

Evidence for a locus (IDDM16) in the immunoglobulin heavy chain region on chromosome 14q32.3 producing susceptibility to type 1 diabetes

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Type 1 diabetes results from autoimmune destruction of pancreatic islet β -cells, possibly initiated or exacerbated by viral infections. Recent studies have demonstrated that antibodies towards enterovirus and autoantibodies towards islet cell components develop in the long preclinical phase of type 1 diabetes. We therefore hypothesised that susceptibility to type 1 diabetes could be influenced by genetic factors controlling production of antiviral antibodies or autoantibodies or both. To search for evidence of linkage or association (linkage disequilibrium) between type 1 diabetes and the immunoglobulin heavy chain (IGH) region, 351 North American and British families with ≥ 2 diabetic children were genotyped for IGH region microsatellites. Using affected sibpair analysis, significant evidence for linkage was obtained for three markers close to the IGH gene cluster (P values 0.004, 0.002, 0.002). No evidence was found for association using family-based methods. To attempt to confirm these findings, a smaller dataset (241 families, 138 with ≥ 2 diabetic children) from Denmark, a more genetically-homogeneous population, was genotyped for one marker only. These families showed no linkage, but significant evidence for association ($P = 0.019$). This study suggests that a locus (assigned the symbol IDDM16) in the IGH region, possibly an IGH gene, influences susceptibility to type 1 diabetes. Genes and Immunity (2002) 3, 338–344. doi:10.1038/sj.gene.6363857

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Introduction

Type 1 diabetes (formerly called insulin-dependent diabetes mellitus, or IDDM) results from autoimmune destruction of the insulin-producing β -cells of the pancreas. For more than 25 years, it has been known that HLA region genes contribute substantially to predisposition to type 1 diabetes.^{1–3} However, theoretical studies in the mid-1980s suggested that genes outside the HLA region may contribute more than half of the genetic susceptibility to this form of diabetes.⁴ Since then, many putative diabetes-predisposing genes outside the HLA region have been localised, including IDDM2 in the insu-

lin gene region⁵ (HLA region predisposition is now collectively referred to as IDDM1), IDDM3,^{6–8} IDDM4,^{6,9–12} IDDM5,^{9,11} IDDM6,¹³ IDDM7,^{14,15} IDDM8,^{7,11} IDDM10,^{16,17} IDDM11,¹⁸ IDDM12,^{19–21} IDDM13,^{21,22} IDDM15,²³ IDDM17,²⁴ IDDM18,²⁵ and other putative localisations currently without an IDDM designation.^{17,26–29} Thus, it is now clear that while HLA region genes contribute the major predisposition to type 1 diabetes, there are numerous other genes with smaller effects on susceptibility. However, particular combinations of these 'minor' non-HLA susceptibility genes may have large effects on diabetes predisposition.

Type 1 diabetes has a long preclinical phase, during which autoantibodies develop towards a variety of islet cell components, such as cytoplasmic islet cell antigens (ICA), insulin (IAA), glutamic acid decarboxylase (GAD), and protein tyrosine phosphatase-2 (IA-2). Presence of autoantibodies (especially multiple antibodies at high titres) is strongly associated with risk of progressing to clinical diabetes, both in relatives of type I diabetics^{30–33} and in the general population.³⁴ It is not clear whether these autoantibodies directly participate in β -cell destruction by binding with β -cell antigens, or whether they arise as a consequence of β -cell destruction following release of self-antigens from destroyed β -cells³⁵ or both. Twin studies suggest that propensity to autoantibody formation is partially under genetic control, since the frequency of autoantibodies was higher among non-diabetic monozygotic co-twins of type 1 diabetics than among non-diabetic dizygotic co-twins of diabetics.^{36,37}

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The factors which initiate the autoimmune destruction of pancreatic β -cells in genetically-susceptible individuals are still unknown, but recent prospective studies provide strong evidence that viruses may be involved. Increased frequencies of antibodies towards enteroviruses such as Coxsackie B have been found in children who subsequently became diabetic³⁸ and mothers at time of delivery of children who subsequently became diabetic.³⁹ Furthermore, temporal association has been demonstrated between appearance of antiviral antibodies and islet cell autoantibodies in siblings of type I diabetics⁴⁰ and in HLA-high-risk children in the general population.⁴¹ One mechanism proposed to explain how virus infection could initiate an autoimmune response is structural similarity ('molecular mimicry') between a viral antigen and a pancreatic β -cell protein.^{42–44}

Production of the heavy chain portion of antibodies is controlled by a cluster of genes in the immunoglobulin heavy chain (IGH) region on chromosome 14q32.3. Genes in this cluster encode the constant regions (C_H domains) of IgG, IgA, IgM, IgE, and IgD isotype heavy chains (including allotype polymorphisms), isotype switch regulation, and the variable (V_H , antigen-binding), diversity, and joining regions of all immunoglobulin heavy chains. We hypothesised that genes controlling production of viral antibodies, or autoantibodies, or both, could influence susceptibility to type 1 diabetes. We here report evidence for linkage and association of type 1 diabetes with genetic markers located at the IGH gene cluster.

