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Research Article

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Evidence for Oligogenic Inheritance of Type 1 Diabetes in a Large Bedouin Arab Family

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Abstract

Based on a genomic search for linkage, a locus contributing to type 1 diabetes in a large Bedouin Arab family (19 affected relatives) maps to the long arm of chromosome 10 (10q25; nonparametric linkage = 4.99; $P = 0.00004$). All affected relatives carry one or two high-risk HLA-DR3 haplotypes that are rarely found in other family members. One chromosome 10 haplotype, the B haplotype, was transmitted from a heterozygous parent to 13 of 13 affected offspring compared to 10 of 23 unaffected siblings. Recombination events occurring on this haplotype place the susceptibility locus in an 8-cM interval between markers D10S1750 and D10S1773. Two adjacent markers, D10S592 and D10S554, showed evidence of linkage disequilibrium with the disease locus. A 273-bp allele at D10S592 was transmitted to 8 of 10 affected offspring compared to 3 of 14 unaffected siblings, and a 151-bp allele at D10S554 was transmitted to 15 of 15 affected offspring compared with 10 of 24 unaffected siblings. D10S554 and D10S592 and the closest flanking markers are contained in a 1,240-kb yeast artificial chromosome, a region small enough to proceed with positional cloning. (*J. Clin. Invest.* 1998. 102:1569–1575.) Key words: genetics • autoimmunity • chromosome 10 • HLA • genetic linkage

Introduction

Type 1 diabetes is an autoimmune disease caused by the destruction of pancreatic insulin-secreting β-cells, a process that may occur subclinically years before the onset of clinical symptoms. The risk to siblings is 15-fold higher than is the population prevalence, but the etiology of the disorder is complex and probably involves multiple genetic and environmental factors. The HLA region at 6p21 accounts for approximately half of the heritable component of susceptibility (1, 2); however, HLA-linked susceptibility is not inherited as a simple Mendelian trait, and the mechanisms of gene action and interaction remain obscure. It has been difficult to identify loci accounting for the remaining half of genetic susceptibility, although at least 14 candidate regions have been reported in studies with large numbers of Caucasian affected sib pair families (3). The general conclusion from family studies is that type 1 diabetes is genetically heterogeneous, with different genetic subtypes having indistinguishable or overlapping clinical characteristics. Heterogeneity may be the consequence of either polygenic inheritance, in which an accumulation of weak effects at many different loci determine disease susceptibility, or oligogenic inheritance, in which a few loci with major effects determine susceptibility. In either case, different loci may be involved in different families. Studies in large multiplex families are important for distinguishing these alternatives, and for mapping and cloning susceptibility genes.

Animal models of immune-mediated diabetes provide examples of both polygenic and oligogenic inheritance. Diabetes in the NOD mouse may be polygenic, with at least 15 loci contributing to disease (4–6). However, there is evidence for oligogenic inheritance in BB rats (7–9) and in LETR rats (10), with one or two non-MHC genes interacting with MHC-linked susceptibility. Interestingly, it appears that different non-MHC genes are involved in different strains. In humans, there is at least one example of a single gene that increases the risk for diabetes independently of HLA; namely, the autoimmune regulator gene (AIRE) on chromosome 21 (11, 12), mutations in which cause autoimmune polyendocrine syndrome type 1 (APS-I).[1] Although APS-I families can usually be distinguished by other clinical features, predominantly candidiasis and hypoparathyroidism, the diabetes that develop in up to 15% of APS-I homozygotes is very similar to HLA-associated type 1 diabetes, including the presence of autoantibodies to glutamic acid decarboxylase (GAD).

If oligogenicity rather than polygenicity applies to human type 1 diabetes, as a general rule or in particular families, studies of large multiplex families from genetically and culturally homogeneous populations should improve the prospects of identifying susceptibility genes. The cooperation of a remarkable Bedouin Arab family from Israel, including 19 affected individuals in three generations, has made it possible to explore the potential of mapping susceptibility genes in a single family with homogenous origins.

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1. *Abbreviations used in this paper:* AIRE, autoimmune regulator gene; APS-I, autoimmune polyendocrine syndrome type 1; GAD, glutamic acid decarboxylase; MODY, maturity onset of diabetes in the young; NPL, nonparametric linkage; YAC, yeast artificial chromosome.

