

A second-generation screen of the human genome for susceptibility to insulin-dependent diabetes mellitus

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During the past decade, the genetics of type 1 (insulin-dependent) diabetes mellitus (IDDM) has been studied extensively and the disorder has become a paradigm for genetically complex diseases. Previous genome screens^{1,2} and studies focused on candidate genes³⁻⁹ have provided evidence for genetic linkage between polymorphic DNA markers and 15 putative IDDM susceptibility loci, designated *IDDM1*–*IDDM15*. We have carried out a second-generation screen of the genome for linkage and analysed the data by multipoint linkage methods. An initial panel of 212 affected sibpairs (ASPs) was genotyped for 438 markers spanning all autosomes, and an additional 467 ASPs were used for follow-up genotyping. Other than the well-established linkage with the HLA region at chromosome 6p21.3, there was only one region, located on chromosome 1q and not previously reported, where the log likelihood ratio (lod) was greater than 3. Lods between 1.0 and 1.8 were found in six other regions, three of which have been reported in other studies. Another reported region¹⁰, on chromosome 6q and loosely linked to HLA, also had an elevated lod. Little or no support was found for most reported IDDM loci (lods were less than 1), despite larger sample sizes in the present study.

IDDM is an autoimmune disorder resulting from cellular infiltration and destruction of the pancreatic islet cells. It is a common chronic disease of children under age 16 (ref. 11), but the underlying causes of susceptibility and the initiators of the pathogenic process remain obscure. Although the fundamental importance of the HLA region on chromosome 6p in IDDM susceptibility was recognized in the 1970s (refs 12,13), the transmission of IDDM fits no simple mendelian pattern and the existence

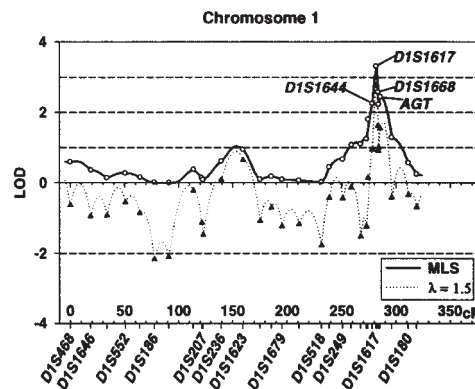


Fig. 1 Multipoint lod map of IDDM and markers on chromosome 1. Ticks on the X-axis represent each marker typed. The solid line is the maximum multipoint lod (MLS), with simultaneous estimation of IBD. The dashed line is the 'exclusion' lod obtained after fixing $\lambda_s = 1.5$ with affected sib sharing probabilities³³ $z_2 = 0.33$, $z_1 = 0.50$ and $z_0 = 0.17$. (The additive model for within-locus effects was assumed.)

of other contributing genes has been suspected^{14,15}. A second IDDM susceptibility gene has been mapped near the insulin gene (INS) on chromosome 11p (refs 3,4,16).

IDDM was the first complex disorder to be studied by genome-wide screening of ASPs (refs 1,2). Despite the modest size of the initial screens (96 and 61 sibpairs, respectively), evidence was reported for possible linkage with a large number of markers. Linked markers were identified by significance ($P < 0.05$) in analyses of allele sharing identical by descent (IBD) in ASPs. Selected markers were followed up in 186 and 190 additional ASPs, and partial genome screens^{7,8,17} (approximately 50 markers) have reported linkage between IDDM and additional markers. Fifteen such 'linkages' have been suggested; the corresponding genes have been designated *IDDM1*–*IDDM15*. *IDDM1* and *IDDM2* refer to the genes in the HLA and INS regions, respectively.

We have carried out a genome-wide screen of all 22 autosomes for IDDM susceptibility, and have also followed up the results from previous studies. Our initial panel consisted of 212 ASPs (Set 1). An additional 467 ASPs (Set 2) were typed for follow-up studies. In the full sample of 679 ASPs, analyses were carried out for previously reported candidate regions^{5,6,9,18-20} (*IDDM1*–*IDDM15*) and markers/regions with moderately increased sharing in our initial screen. Genotypes were determined for 438 simple tandem repeat polymorphisms (STRPs) of known map location. Multipoint analyses, which had not previously been applied to a genome-wide screen for IDDM, were carried out on the complete data set.

