sex-average), .318 (male) .472 (female).

Am. J. Hum. Genet. 60:174–187, 1997

Table 3

IBD and Statistics for Markers in IDDM5 and IDDM8 Regions

Locus	IBD1	IBD0	%IBD	*z*	*P* value
			A. All Families		
ESR	500.2	423.0	54.5	1.73	.002
D6S264	399.6	371.6	52.0	.26	NS
D6S446	366.9	343.8	52.4	.31	NS
D6S281	389.3	324.8	55.1	1.49	.004
			B. French Families		
ESR	101.2	97.8	51.6	.06	NS
D6S264	91.6	101.1	47.5	.00	NS
D6S446	88.9	90.1	49.7	.00	NS
D6S281	101.3	86.5	55.1	.50	NS
			C. U.S. Families		
ESR	241.9	174.2	58.8	2.81	.0002
D6S264	185.2	161.7	52.4	.18	NS
D6S446	166.0	139.3	55.8	.85	.02
D6S281	165.7	138.6	54.5	.47	NS
			D. Danish Families		
ESR	136.9	139.5	49.5	.00	NS
D6S264	109.0	98.1	53.4	.17	NS
D6S446	109.3	108.3	50.7	.01	NS
D6S281	112.2	92.1	55.1	.48	NS

NOTE.—Abbreviations as in table 1.

sought in other mulitplex IDDM families. Since the *IDDM15* region is linked to HLA in male meioses, the accuracy of the estimated recombination distances will be a key factor in the statistical evaluation of linkage. We have addressed this problem by obtaining recombination estimates from closely verified genotypes from the CEPH panel of 59 reference families (466 meioses). No data are available in the literature to evaluate the precise region of *IDDM15* for linkage to disease susceptibility in other family panels. From published data, the closest tested marker is D6S300, which is located 5 cM proximal to D6S283; it showed no evidence of linkage in sex-combined data from 96 U.K. families (Davies et al. 1994). The other previously reported non-MHC susceptibility loci on chromosome 6 map to different regions of 6q. The most closely linked marker for *IDDM5*, ESR, is located distal to *IDDM15*, at 6q25, and exhibits >30 cM recombination with the markers linked to *IDDM15*. The other non-MHC chromosome 6 locus, *IDDM8*, is located in an even more distal region at 6q27.

Interestingly, it has been suggested that chromosome 6 contains a gene responsible for transient neonatal diabetes mellitus (TND), since several cases of TND reported, to date, have been associated with paternal isodisomy of chromosome 6 (Abramowicz et al. 1994; Temple et al. 1995). This observation would favor the hypothesis of maternal imprinting at this gene (Ledbetter and Engel 1995), as does the observation of a family with three half-sibs affected by TND having the same father but different mothers (Sequel et al. 1982). Although it is presently not possible to determine whether one of the putative IDDM susceptibility genes on chromosome 6 is identical to this TND gene, our data provide no evidence of different IBD frequencies in male and female meioses in any of these regions. Comparative mapping data suggest that all the human IDDM susceptibility regions on chromosome 6 map outside the 11-cM region near H2 that has been reported by Ikegami et al. (1995) to contain a non-MHC susceptibility gene for diabetes in the NOD mouse.

Luo et al. (1996) recently provided new data that confirm the evidence of linkage for *IDDM5* and *IDDM8*. When data from their study was combined with previously published results, they obtained significance of 2×10^{-6} and 1×10^{-6}, respectively. Our analysis of families from France, the United States, North Africa, and Denmark also supports linkage to *IDDM5* ($P = .002$) and *IDDM8* ($P = .004$). These results are not independent of those of Luo et al. (1996) because families from the HBDI panel (U.S. families) have been incorporated in both studies. When a combined data set was produced with duplicate families counted only once, we obtained *P* values of .0001 at ESR and .00004 at D6S281. Luo et al. (1996) also found evidence for heterogeneity among data sets for linkage to the *IDDM5* region. With the addition of our family panels, evidence for heterogeneity by source was marginally significant ($P = .04$) on the basis of contingency table analysis of data in table 4 (after removal of North Africa families because of their small number). There is no evidence of heterogeneity for linkage in the *IDDM8* region in our data, nor in those presented by Luo et al. (1996).