Methods

The Bedouin Arab family shown in Fig. 1 was identified through ICA screening studies in Israel. The ICA$^+$ proband (G34) progressed to diabetes at age 6 mo. The extended pedigree includes 248 individuals, of whom 19 are reported to have (16 living) or to have had (2 deceased) insulin-dependent diabetes (autoimmune type 1 diabetes) (13). Following informed consent, we obtained blood samples from 108 family members, including 16 of 17 living affected relatives and the spouses and one or more offspring of two deceased affected relatives. An initial screening panel of 13 DNA samples from 10 affected relatives and three unaffected parents was used in a genome-wide search for linkage. Of the seven living affected relatives who were not included in the genome-wide screen, one (E48) was unavailable, whereas three (F80, E46, and E47) were sampled and three (E45, F61, and G30) developed diabetes after marker typing was in progress.

GAD autoantibodies were measured by radioassay using in vitro–transcribed and translated human GAD65 (14). Insulin autoantibodies were measured by competitive radioassay (15). HLA-DRB1, −DQA1, and −DQB1 alleles were amplified using PCR and typed with sequence-specific oligonucleotide probes (16, 17). Microsatellite marker typing was performed by Dr. Eric Lander (83 MIT markers) and by Research Genetics Inc. (272 markers; Huntsville, AL). Genotyping data from the Centre d'Etudes du Polymorphisme Humain (CEPH) family resource (18) were used to develop a genetic map of the 355 markers. A subset of 309 well-ordered and evenly spaced ($\sim$ 10 cM) markers was included in multipoint linkage analyses.

Additional chromosome 10 markers were typed in family members and in yeast artificial chromosomes (YACs) (obtained from Research Genetics Inc.) at the Barbara Davis Center laboratory. Marker typing was done by PCR amplification of genomic DNA using fluorescently labeled primers and an automated DNA fragment analyzer (PE Applied Biosystems, Foster City, CA).