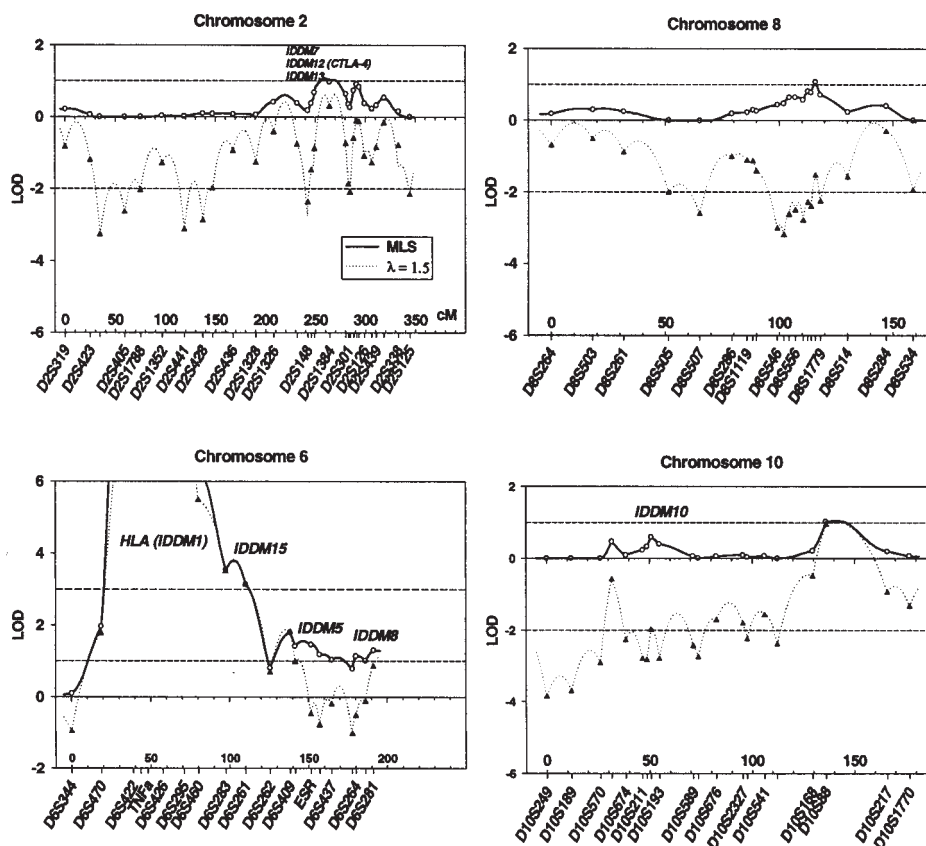
Table 1 • Multipoint IBD and lod results

Marker	Location (cM from pter)	N ^a	Multipoint		
			IBD	MLS ^b	MLS ^c
<i>D1S1644</i>	278	851	54.7%	2.25	1.78
<i>D1S1617</i>	281	798	55.6%	3.31	2.84
<i>D1S1668</i>	283	651	54.9%	2.58	2.16
<i>D1S103</i>	284	874	54.5%	2.21	1.83
<i>D1S1656</i> ^d	284	953	54.5%	2.22	1.90
<i>AGT</i> ^e	285	712	54.8%	2.44	2.06

^aNumber of informative transmissions to ASPs. ^bIn families with more than two affected sibs, the first offspring in the file list was paired with each of the others, so that only fully independent ASPs were used for analysis. ^cAll possible ASPs were used for analysis. ^dWhen *D1S1656* was genotyped in the Set 1 families, we found IBD of 59% with $N = 328$; this was the original suggestion of linkage with IDDM on chromosome 1q, and led us to type the entire set of families with more markers in the region. For this follow-up, *D1S1656* was also retyped in the Set 1 families, and yielded IBD of 54% with $N = 297$. ^eAngiotensinogen precursor.