Results

Dataset 1

Affected sibpair analysis using 351 families with two or more diabetic children (Canadian, British, and American subjects) showed significant evidence for linkage of type 1 diabetes to the three genetic markers surrounding the IGH gene cluster (see Table 1). These markers included D14S292 ($P = 0.004$), D14S542 ($P = 0.002$) and D14S293 ($P = 0.002$). D14S1007, located more than 2 Mb distal to the IGH region at the extreme qter end of chromosome 14, did not show significant evidence for linkage to diabetes ($P = 0.211$). Analysis of linkage in subsets of families categorized by gene sharing in the HLA region suggested stronger evidence for linkage to D14S293 (as indicated by mean sharing at that marker) in families with >50% shar-

ing at HLA (mean D14S293 sharing 0.56, $P = 0.0002$) than in families with $\leq 50\%$ sharing at HLA (mean D14S293 sharing 0.50, $P = 0.56$) (Table 1). Diabetic sibpairs with increased sharing of HLA genes are known to have a significantly higher frequency of HLA-DR3/4 (the HLA genotype with the greatest risk of developing diabetes) than diabetic sibpairs without increased HLA sharing. Therefore, we further analysed families subdivided by whether or not the first affected child had the genotype HLA-DR3/4 (Table 2). Results of this analysis showed a tendency for stronger linkage evidence in HLA-DR3/4-index families than in non-HLA-DR3/4-index families: D14S292 sharing 0.54 ($P = 0.042$) vs 0.53 ($P = 0.083$), respectively; D14S542 sharing 0.55 ($P = 0.026$) vs 0.52 ($P = 0.146$); D14S293 sharing 0.55 ($P = 0.014$) vs 0.52 ($P = 0.173$). Association analysis in all families produced no significant evidence for linkage disequilibrium between any marker and a putative diabetes susceptibility locus.

Dataset 2

In an attempt to confirm findings from dataset 1, an independent set of 241 Danish families was typed after dataset 1, for one marker only (to reduce the probability of false-positive results). Affected sibpair analysis of the 138 multiplex Danish families showed no significant evidence for linkage to D14S542, although there was a slight elevation of gene sharing (sharing 0.516; $P = 0.266$). However, as seen in Table 3, AFBAC association analysis using all 241 Danish families (first diabetic child only) revealed a significant association between D14S542 and type 1 diabetes ($P = 0.0191$). The largest χ^2 contribution to the association effect was decreased transmission of the 92 bp allele to the diabetic child; this allele comprised 11% of the transmitted group of alleles compared with 18% of the non-transmitted group (Table 3). Subdividing the Danish families by presence/absence of HLA-DR3/4 in the index diabetic child showed evidence for decreased transmission of the 92 bp allele in both the DR3/4 subset (8% transmitted compared with 16% non-transmitted) and the non-DR3/4 subset (13% compared with 20%) (data not shown). Examination of the heterogeneous families of dataset 1 showed a non-significant trend in the same direction—the D14S542 allele with the largest effect was also the 92 bp allele, with decreased transmission to

Table 1 Results of affected sibpair linkage analysis for dataset 1^a (all families and HLA-sharing subgroups)

Marker (cen-qter)	Mb ^c	All families			Families with >50% HLA sharing ^b			Families with $\leq 50\%$ HLA sharing ^b		
		No. Affected sibpairs	Mean sharing	<i>P</i> value	No. affected sibpairs	Mean sharing	<i>P</i> value	No. affected sibpairs	Mean sharing	<i>P</i> value
D14S292	105.43	321	0.540	0.0042	208	0.537	0.0272	110	0.545	0.035
D14S542 ^d		395	0.542	0.0022	254	0.540	0.0135	134	0.545	0.041
D14S293	106.14	390	0.540	0.0018	255	0.561	0.0002	129	0.497	0.557
IGH	105.38– 106.65									
D14S1007 qter	108.98 109.00	402	0.512	0.211	258	0.516	0.203	130	0.514	0.300

^aComprised of 351 multiplex families: 125 Canadian, 98 British, 128 American. ^bNot all families had HLA sharing data, therefore number of affected sibpairs is greater for all families than for the sum of families with >50% and $\leq 50\%$ HLA sharing. ^cMegabases from pter, according to Location Database (LDB). ^dAccording to NCBI STS maps, D14S542 is located between D14S292 and D14S293.