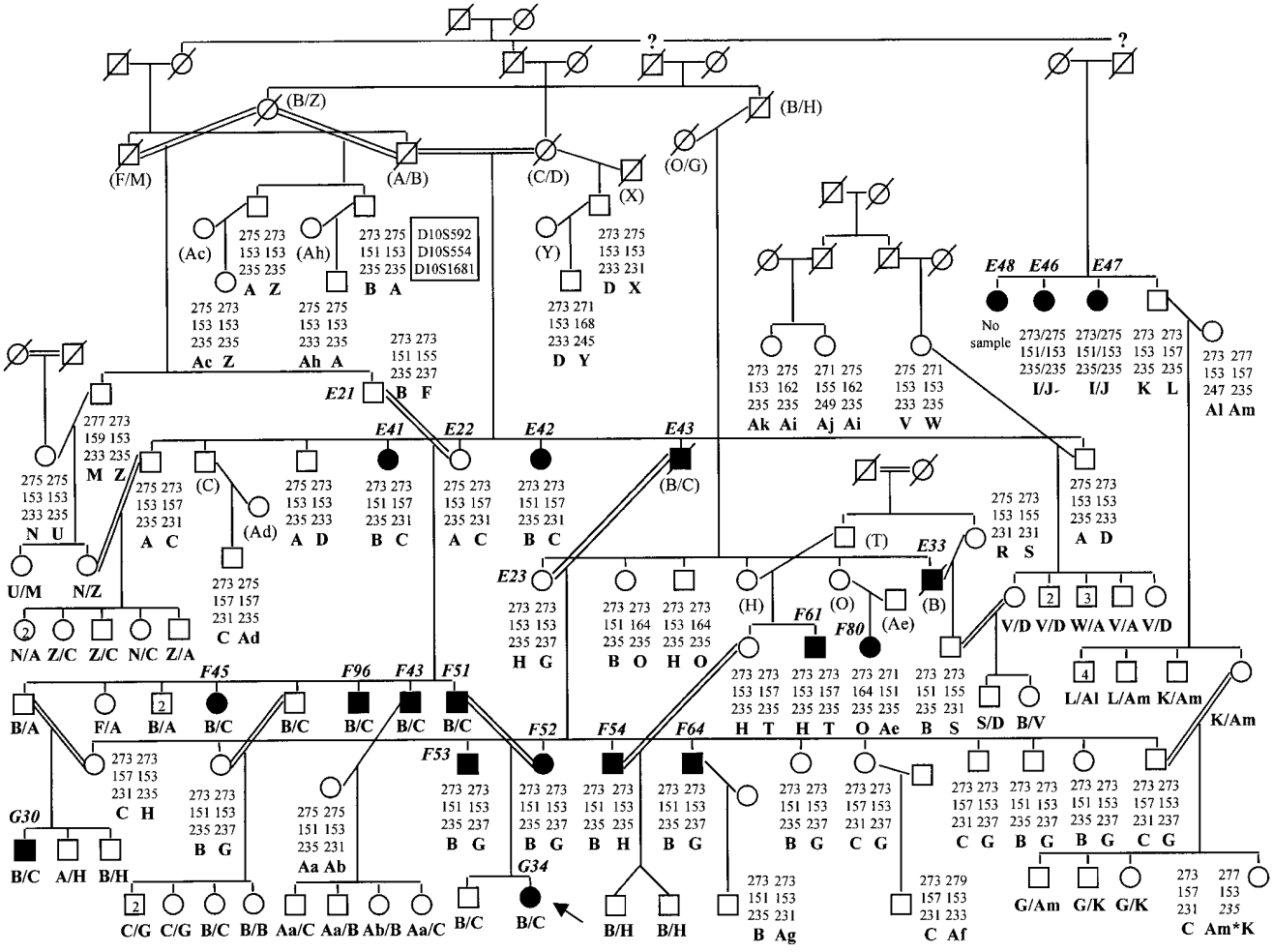


Figure 1. Extended pedigree showing 10q haplotypes of a Bedouin Arab family with 19 relatives affected with type 1 diabetes (*shaded symbols*). The arrow points to the proband. Identification numbers are given in boldface italics for all affected individuals and for three unaffected parents who join different branches of the family. A 13-member screening panel, including 10 closely related living affected individuals (E41, E42, F96, F43, F51, F52, F53, F54, F64, and G34) and connecting unaffected relatives (E21, E22, and E23), was used in a genome-wide screen for linkage. Note that F61, F45, and G30 developed diabetes after this screen. A three-locus 10q haplotype, showing alleles for D10S592, D10S554, and D10S1681, is given for the subset of typed individuals with data missing for one or both parents. Haplotype identifiers assigned to each of 36 independent haplotypes are shown in bold below each three-locus haplotype. The haplotype identifiers indicate haplotypes transmitted from typed parents to typed offspring. Inferred haplotypes are given in parentheses for deceased or otherwise unavailable family members. One offspring (*lower right*) inherited a recombinant three-locus haplotype (**Am*K**). The identity and independence of different haplotypes were inferred by a comparison of alleles for 20–30 contiguous markers (see Fig. 4).

Multipoint parametric and nonparametric linkage (NPL) analyses were performed using GENEHUNTER (19). GENEHUNTER was also used in combination with CRIMAP (20) to construct 10q haplotypes (see Fig. 1), most of which could be inferred without ambiguity (phase probabilities > 0.99). Due to the limitations of genetic analysis software, linkage analysis could not be performed on the complete pedigree structure, which included 17 inbreeding loops. For example, the computational time estimated from test runs using SIMIBD (21) was 83 days for one two-point analysis. Thus, the 13-member screening panel was split into two branches for initial multipoint analysis, and the complete kindred was split into four affected branches for the 10q analysis. In general, the results of nonparametric linkage analysis were relatively insensitive to the method of splitting, but parametric linkage analysis was less robust.

Allele frequencies for nine independent 10q haplotypes transmitted to diabetic offspring were compared with 27 other independent 10q haplotypes by using a Fisher exact test applied to each of a series of 2×2 tables (allele_k versus all other alleles for each allele k at each marker). A McNemar test (transmission disequilibrium test [22]) was used to compare transmission frequencies for disease-associated marker alleles to the expected frequency of 0.5. A contingency chi-square was used to compare transmission frequencies between affected and unaffected offspring.

Results

Clinical, autoantibody, and HLA results confirm that diabetes in this family is an HLA-associated autoimmune disorder with early onset (mean of 22 yr, range of 0.5–45 yr), ketosis, and insulin dependence. One family member (E41; Fig. 1) is symptomatic for both type 1 diabetes and celiac disease. GAD65 autoantibodies were present in nearly all affected relatives. GAD65 autoantibodies and/or insulin autoantibodies (IAA) were present in many unaffected relatives.