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Fig. 2 Multipoint lod map of IDDM and markers on chromosomes 2, 6, 8 and 10; each has at least one site with lod greater than 1. For details, see Fig. 1 legend.



Multipoint maps of all 22 autosomes (Figs 1–3), for combined Set 1 and Set 2 data, show that there were nine regions (on five chromosomes: 1, 2, 6, 8, 10) where the multipoint lod (MLS) was greater than 1 (Figs 1,2). Only the HLA region (chromosome 6p21.3) yielded significant evidence of linkage by the stringent criterion of lod greater than 3.6 (ref. 21); here the highest lod was 34.2.

A second, previously unrecognized region on chromosome 1q near the marker *DIS1617* showed suggestive evidence of linkage (lod=3.31), with multipoint lod greater than 2 at six markers spanning 7.6 cM (Table 1). The markers showing strongest evidence for linkage are located in a region representing a 40-cM gap in one genome screen¹, or covered by small samples of sib-

pairs in another². No significant linkage disequilibrium²² with IDDM was found in this region.

We also obtained elevated lods for a second region on chromosome 6, at 6q21 and loosely linked to HLA, where a previous study¹⁰ placed *IDDM15*. The large effect at HLA extends into this region (Fig. 2); thus to evaluate results for *IDDM15* (*D6S283*) it is necessary to take linkage with HLA (6p21.3) into account. We determined the component of the lod at *D6S283* that would be predicted, solely as a consequence of linkage with HLA. The observed IBD at HLA (TNF) was 0.73, and the recombination fraction with *D6S283* was 0.38 (yielding predicted lod 1.24) or 0.35 (predicted lod 2.09), depending on whether we used the

Table 2 • Linkage results at previously reported IDDM loci

Locus	Chromosome	Defined by marker	Marker tested	Set 1		Set 2		Total (Multipoint)		
				N	IBD (%)	N	IBD (%)	N	IBD (%)	MLS
<i>IDDM1</i>	6p21.3	<i>HLA-DQB</i>	<i>TNF</i>	264	64 ^a	354	77 ^a	618	73.0	32.50
<i>IDDM2</i>	11p15.5	<i>INS VNTR</i>	<i>TH</i>	253	53 ^d	354	54 ^d	607	53.2	0.60
<i>IDDM3</i>	15q26	<i>D15S107</i>	<i>D15S107</i>	252	57 ^c	254	48 ^d	506	50.7	0.03
<i>IDDM4</i>	11q13	<i>FGF3</i>	<i>FGF3</i>	262	56 ^d	516	51 ^d	778	52.4	0.43
<i>IDDM5</i>	6q25	<i>ESR1</i>	<i>ESR1</i>	291	57 ^b	561	52 ^d	852	54.1	1.46
<i>IDDM6</i>	18q21	<i>D18S64</i>	<i>D18S39</i>	302	50 ^d	—	—	302	50.0	0.00
<i>IDDM7</i>	2q31	<i>D2S152</i>	<i>D2S152</i>	326	56 ^c	327	51 ^d	653	52.7	0.72
<i>IDDM8</i>	6q27	<i>D6S264</i>	<i>D6S264</i>	242	49 ^d	488	55 ^c	730	54.0	1.14
<i>IDDM9</i>	3q21–q25	<i>D3S1576</i>	<i>D3S1576</i>	244	50 ^d	299	52 ^d	543	52.1	0.23
<i>IDDM10</i>	10 cen	<i>D10S193</i>	<i>D10S193</i>	283	49 ^d	326	53 ^d	609	52.5	0.40
<i>IDDM11</i>	14q24.3	<i>D14S67</i>	<i>D14S48</i>	236	56 ^c	197	49 ^d	433	52.5	0.28
<i>IDDM12</i>	2q33	<i>CTLA4^e</i>	<i>D2S1391</i>	259	60 ^a	326	48 ^d	585	53.5	0.84
<i>IDDM13</i>	2q34	<i>D2S301</i>	<i>D2S301</i>	200	55 ^d	218	43 ^d	418	52.6	0.36
<i>IDDM14*</i>										
<i>IDDM15</i>	6q21	<i>D6S283</i>	<i>D6S283</i>	282	52 ^d	490	59 ^a	772	57.1 ^f	3.51 ^f