The statistical method that is described here can be applied generally to discriminate between the hypotheses of a single susceptibility locus or linkage between two susceptibility loci. The power to detect the presence of two susceptibility loci will depend on the distances between them and the relative size of their effects. Although neither *IDDM5* nor *IDDM8* is in a region that exhibits linkage to HLA, the distance between markers for these loci is 27 cM in CEPH families (data not shown), and *IDDM5* resides 35 cM from the *IDDM15* region. Two-locus analysis was not performed with *IDDM5* and *IDDM8*, because the marker loci were less informative than HLA, which reduces the amount of data available, and neither region exhibited very strong evidence of linkage in our family panel alone. Since

Table 4

Cumulated Data at Markers in IDDM5 and IDDM8 Regions

Families	IBD1	IBD0	%IBD	*z*	*P* value	Study
			A. ESR			
French	63 (51)	68 (58)	48.1 (46.8)	.04 (.10)	.33 (.25)	0
U.S.	225 (176)	166 (117)	57.5 (60.1)	1.93 (2.58)	.001 (.0003)	0
North African	15 (14)	6 (4)	71.4 (77.8)	.84 (1.21)	.02 (.009)	0
Danish	86 (75)	79 (72)	52.1 (51.0)	.16 (.01)	.29 (.40)	0
FL53	50	45	52.6	.16	.30	2
ITA46	56	33	62.9	1.29	.007	2
UK96	95	59	61.7	1.83	.002	1
UK102	65	73	47.1	.10	.25	1
All	655 (582)	529 (461)	55.3 (55.8)	2.91 (3.05)	.0001 (.00009)	
			B. D6S281			
French	53 (42)	50 (39)	51.5 (51.9)	.02 (.02)	.38 (.37)	0
U.S.	150 (113)	121 (91)	55.4 (55.4)	.67 (.52)	.04 (.06)	0
North African	7 (5)	2 (2)	77.8 (71.4)	.60 (.28)	.05 (.13)	0
Danish	63 (55)	53 (45)	54.3 (55.0)	.19 (.22)	.18 (.16)	0
FL53	53	30	63.9	1.38	.006	2
ITA46	48	34	58.5	.52	.06	2
UK96	65	39	62.5	1.41	.005	1
All	439 (381)	329 (280)	57.2 (57.6)	3.42 (3.35)	.00004 (.00004)	

NOTE.—0 = this study; 1 = Davies et al. (1994); 2 = Luo et al. (1996). FL, ITA, UK = families from Florida, Italy, and United Kingdom studied in the above references, respectively. Calculations were done in all the families, or in the families which contained only two affected siblings (in parentheses). Abbreviations as in table 1.

multilocus data is not available in the literature, two-locus analysis of the combined data sets cannot be undertaken at present. Although such analysis may be useful in the future, it should be noted that the situation described here for linkage between *IDDM15* and HLA is quite different because of the large contribution of the MHC in IDDM. Finally, it should be noted that the evidence of linkage and the IBD probabilities declined at intervening markers spanning the regions of *IDDM15-IDDM5* and *IDDM5-IDDM8* (data not shown), a pattern that has been considered characteristic of distinct susceptibility loci in other studies (Luo et al. 1995, 1996).

Acknowledgments

We gratefully acknowledge the aid of the Danish Study Group of Diabetes in Childhood, who aided in the collection of some of the Danish families, and the HBDI, for providing access to U.S. families. G. M. Lathrop hold a Wellcome Trust Principal Fellowship.