Table 2 Results of affected sibpair linkage analysis for Dataset 1^a (HLA-DR3/4 subgroups)

Marker (<i>cen-qter</i>)	Mb ^c	Families with HLA-DR3/4 index diabetic ^b			Families without HLA-DR3/4 index diabetic ^b		
		No. affected sibpairs	Mean sharing	<i>P</i> value	No. affected sibpairs	Mean sharing	<i>P</i> value
D14S292	105.43	128	0.541	0.0416	188	0.528	0.083
D14S542 ^d		149	0.545	0.0258	218	0.522	0.146
D14S293	106.14	144	0.549	0.0136	221	0.517	0.173
IGH	105.38– 106.65						
D14S1007	108.98	147	0.521	0.1866	227	0.490	0.692
qter	109.00						

^aComprised of 351 multiplex families: 125 Canadian, 98 British, 128 American. ^bNot all families had HLA-DR3/4 data, therefore total number of sibpairs differs from those in Table 1. ^cMegabases from pter, according to Location Database (LDB). ^dAccording to NCBI STS maps, D14S542 is located between D14S292 and D14S293.

Table 3 Results of AFBAC association analysis for D14S542 in Danish families

Allele size (bp)	Transmitted No. (Freq.)	Non-transmitted No. (Freq.)	χ^2
86	28 (0.07)	23 (0.05)	0.49
88	91 (0.21)	105 (0.24)	1.00
90	174 (0.40)	151 (0.35)	1.63
92	48 (0.11)	78 (0.18)	7.14
94	38 (0.09)	41 (0.10)	0.11
96	34 (0.08)	19 (0.04)	4.25
<86, >96	17 (0.04)	13 (0.03)	0.53
Total alleles ^a	430	430	
Summed χ^2			15.15
<i>P</i> Value			0.019

^aNot all families had both parents typed.

the diabetic child (15% transmitted *vs* 18% non-transmitted) (data not shown).

Discussion

The results of this study suggest, by evidence for linkage in an ethnically heterogeneous dataset and for linkage disequilibrium in a more homogeneous dataset, that a type 1 diabetes susceptibility locus is located in or near the IGH cluster. This diabetes gene has been assigned the symbol *IDDM16* by the Human Gene Nomenclature Committee (<http://www.gene.ucl.ac.uk/cgi-bin/nomenclature/searchgenes.pl>).

Immunoglobulin molecules have a central role in immune response to foreign and self antigens. In the cellular immune system, the Fc-receptors of circulating macrophages (a type of antigen presenting cell, APC) detect antigen-antibody complexes by recognising the Fc portion of the constant region of immunoglobulin heavy chains. The complex is internalised, then processed antigen is presented on the APC surface in conjunction with HLA class II molecules and recognised by T cell receptors, inducing T cell proliferation. In the humoral immune system, B cells are stimulated to produce antibodies when immunoglobulins expressed on their surface detect antigen. B cells can also function as APCs—the B cell antigen receptor (BCR, a complex of membrane

immunoglobulin and signalling subunits) takes up antigen, which is processed and presented in the context of HLA to T cells. Immunoglobulins also participate in cell killing by binding cellular antigen and activating the complement cascade. It is therefore conceivable that genetically-controlled differences in immunoglobulin structure and function could alter a person's immune response to foreign (eg viral) or self (eg pancreatic β -cell) antigens, or both.

Between 1984 and 1991, more than 20 studies examined serologically-detected Gm allotypes, variants on the constant region of IgG heavy chains, in type 1 diabetes (reviewed in references 45–47). Although none of these studies found direct evidence of linkage or association with diabetes, many reported evidence of IGH region involvement through interaction with HLA genes. In most datasets, the Gm(1) frequency was higher in HLA-DR3/4 diabetics than in those who were not DR3/4.^{45,48–52} Two discrepant datasets showed other significant Gm-HLA effects.^{53,54} Linkage studies have been less common, but overall have suggested increased joint sharing of Gm and HLA haplotypes in diabetic sibpairs.^{45,55–57} In one of the few studies examining Gm allotypes for all three polymorphic IgG subclasses, the strongest interaction was a lower frequency of Gm(23) in HLA-DR3/4 diabetics *vs* non-DR3/4 diabetics or normal controls.⁴⁵ Since haplotypes positive for Gm(1) are negative for Gm(23), this finding was consistent with previous studies and suggested that negativity for Gm(23), an IgG2 subclass marker, increases susceptibility to type 1 diabetes in HLA-DR3/4 individuals.