For comparison with published results, we give MLS precisely at the marker indicated. In the text, we describe 'regional' maximum multipoint lods, which are slightly higher in some cases. *No published information is available on location or linkage for *IDDM14*. ^a $P < 0.001$, ^b $P = 0.01$, ^c $P < 0.05$, ^dNS ($P > 0.05$) uncorrected P -values. ^eTDT for G allele at position 49: 398 transmitted, 352 not transmitted, $\chi^2 = 2.8$, $P > 0.05$. ^fMLS is 1.71 – 2.27 after adjustment for linkage with HLA (see text).

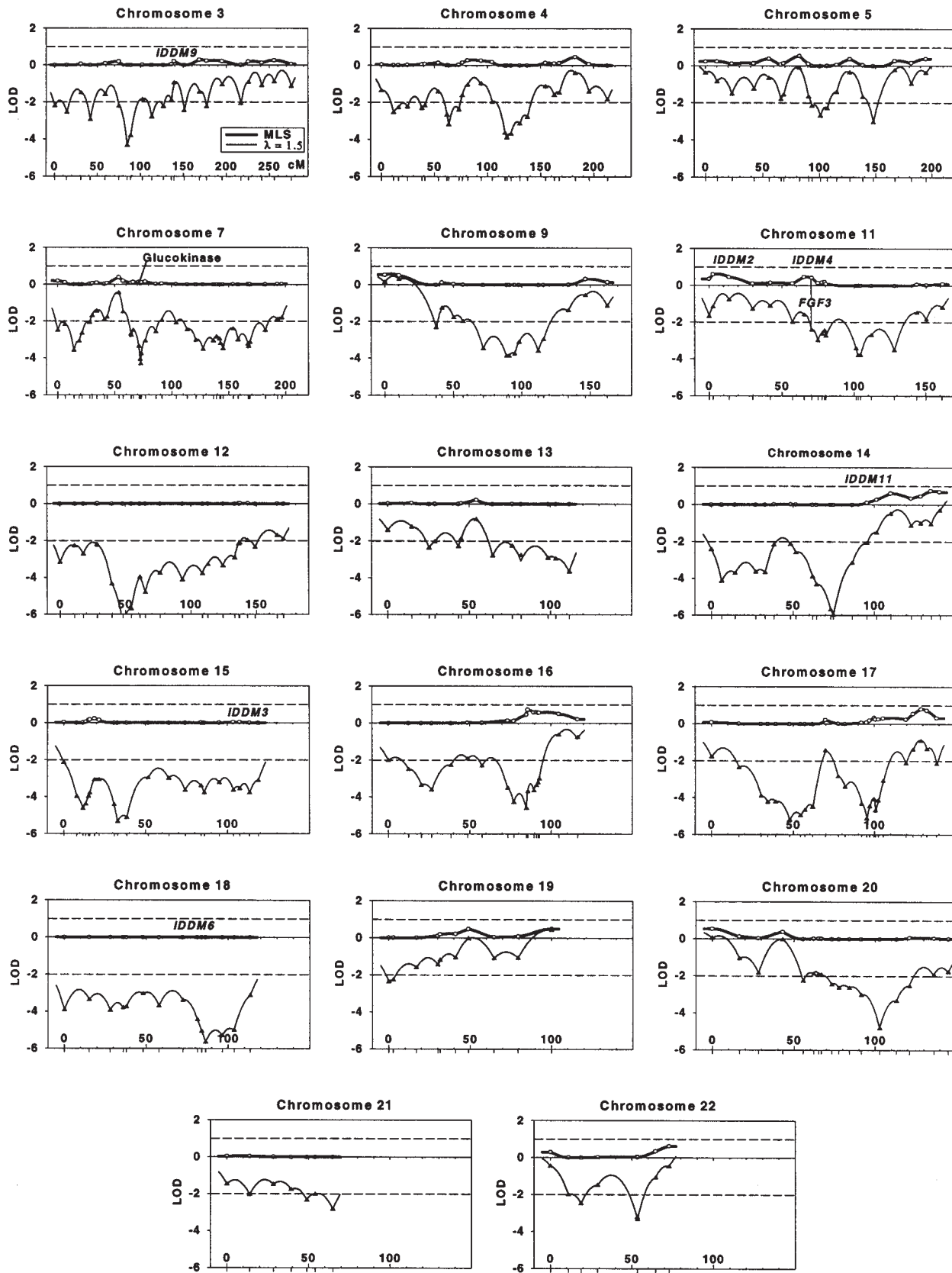


Fig. 3 Multipoint lod map of IDDM and markers on 17 chromosomes where there was no region with lod greater than 1. The lower line in each panel shows the exclusion lod. For details, see Fig. 1 legend.

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Kosambi or the Haldane mapping function, respectively. As the total lod at *D6S283* is estimated as 3.51 or 3.80, respectively, the 'adjusted' lod score is 2.27 or 1.71, respectively. These values define realistic estimates of the lod range at *D6S283*, after correction for that due to linkage with HLA.

Only chromosome 1q, HLA and possibly chromosome 6q21 had lods greater than 1.8 (Figs 1,2). We found lods minimally greater than 1 in three regions where linkage to IDDM has not previously been reported; chromosomes 1, 8 and 10 had maximum multipoint lods of 1.04, 1.08 and 1.05, respectively.

Linkage has been reported for the remaining regions with lods between 1.0 and 1.8 (chromosomes 6q and 2q; Table 2). On chromosome 6q, we observed maximum multipoint lods of 1.8 (12-cM proximal to *ESR1* at 6q25) and 1.3 (for *D6S281* at 6qter). These values are the regional maxima; lower lods were seen exactly at *ESR1* and *D6S264*, the markers originally used to define *IDDM5* and *IDDM8*, respectively. On chromosome 2q, previous studies have indicated three loci: *IDDM7*, *IDDM12* and *IDDM13* (refs 23, 6, 5). The maximum lod in this region is approximately 1.07 (Fig. 2), and evidence for distinct loci is equivocal. The majority of chromosomes in this genome screen show no regions where lods are even modestly elevated (Fig. 3).