All of 16 affected relatives with known or inferred HLA haplotypes carried either the DR3-DQ2 haplotype, DRB1 *0301-DQA1*0501-DQB1*0201, which is associated with type 1 diabetes in most other populations, or an unusual DR3-DQ5 haplotype, DRB1*0301-DQA1*0102-DQB1*0502. The DQ5 molecule has been reported on a DR2 haplotype (DRB1 *1601-DQA1*0102-DQB1*0502) in Sardinia, where it is also associated with high diabetes risk (23). Neither of the HLA-DR3 haplotypes was present on any of 18 HLA haplotypes carried by spouses with no known genetic relationship to a family member with diabetes.

Results of the genome screen for linkage are shown in Fig. 2. A predominant peak in the NPLs is seen for the long arm of chromosome 10, with the maximum NPL occurring at D10S1237 ($P < 0.002$). Closer inspection of the data corresponding to two lower peaks on 17q (D17s928) and 20q (D20s171) revealed that diabetes segregates with different marker alleles in the two different branches of the family. These peaks are therefore interpreted as false linkages caused by pedigree splitting.

A high-resolution map of the candidate region on 10q was constructed using the CEPH genotyping database and the Massachusetts Institute of Technology (MIT) physical mapping database to identify polymorphic markers and YACs (Fig. 3). The evidence for linkage increased substantially ($P = 0.00004$) with the higher density of markers and the inclusion

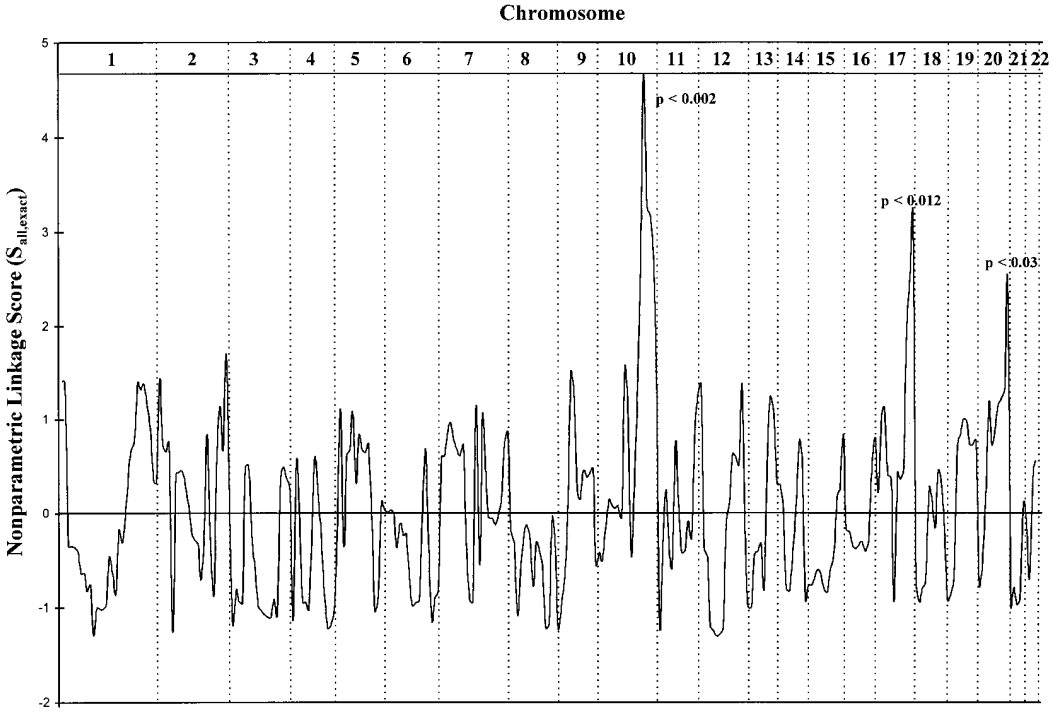


Figure 2. Results of an initial genome-wide (309 markers) multipoint NPL analysis using GENEHUNTER (19). Exact *P* values are given for peak NPLs on 10q, 17q, and 20q. The maximum NPL and lowest *P* value (0.002) correspond to marker D10S1237 at 10q25. The information content for this marker was 0.74, similar to 0.78 and 0.85 for the flanking markers. 10 of 10 affected individuals in the genome-wide screening panel inherited a 404-bp allele for D10S1237. The evidence for linkage to this region was strengthened with the analysis of additional microsatellites and additional family members (see Table I). The two lesser peaks on 17q and 20q are most likely spurious linkages because different marker alleles were transmitted to affected relatives in different sibships.