References

Abramowicz M, Andrien M, Dupont M, Dorchy H, Parma J, Duprez L, Ledley F, et al (1994) Isodisomy of chromosome 6 in a newborn with methylmalonic acidemia and agenesis of pancreatic beta cells causing diabetes mellitus. J Clin Invest 94:418–421

Bain S, Prins J, Hearne C, Rodrigues N, Rowe B, Pritchard L, Richie R, et al (1992) Insulin gene region-encoded susceptibility to type 1 diabetes is not restricted to HLA-DR4-positive individuals. Nat Genet 2:212–215

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

Bennett ST, Lucassen AM, Gough SC, Powell EE, Undlien DE, Pritchard LE, Merriman ME, et al (1995) Susceptibility to human type 1 diabetes at *IDDM2* is determined by tandem repeat variation at the insulin gene minisatellite locus. Nat Genet 9:284–292

Copeman J, Cuccia F, Hearner C, Cornall R, Reed P, Ronningen K, Undlien D, et al (1995) Linkage disequilibrium mapping of a type I diabetes susceptibility gene (*IDDM7*) to chromosome 2q31-q33. Nat Genet 9:80–85

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

Cox D, Snell E (1989) Analysis of binary data, 2d ed. Chapman & Hall, London

Dausset J, Cann H, Cohen D, Lathrop M, Lalouel JM, White R (1990) Centre d'etude du polymorphisme humain (CEPH):

collaborative genetic mapping of the human genome. Genomics 6:575–577

Davies JL, Kawaguchi Y, Bennett ST, Copeman JB, Cordell HJ, Pritchard LE, Reed PW, et al (1994) A genome-wide search for human type 1 diabetes susceptibility genes. Nature 371:130–136

Dib C, Faure C, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, et al (1996) A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature 380:152–154

Field L, Tobias R, Magnus T (1994) A locus on chromosome 15q26 (*IDDM3*) produces susceptibility to insulin-dependent diabetes mellitus. Nat Genet 8:189–194

Field L, Tobias R, Thomson G, Plon S (1996) Susceptibility to insulin-dependent diabetes mellitus maps to a locus (DDM11) on human chromosome 14q24.3-q31. Genomics 33:1–8

Ghosh S, Palmer SM, Rodrigues NR, Cordell HJ, Hearne CM, Cornall RJ, Prins J-B, et al (1993) Polygenic control of diabetes in non-obese diabetic mice. Nat Genet 4:404–409

Gu L, Dene H, Deng AY, Hoebee B, Bihoreau M-T, James M, Rapp JP (1996) Genetic mapping of two blood pressure quantitative trait loci on rat chromosome 1. J Clin Invest 97:777–788

Gyapay G, Morissette J, Vignal A, Dib C, Fizames C, Millasseau P, Marc S, et al (1994) The 1993–1994 Généthon human genetic map. Nat Genet 7:246–251

Hashimoto L, Habita C, Beressi JP, Delepine M, Besse C, Cambon Thomsen A, Deschamps I, et al (1994) Genetic mapping of a susceptibility locus for insulin-dependent diabetes mellitus on chromosome 11q. Nature 371:161–164

Hyer RN, Julier C, Buckley JD, Trucco M, Rotter J, Spielman R, Barnett A, et al (1991) High-resolution linkage mapping for susceptibility genes in human polygenic disease: insulin-dependent diabetes mellitus and chromosome 11q. Am J Hum Genet 48:243–257

Ikegami H, Makino S, Yamato E, Kawaguchi Y, Ueda H, Sakamoto T, Takekawa K, et al (1995) Identification of a new susceptibility locus for insulin-dependent diabetes mellitus by ancestral haplotype congenic mapping. J Clin Invest 96:1936–1942

Julier C, Hyer RN, Davies J, Merlin F, Soularue P, Briant L, Cathelineau G, et al (1991) Insulin-IGF2 region on chromosome 11p encodes a gene implicated in HLA-DR4-dependent diabetes susceptibility. Nature 354:155–159

Kennedy G, German M, Rutter W (1995) The minisatellite in the diabetes susceptibility locus *IDDM2* regulates insulin transcription. Nat Genet 9:293–298

Knapp M, Seuchter SA, Baur MP (1994) Linkage analysis in nuclear families. 2. Relationship between affected sib-pair tests and LOD score analysis. Hum Hered 44:44–51

Kreutz R, Hubner N, James MR, Bihoreau MT, Gauguier D, Lathrop GM, Ganten D, et al (1995) Dissection of a quantitative trait locus for genetic hypertension on rat chromosome 10. Proc Natl Acad Sci USA 92:8778–8782