In view of the reports of more than a dozen IDDM loci, we examined our data for evidence in the corresponding regions of the genome. Markers at or near the previously reported IDDM loci were typed in the full set of 679 ASPs, except for *IDDM6*, which was typed only in Set 1 families. Set 1 families largely overlap with families used in previous genome screens. The sample of Set 2 families does not overlap with those studied in early large-scale genome screens^{1,2}; the overlap with other studies^{9,10,17,23} ranges from 25 to 90 families in the 428 Set 2 families (467 ASPs).

Results were obtained for all but one of the 15 named IDDM loci. (*IDDM14* has not been identified in published material; Table 2). For seven of the 13 loci, the IBD was not significant in Set 1 families (nominal $P > 0.05$). The remaining six loci (*IDDM1*, 3, 5, 7, 11, 12) had significant IBD (nominal $P < 0.05$) in Set 1 families, consistent with previous reports. For all of these IDDM loci except HLA, however, the IBD was much lower in the Set 2 follow-up, leading to non-significant IBD in the total. (For *IDDM15*, discussed above, IBD was significant in Set 2 but not in Set 1.)

As the families in Set 2 show little overlap with those studied by others, and as the sample size of Set 2 is more than twice that of Set 1, these results constitute a marked non-replication. Furthermore, when we combined Set 1 and Set 2 data for multipoint analyses, we failed to replicate the previous results for most markers, despite the inclusion of families (mostly from Set 1) that suggested linkage in previous studies. Removing the 'overlap' families, if it were possible, would further weaken the evidence for linkage.