The studies summarised above investigated Gm allotypes (IgG constant region variants). However, it is possible that the observed effects could be due to nearby genes in linkage disequilibrium with Gm loci—for example, IgA, IgM, IgE, or IgD heavy chain constant region genes, or IGH *variable* region genes. Since 1991, there have been very few new studies of IGH markers in type 1 diabetes. A Finnish study found no evidence for linkage to IGH variable region markers, but significant association for two of the four markers ($P=0.02$ and 0.004).⁵⁸ This evidence for association but not for linkage is similar to our findings using the Danish dataset. Risch and Merikangas⁵⁹ have shown theoretically that association (linkage disequilibrium) analysis is more powerful than linkage analysis for detecting susceptibility genes

for complex multigene disorders, provided that the marker is very near the susceptibility locus. However, studies with large sample sizes (such as our dataset 1 with 351 families) may be capable of detecting linkage. Other investigators analysing an even larger dataset of 616 families²⁷ also detected (but did not comment on) evidence for linkage of type 1 diabetes to distal chromosome 14q, with a maximum lod score (MLS) approaching 1.0 (see figure 3 in reference 27). Interestingly, D14S292 has shown evidence for linkage to another autoimmune disorder, multiple sclerosis (MLS 1.4).⁶⁰

Previous studies using Gm serological markers detected only HLA-dependent linkage or association with diabetes. In the present study, evidence for linkage to D14S293 appeared stronger in families with >50% HLA sharing (Table 1). This finding is consistent with the Gm studies showing increased joint sharing of Gm and HLA haplotypes in diabetic sibpairs, suggesting possible synergy between susceptibility genes in the IGH and HLA regions. Similarly, in the present study there was stronger evidence for IGH linkage in HLA-DR3/4-index families (Table 2), consistent with previous results showing a lower frequency of the IgG2 allotype Gm(23) in DR3/4 diabetics than in non-DR3/4 diabetics or in the general population. However, in the Danish families, the D14S542 association appeared to be independent of whether or not the index diabetic child was DR3/4.

Gm allotypes are known to be associated with differences in serum concentrations of IgG subclasses in normal individuals, and studies have consistently shown that individuals who are Gm(23)-negative have lower IgG2 levels than individuals positive for that allotype.⁶¹⁻⁶⁴ For example, Nahm *et al*⁶¹ showed that 90% of blood donors with low levels of IgG2 were Gm(23)-negative, compared with only 30% of unselected donors. It is also known that Gm allotypes influence IgG subclass response to specific pathogen antigens. Individuals who lack Gm(23) have lower IgG2 responses to H. influenza type b polysaccharide, which may compromise ability to control infection by this pathogen, since IgG2 is thought to be important in the immune response to bacterial polysaccharide antigens.⁶⁵ It is possible that Gm(23)-negative individuals may also have deficient IgG2 antibody responses to diabetogenic viruses, resulting in an increased predisposition to type 1 diabetes, particularly in conjunction with high risk HLA types.

An alternative explanation for IGH involvement in diabetes susceptibility involves propensity to production of autoantibodies towards islet cells (ICA), insulin (IAA), glutamic acid decarboxylase (GAD), and islet cell antigen 512 (IA-2). Association between IAA and Gm type has been reported in Japanese newly-onset type 1 diabetics.⁶⁶ Recent studies have shown increased frequencies of autoantibodies in blood taken from infants and young children who subsequently develop diabetes, suggesting that the autoimmune process may begin very early.³¹⁻³³ Among relatives of type 1 diabetics, those with anti-GAD antibodies who progressed to develop diabetes ('progressors') were more likely to have anti-GAD of subclass IgG1 and/or IgG3. On the other hand, relatives with anti-GAD antibodies who did not progress to develop diabetes ('non-progressors') were more likely to have IgG2 and/or IgG4 antibodies.⁶⁷ Thus, a higher risk of diabetes was associated with specific IgG subclasses of anti-GAD, ie IgG1 and IgG3, which in turn would be more

likely to occur in Gm(23)-negative persons. Another recent study found that progressors had lower levels of specific isotypes (IgM and IgE) of anti-GAD antibodies than non-progressors.⁶⁸ If autoantibodies actively participate in the process of pancreatic β -cell destruction, then the IgG subclass and/or Ig isotype predominance of anti-GAD could influence risk of developing diabetes.