Lathrop GM, Ott J (1990) Analysis of complex diseases under oligogenic models and interfamilial heterogeneity by the LINKAGE programs. Am J Hum Genet Suppl 47: A188

Ledbetter D, Engel D (1995) Uniparental disomy in humans: development of an imprinting map and its applications for prenatal diagnosis. Hum Mol Genet 4:1757–1764

Lernmark A, Ducat L, Eisenbarth G, Ott J, Permutt A, Rubenstein P, Spielman R (1990) Family cell lines available for research. Am J Hum Genet 47:1028–1030

Lucassen AM, Julier C, Beressi JP, Boitard C, Froguel 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 and associated VNTR. Nat Genet 4:305–310

Lucassen AM, Screaton GR, Julier C, Elliott TJ, Lathrop M, Bell JI (1995) Regulation of insulin gene expression by the IDDM associated, insulin locus haplotype. Hum Mol Genet 4:501–506

Luo D-F, Bui MM, Muir A, Maclaren NK, Thomson G, She J-Y (1995) Affected-sib-pair mapping of a novel susceptibility gene to insulin-dependent diabetes mellitus (*IDDM8*) on chromosome 6q25-q27. Am J Hum Genet 57:911–919

Luo D-F, Buzzetti R, Rotter JI, Maclaren N, Raffel L, Nistico L, Giovannini C, et al (1996) Confirmation of three susceptibility genes to insulin-dependent diabetes mellitus: *IDDM4, IDDM5,* and *IDDM8*. Hum Mol Genet 5: 693–698

Macaubas C, Hallmayer J, Kalil J, Kimura A, Yasunaga S, Grumet F, Mignot E (1995) Extensive polymorphism at a (CA)n microsatellite located in the HLA-DQA1/DQB1 class II region. Hum Immunol 42:209–220

Martin M, Mann D, Carrington M (1995) Recombination rates across the HLA complex: use of microsatellites as a rapid screen for recombinant chromosomes. Hum Mol Genet 4:423–428

McGinnis R, Spielman R (1995) Insulin 5′ flanking polymorphism. Length of class 1 alleles in number of repeat units. Diabetes 44:1296–1302

Nedospasov S, Udalova I, Kuprash D, Turetskaya R (1991) DNA sequence polymorphism at the human tumor necrosis factor (TNF) locus: numerous TNF/lymphotoxin alleles tagged by two closely linked microsatellites in the upstream region of the lymphotoxin (TNF-beta) gene. J Immunol 147: 1053–1059

Owerbach D, Gabbay K (1995) The HOXD8 locus (2q31) is linked to type I diabetes. Diabetes 44:132–136

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

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

Rotter JI, Landam EM (1984) Measuring the genetic contribution of a single locus to a multilocus disease. Clin Genet 26: 529–542

Sequel A, Coffey J, Killelea D (1982) Transient neonatal

diabetes mellitus in half sisters. Am J Dis Child 136: 626–627

She JX, Bui MM, Tian XH, Muir A, Wakeland EK, Zorovich B, Zhang LP, et al (1994) Additive susceptibility to insulin-dependent diabetes conferred by HLA-DQB1 and insulin genes. Autoimmunity 18:195–203

Temple I, James R, Crolla J, Sitch F, Jacobs P, Howell W, Betts P (1995) An imprinted gene(s) for diabetes. Nat Genet 9:110–112

Thomson G, Robinson WP, Kuhner MK, Joe S, Klitz W (1989) HLA and insulin gene associations with IDDM. Genet Epidemiol 6:155–160

Undlien DE, Bennett ST, Todd JA, Akselsen HE, Ikaheimo I, Reijonen H, Knip M, et al (1995) Insulin gene region-encoded susceptibility to IDDM maps upstream of the insulin gene. Diabetes 44:620–625

van der Auwera BJ, Heimberg H, Schrevens AF, van Waeyenberge C, Flament J, Schuit FC (1993) 5′ insulin gene polymorphism confers risk to IDDM independently of HLA class II susceptibility. Diabetes 42:851–854