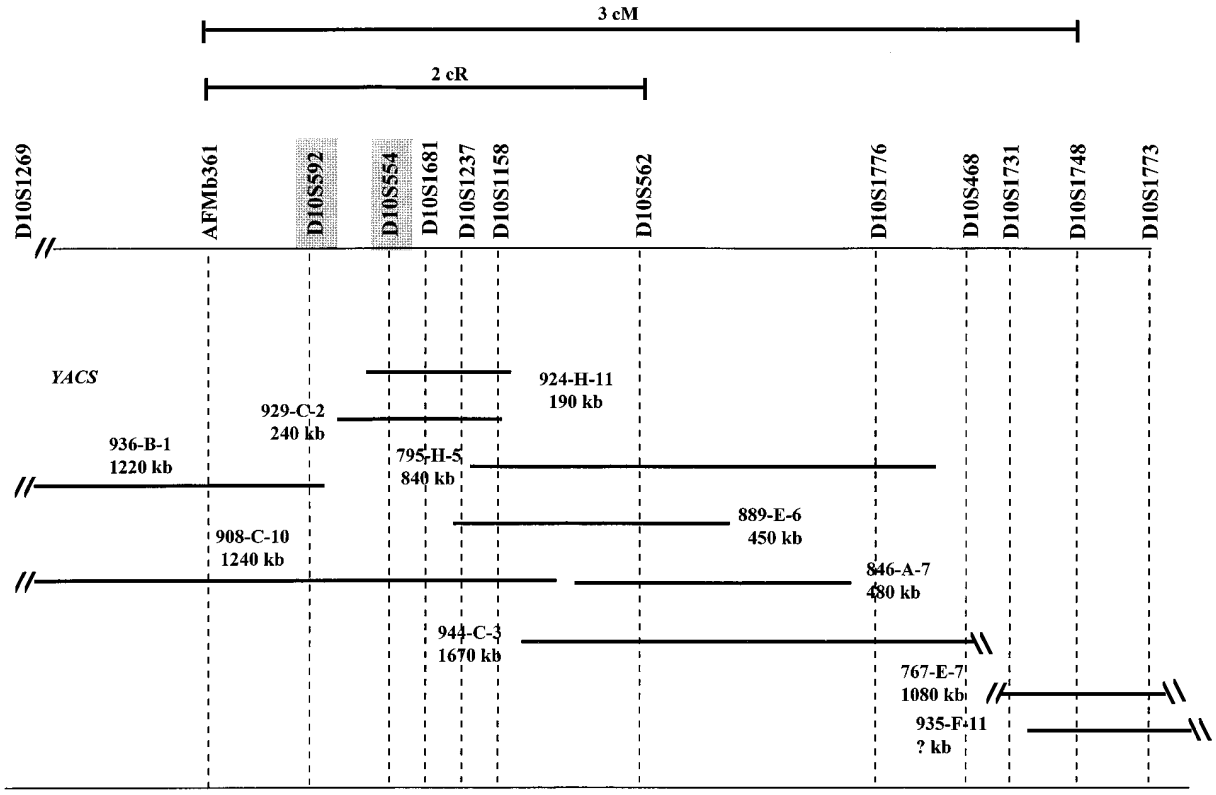


Figure 3. A high density physical and genetic map of a 2–3-cM region surrounding D10S1237. Candidate markers and YACs were identified using a combination of resources, including genetic data from the CEPH collaboration (18) and the CEPH and MIT physical maps (31, 32). Ambiguities and inconsistencies in the published maps were resolved by duplicate typing in our laboratory. Genetic distances in centimorgans (cM) are based on data from the CEPH reference families (CEPH public database, vol. 8). Distances in centiRads (1 cR ~ 55 kb) are based on the MIT radiation hybrid map (33).