A third possible explanation for IGH effects on diabetes predisposition involves both response to pathogens and propensity to autoantibody formation. In this hypothetical scenario, minor leakage of non-tolerized pancreatic β -cell antigens could occur as a result of random virus-induced β -cell damage or developmental turnover of β -cells. These self antigens would be presented with HLA to T-helper cells, leading to low levels of autoantibody production, but the number of self molecules being presented would not be sufficient to initiate cytotoxic T cell destruction of β -cells. However, in situations where antigen release from β -cells is increased, eg following repeated or chronic viral infection, intensified autoantibody production would significantly increase the likelihood of a cytotoxic T cell attack. In addition, cross-reacting antiviral antibodies (molecular mimicry with self antigens) may also participate. Both the autoantibody response to self antigens and the antibody response to virus could be influenced by IGH region genes controlling Ig isotype or IgG subclass profile. Thus, interindividual differences at IGH region genes (in addition to other genes controlling autoimmune and antiviral response) would contribute to diabetes susceptibility.

In summary, the present study suggests that one or more loci in the IGH region, possibly IGH genes themselves, confer susceptibility to type 1 diabetes. More studies are required to elucidate the precise identity of *IDDM16*, how it alters diabetes predisposition, and the nature of its possible interaction with HLA.

Materials and methods

Subjects

Dataset 1: This dataset consisted of 351 families having two or more children with type 1 diabetes, generally onset less than 18 years of age. Families were ascertained through Pediatric Diabetes Centres across Canada ($n = 125$), through the British Diabetes Association (BDA) Warren Repository⁶⁹ ($n = 98$), and through the Human Biological Data Interchange (HBDI) in Philadelphia⁷⁰ ($n = 128$). These are the same BDA and HBDI families studied in Field *et al* (1996),¹⁸ while most of the 125 Canadian multiplex families are newly collected (only 29 were in Field *et al*, 1996). All families were of European ancestry and were nuclear (with the exception of two American families, which had affected sibling pairs in two generations). Genetic markers were typed on both parents and at least two affected children per family.

Dataset 2: This replication dataset was genotyped for one marker only, to attempt to confirm results from dataset 1. It consisted of 241 Danish families collected in collaboration with the Danish Study Group of Diabetes in Childhood and the Danish IDDM Epidemiology and Genetics Group.²¹ All families were nuclear. The 138 multiplex families contained two or more children with type 1 dia-

betes (168 affected sibpairs), and the remaining 103 families contained one diabetic child.

All subjects or their legal guardians gave informed consent for genetic studies, in accordance with requirements of the appropriate local Ethics Review Board.

Genetic marker typing

HLA region gene sharing in Canadian families was determined in our laboratory by typing HLA loci using standard serological or molecular methods,^{71–73} or by typing microsatellite DQCAR at the HLA-DQ locus.⁷⁴ HLA genotype information for British and American families was provided by the BDA and HBDI Repositories. Danish families had been previously HLA typed. Microsatellite markers were typed by standard methods.⁷⁵ Briefly, polymerase chain reaction (PCR) was performed on microtitre plates using 20 ng genomic DNA, 15 pmoles of each primer, 0.5 units Taq polymerase, 50 mM KCl, 10 mM Tris (pH 8.3), 1.5 mM MgCl₂, 0.017% BSA, 0.05% Non-ident P-40, 0.05% Tween, 200 μM each dGTP, dATP, and dTTP, 2.5 μM dCTP, and 1 μCi of [α-³²P]dCTP, in a final reaction volume of 10 μl. Samples were overlaid with 15 μl mineral oil to prevent evaporation, then PCR amplified in a Techne PHC-3 or Ericomp thermocycler using 27 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 2 min, and extension at 72°C for 1 min, starting with an initial denaturation at 94°C for 6 min and ending with a final extension at 72°C for 7 min. Then 2 μl of each reaction product plus 2 μl Stop Soln was transferred to a 6% sequencing gel for electrophoresis with M13mp18 sequencing ladder size markers, and the product visualised by autoradiography (with 3 to 48-h exposures) of the dried gel. Four microsatellite markers were typed in dataset 1: D14S292, D14S293, D14S542, and D14S1007, and only one marker (D14S542) was typed in dataset 2. Primer sequences were obtained from the Genome Database (GDB). According to the Location Database (LDB), the relative locations of the markers are (with distances in megabases from pter): D14S292 (105.43), D14S293 (106.14), IGH genes (105.38–106.65), D14S1007 (108.98), qter (109.00) (D14S542 is not placed). Current STS maps from the National Center for Biotechnology Information (NCBI) place D14S292 and D14S293 1.1 Mb apart with D14S542 between them.