Excluding HLA and INS, the 12 new IDDM loci^{5-10,17,19,20,23-25} fall into 9 chromosomal regions. In three of these we found lods greater than 1: chromosome 2q31-q34 (lod 1.07; *IDDM7*, 12, 13); chromosome 6q21 (lod 1.7-2.3; *IDDM15*); chromosome 6q25-qter (lod 1.3-1.8; *IDDM* 5, 8). For the six remaining IDDM loci identified in previous genome screens (*IDDM3*, 4, 6, 9, 10, 11), located on six other chromosomes, we found weak or negligible support (maximum multipoint lod: 0.43). In summary, we found little or no support for linkage in six of nine previously reported chromosomal regions, and only modest (non-significant) support for the rest: three regions (6 markers) on chromosomes 2q and 6q.

The multipoint results allowed us to exclude even modest contributions to IDDM ($\lambda_s \geq 1.5$) at six of the 12 new IDDM loci. Lods of less than -2 were found for *IDDM3/D15S107* (lod = -3.6), *IDDM4/FGF3* (-2.43), *IDDM6/D18S39* (-3.4),

IDDM9/D3S1576 (-2.26) and *IDDM10/D10S193* (-2.8). The lod at *IDDM13/D2S301* is also strongly negative (-1.9).

In addition to the basic analysis of data from genome screens, some investigators have reported results from 'conditional' analyses. Here we do not present such analyses. Typically, conditional analyses of one marker are done by dividing the total sample into subsets defined by variation at some other marker (for example, HLA). Although such stratification procedures have been used in a number of studies^{1,2,5,7,17} with the goal of strengthening evidence for linkage, the results have been difficult to interpret confidently.

For this procedure to enhance the power to detect linkage, the tested locus must show a large difference in IBD among subsets; when is this expected to happen? If the joint effect of two or more loci is 'non-additive', the differences seen after stratification are not large when the individual locus effects are of plausible size (those considered in ref. 26, for example). In fact, non-additive joint effects that are exactly multiplicative produce no differences among subsets. On the other hand, genetic effects that are additive can produce such differences²⁶. Again, however, given plausible effects of the individual loci, the differences are not large enough to increase substantially the power to detect linkage. In the present case, non-additive interactions are suggested by recurrence risk patterns in relatives²⁷.

In practice, conditional analyses with modest sample sizes can produce inconsistent results, as illustrated by the first genome screens in IDDM^{1,2}. Two groups reported that the evidence for linkage at *FGF3* (the defining marker for *IDDM4*) was appreciably stronger after they 'conditioned' on HLA sharing (or HLA type). One group, however, found increased evidence in ASPs that shared at HLA, and the other group found the increase in ASPs that did not share at HLA. These contradictory findings cannot both be interpreted as strengthening the evidence for linkage with *FGF3*.

Once linkage is established, it is possible in principle that conditional analyses, or other ways of examining joint distributions across loci, will help in characterizing complex inheritance patterns. On theoretical and empirical grounds, however, this procedure is unlikely to enhance the power to detect linkage with IDDM in the first place.

What do the present results suggest about the use of linkage methods to identify IDDM loci? In this largest genome screen for IDDM to date, we found one new region with a multipoint lod of 3.3 on chromosome 1q. This evidence for linkage with IDDM is considerably stronger than that in our data for any of the other new IDDM loci identified, including *IDDM4*, 5 and 8, described as confirmed^{19,20}. Additional results from tests for linkage and for linkage disequilibrium²² will be required to establish whether the present results, or those for any of the other putative IDDM loci, can be consistently replicated.

Despite our use of larger samples (Sets 1 and 2 combined) and a dense marker map, we did not find significant evidence for most previously identified IDDM susceptibility loci. Although the effect of the HLA region is easily detected, it remains unclear how many additional IDDM susceptibility loci are detectable by IBD methods in a study of the present size. For example, the only other known susceptibility locus (*IDDM2*) gave results that were not significant in our material (IBD = 53%, lod = 0.60). This is not surprising, in view of the modest contribution of this locus to IDDM; $\lambda_s = 1.1$ from association data¹⁵, $\lambda_s = 1.1$ in present IBD data.