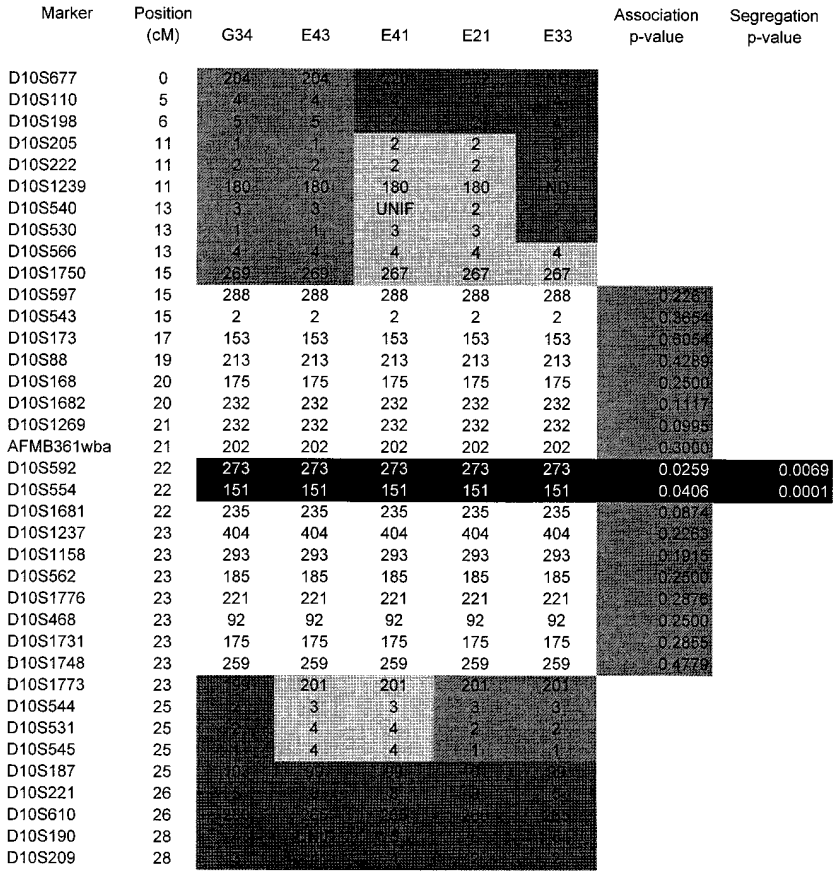
of data for additional affected relatives and all unaffected siblings (Table I).

Due to the large sibship sizes, availability of genetically distant family members, and high (0.25 cM) density of mapped markers, it was possible to reconstruct ancestral 10q haplotypes for affected relatives and their unaffected siblings for comparison with each other and with 10q haplotypes from unrelated spouses. Haplotypes identical by descent from a recent common ancestor were distinguished from other haplotypes by comparing marker alleles for 20–30 adjacent marker loci. Three haplotypes found in affected relatives from three colineal sibships (F51 and sibs, F52 and sibs, and E33 [inferred from sibs and offspring]; see Fig. 1) are undoubtedly identical by descent, with alleles in common for at least 22 adjacent markers in a 12-cM interval (Fig. 4). This high-risk haplotype, designated the B haplotype, was transmitted from a heterozygous parent to 13 of 13 affected offspring ($P = 0.0003$; excludes G34) who inherited B from one of two heterozygous parents in six sibships compared with 10 of 23 unaffected siblings ($P < 0.0002$). Assuming that the disease-causing gene lies within the region shared by 14 affected offspring (all affected relatives except for F80 and F61, and possibly E46 and E47), the ancestral recombinants and observed recombinants (G34 and E43) involving the B haplotype delineate an 8-cM candidate region between markers D10S1750 and D10S1773 (Fig. 4). Several GAD autoantibody-positive, nondiabetic relatives do not carry the B haplotype, suggesting that the 10q locus is associated with progression to diabetes rather than expression of autoantibodies.