Linkage analysis

Analysis of linkage between type 1 diabetes and IGH region markers was performed by estimating the mean proportion of marker genes which pairs of affected siblings shared identical-by-descent (IBD) from their parents, using the SIBPAL Ver 3.1 program in the SAGE package.⁷⁶ Linkage is suggested when sharing is significantly greater than the 50% sharing expected by chance in siblings. When a parent is uninformative (homozygous) at the marker locus, the mean sharing estimate incorporates the 0.5 random probability of sharing. In the presence of linkage, this procedure will bias the sharing estimate down towards 0.5 as the informativeness of the marker locus decreases (thus, the estimated mean sharing will be less than if only informative meioses had been scored). However, the variance of the mean sharing estimate will also be biased, becoming smaller as the number of uninformative parents increases, so that the *P* value for significance of the observed mean sharing estimate being greater than 50%

is approximately the same whether or not information from uninformative parents is used.

Linkage heterogeneity analysis

The HLA region contains the major diabetes-predisposing locus/loci identified to date. To evaluate the possibility of heterogeneity between subsets of families defined by strength of linkage to HLA, families in dataset 1 were categorized into two groups: (1) families in which the affected pair/s of siblings shared less than or equal to 50% of genes at the HLA-DR,DQ loci (usually zero or one genes shared), and (2) families in which the affected sib pair/s shared greater than 50% of HLA genes (usually two genes shared). When there was more than one pair of affected sibs, the average sharing among all pairs was used to categorise the family. Families for which HLA data was incomplete (one British and seven American) were excluded from all analyses involving HLA. The Danish families of dataset 2 were not submitted to HLA-dependent linkage analyses, since the high degree of genetic homogeneity in this population frequently made it impossible to unequivocally determine the number of HLA haplotypes shared between diabetic siblings (parents were homozygous). Finally, to further evaluate HLA-dependent effects, families of datasets 1 (which showed significant linkage) were also categorised into two groups according to whether the first diabetic child (the index case) was HLA-DR3/4 *vs* not HLA-DR3/4.

Association analysis

To investigate the possibility of association arising from linkage disequilibrium between a marker locus and an diabetes-predisposing locus (indicating very close proximity between the two loci), we compared the frequencies of alleles transmitted from parents to the first diabetic child with the frequencies of alleles not transmitted to the diabetic child, using the 'simplex' routine in the AFBAC computer program.⁷⁷ Association was tested with a $2 \times n$ contingency χ^2 , where *n* is the number of alleles. Since many uncommon alleles can occur when typing microsatellite markers, alleles with expected frequencies less than 5% in both transmitted and non-transmitted groups were combined into an 'other alleles' category prior to statistical testing. The non-transmitted alleles form a control group of representative alleles from the general population.⁷⁷ Unless there have been large amounts of recent population admixture, AFBAC analysis detects only associations due to linkage with disequilibrium between marker loci and the susceptibility locus, not spurious associations due to population stratification. Families of dataset 2 (which showed significant association) were also categorised into two groups according to whether the first diabetic child was HLA-DR3/4 or not.

As recommended by one authority,⁷⁸ exact *P* values are reported, rather than *P* values adjusted for multiple testing. Since this study arose to test a prior hypothesis, and previous studies had suggested effects of the IGH region in type 1 diabetes, we did not apply genomewide significance criteria in evaluating the linkage and association results.

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Electronic Database Information

URLs for the data in this article are as follows:

Genome Database (GDB), <http://www.gdb.org> (microsatellite primer sequences)
Location Database (LDB), <http://cedar.genetics.soton.ac.uk> (relative map locations of markers)
National Center for Biotechnology Information (NCBI) <http://www.ncbi.nlm.nih.gov> (relative map locations of markers)
HUGO Gene Nomenclature Committee, <http://www.gene.ucl.ac.uk/cgi-bin/nomenclature/searchgenes.pl> (assignment of *IDDM16* symbol)

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