The contribution of HLA was originally estimated at $\lambda_s = 3.2$ (ref. 15), consistent with our current results for the closely linked marker at TNF ($\lambda_s = 3.6$). The overall λ_s for sibship aggregation of IDDM is approximately 15 (ref. 15). Thus HLA accounts for only a fraction of the total familial aggregation, suggesting that additional loci also contribute. Our results suggest that individually these loci are not likely to have substantial

effects (for example, $\lambda_s = 2$), so it will be difficult to detect them by linkage. Assuming $\lambda_s = 2$ for putative additional loci, our results exclude ($\text{lod} < -2$) 85% of the genome. In contrast, only 35% of the genome could be excluded for $\lambda_s = 1.5$ (Figs 1–3). Genes with effects this small, however, will not be easily and reliably found by conventional linkage methods.

Methods

DNA samples from IDDM families. The full set of family material consisted of 679 ASPs in 616 nuclear families. DNA was obtained from the Human Biological Data Interchange²⁸, the British Diabetic Association-Warren Registry²⁹ and material collected at The Children's Hospital of Philadelphia³⁰. A two-stage approach³¹ was employed to test for linkage. The full genome screen was carried out using 212 ASPs in 188 families (Set 1 families: 101 from BDA, 65 from HBDI, 22 from Philadelphia). The 166 Set 1 families from BDA and HBDI include all the families studied by Davies *et al.*¹, and 23 HBDI families studied by Hashimoto *et al.*², as well as most of those studied in other previous analyses^{7–9,17}. Markers showing evidence (nominal $P < 0.05$) of linkage in the initial genome screen were typed in a second set of 467 ASPs in 428 families (Set 2 families: 149 from BDA, 279 from HBDI). It is difficult to establish the exact degree of overlap between the large sample of Set 2 families and those studied previously. From available information, there is no overlap with the full genome screens^{1,2}, and variable overlap (25 to 90 families, depending on the study) among the 428 in Set 2. The numbers of families with two, three, four, five and six affected offspring were 534, 56, 2, 1 and 1, respectively.

Genotypes. The genome-wide screen consisted of genotypes for 438 simple tandem repeat polymorphisms (STRPs) of known map location on the 22 autosomes. Primers were obtained from Research Genetics and participating labs. The mean interval between markers was 7.1 cM, and most had heterozygosity greater than 0.7. Genotypes were determined by standard methods¹⁸. Genotypes were scored from autoradiograms in blind duplicate fashion; discrepancies were resolved by a third reader or by retyping. Selected markers yielding evidence for significantly increased sharing were retyped in more than one of the collaborating laboratories without knowledge of genotypes obtained previously.

Approximately 50 markers were followed up by typing of Set 2 families because of elevated sharing ($\text{IBD} > 0.55$) in Set 1, regardless of lod value. In most cases additional closely linked markers were typed as well. Except for DIS1656, none of these resulted in significant evidence for linkage in the total material, so they are not reported separately. They account, however, for regions of high marker density (Figs 1–3).

Analysis. For each family, the complete set of marker data was screened for families that consistently yielded genotypes violating mendelian requirements. After inconsistencies were removed or corrected, the computer program ASPEX³² was used to construct marker maps from the typing data, and to carry out multipoint lod analysis based on the intermarker distances. In families with more than two affected sibs, the first offspring in the file list was selected (arbitrarily) and paired with each of the others, so that only fully independent ASPs were used for analysis. No untyped parents were used. In the alternative analysis (of chromosome 1q results only; Table 1), multipoint lods were estimated when all possible ASPs were formed, resulting in a maximum multipoint lod of 2.84, also at marker DIS1617. When meioses from parents without genotype data were included, the maximum lods in this region of chromosome 1 were approximately 0.1 lower than those shown in Table 1.

In Table 2, we report IBD for single markers, for comparison with published results. For the total material, we report IBD and lods as multipoint estimates, since these provide the best available means for assessing linkage for a region. Maximum multipoint lods were obtained by estimating IBD. Multipoint exclusion mapping is presented for a fixed λ_s value of 1.5 (N.R., unpublished observation; ref. 33).

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