The 151-bp allele at D10S554, a relatively rare variant present on the B haplotype and on two other haplotypes from

Table I. Multipoint NPLs for 15 Markers in a 2–3 cM Region at 10q25

Marker	NPL	P_{exact}
D10S168	3.9579	0.00073
D10S1682	3.9884	0.00069
D10S1269	3.9905	0.00069
AFMb361	3.9929	0.00067
D10S592	4.4881	0.00018
D10S554	4.9861	0.00004
D10S1681	4.9183	0.00007
D10S1237	4.8181	0.00007
D10S1158	4.8201	0.00007
D10S562	4.8224	0.00007
D10S1776	4.8284	0.00007
D10S468	4.8328	0.00007
D10S1731	4.8143	0.00007
D10S1748	3.6799	0.00134
D10S1773	3.1431	0.00442



Marker	Position (cM)	G34	E43	E41	E21	E33	Association p-value	Segregation p-value
D10S677	0	264	264					
D10S110	5	4	4					
D10S198	6	5	5					
D10S205	11	1	1	2	2			
D10S222	11	2	2	2	2	2		
D10S1239	11	180	180	180	180	ND		
D10S540	13	3	3	UNIF	2			
D10S530	13	1	1	3	3			
D10S566	13	4	4	4	4	4		
D10S1750	15	269	269	267	267	267		
D10S597	15	288	288	288	288	288	0.2261	
D10S543	15	2	2	2	2	2	0.3654	
D10S173	17	153	153	153	153	153	0.5054	
D10S88	19	213	213	213	213	213	0.4289	
D10S168	20	175	175	175	175	175	0.2500	
D10S1682	20	232	232	232	232	232	0.1117	
D10S1269	21	232	232	232	232	232	0.0995	
AFMB361wba	21	202	202	202	202	202	0.3000	
D10S592	22	273	273	273	273	273	0.0259	0.0069
D10S554	22	151	151	151	151	151	0.0406	0.0001
D10S1681	22	235	235	235	235	235	0.0874	
D10S1237	23	404	404	404	404	404	0.2263	
D10S1158	23	293	293	293	293	293	0.1915	
D10S562	23	185	185	185	185	185	0.2500	
D10S1776	23	221	221	221	221	221	0.2876	
D10S468	23	92	92	92	92	92	0.2500	
D10S1731	23	175	175	175	175	175	0.2855	
D10S1748	23	259	259	259	259	259	0.4779	
D10S1773	23	201	201	201	201	201		
D10S544	25	3	3	3	3	3		
D10S531	25	4	4	2	2	2		
D10S545	25	4	4	1	1	1		
D10S187	25	20	20	20	20	20		
D10S221	26	3	3	3	3	3		
D10S610	26	230	230	230	230	230		
D10S190	28	4	4	4	4	4		
D10S209	28	5	5	5	5	5		

Figure 4. Alleles carried by the high-risk B haplotype, which was present in affected individuals from at least six of nine sibships with one affected sibling. The distance of each marker from D10S677 (top marker) is given in centimorgans (cM). The haplotype shown for the deceased affected relative, E33, was inferred from the marker data from a spouse, one child, and three siblings (Fig. 1). The haplotypes for 3 colineal sibships (shown as the haplotypes for E41, E21, and E33) are undoubtedly identical by descent for a 12-cM region between D10S530 and D10S531, based on common alleles for at least 22 markers and consistent with what is known about the genetic relatedness of these branches of the family. The darkly shaded and unshaded alleles correspond to markers within an 8-cM candidate region defined by all observed and historical recombinants. Minimum *P* values for disease-marker association tests are given for 18 markers in the candidate region defined by the recombinants. For most of these markers, the allele corresponding to the minimum *P* value was also carried by the B haplotype. The column at the far right gives *P* values from a comparison of the transmission frequencies in affected vs. unaffected offspring for the two markers with an allele in increased frequency on the nine nonidentical haplotypes found in affected relatives (see Fig. 1). A 1–2-cM candidate region (darkly shaded) is defined by the combination of results from linkage and association analyses. (ND, not done; UNIF, uninformative).

distantly-related affected relatives (E46, E47, and F80), was transmitted to 15 of 15 affected offspring ($P = 0.0001$; excludes G34) (Fig. 1) compared with 10 of 24 unaffected siblings ($P = 0.0001$). Although fewer parents were informative for D10S592 ($P = 0.096$ for McNemar test), it is remarkable that the 273-bp allele at D10S592 was transmitted to only 3 of 14 unaffected offspring of heterozygous parents compared to 8 of 10 affected siblings ($P = 0.007$). A comparison of marker allele frequencies for a total of nine nonidentical haplotypes found in affected relatives with 27 nonidentical haplotypes found only in unaffected relatives revealed significantly increased frequencies of both the 273-bp allele at D10S592 ($P = 0.026$) and the 151 bp allele at D10S554 ($P = 0.041$) on haplotypes inherited by affected offspring. A similar trend was seen for a 235-bp allele at D10S1681, although the difference was not statistically significant ($P = 0.087$). Each independent haplotype was designated by a different letter (Fig. 1) and counted only once in this analysis. For all other markers, there was no evidence for an allele in increased frequency on haplotypes found in affected relatives. The total set of 36 nonidentical haplotypes showed no evidence for allelic associations between loci, indicating that the association between diabetes and marker alleles at D10S592 and D10S554 is not an artifact of linkage disequilibrium for the markers with each other. Taken together, these results suggest that the susceptibility locus is most likely in a 1–2-cM interval between AFMb361wba and D10S1237. The 2 loci (D10S554 and D10S592) in apparent linkage disequilibrium with a disease-causing allele are present in a 1,240-kb YAC, together with flanking markers (Fig. 2).

Discussion

The results of this study demonstrate the empirical power and efficiency of mapping complex disorders by studying large multiplex families from homogenous populations. The combined results from linkage and association studies provide compelling and internally consistent evidence for a diabetes susceptibility locus mapping to 10q25. The pattern of linkage disequilibrium, which is restricted to adjacent markers < 1 cM apart, is consistent with the population history of Bedouin Arab tribes, each of which, according to legend, is descended from a single male ancestor who lived ~ 100 generations ago (24). Finally, the ability to identify high- and low-risk haplotypes (e.g., the high-risk B haplotype and the low-risk A haplotype, which was not transmitted to an affected offspring in 7 opportunities) will facilitate gene identification by positional cloning, because sequences differing between the A and B haplotypes are prime candidates for disease-associated mutations.

The linkage statistics and *P* values from nonparametric linkage analysis are conservative, because the complex pedigree structure made it difficult to account for the transmission of an identical haplotype (the high-risk B haplotype) to affected relatives in both branches of the screening panel and to an affected relative (E33) in a more distantly related branch of the family. It is also remarkable that two other affected relatives (E46 and E47) who could not be haplotyped have identical genotypes for D10S592, D10S554, and D10S1681, with an allele in common with the B haplotype for all three markers.

Parametric linkage analysis under different models did not allow a distinction between a common recessive susceptibility allele most often found on a haplotype carrying the 273-bp allele at D10S592, and a less common dominant susceptibility allele most often found on a haplotype carrying the 151-bp allele at D10S554. However, in either case, the penetrance associated with 10q-linked susceptibility is high (0.43 for the recessive model and 0.52 for the dominant model; data not shown). When combined with the strong evidence for an association between diabetes and HLA-DR3 alleles, the conclusion of oligogenic inheritance of diabetes with a predominant influence of HLA-linked and 10q-linked susceptibility appears valid for this family. Potential candidates for the 10q locus include 8 expressed sequence tags, CASPase7 (which is involved in apoptosis downstream of Fas), and ADRB1 (the beta-adrenergic receptor).

6 of 14 or more IDDM loci reported in studies of Caucasian affected sib pairs (25) meet the suggested criterion for confirmed linkages (26). None of these loci corresponds to the locus at loq25. The six loci are HLA/IDDM1, INS/IDDM2, IDDM4 at 11q13, IDDM5 at 6q25, IDDM8 at 6q27, and CTLA-4/IDDM12 on 2q33. It is clear that the relative risks associated with each locus are small, but it is not possible to determine if this is because of polygenicity or oligogenicity with genetic heterogeneity. The polygenic model predicts that diabetes in the majority of cases depends on the combined effects of multiple genes, whereas the oligogenic model implies that different loci have a major effect on susceptibility, but the relevance of each locus is restricted to a relatively small subset of cases. In either case, disease gene mapping in most Caucasian populations is likely to be very difficult. However, if genes with major effect can be identified in remarkable families with many affected relatives, the influence of these genes can then be studied in the general population.

One disadvantage of this approach is that multiplex families may be relatively uninformative for linkage due to a markedly increased frequency of high-risk alleles, as exemplified by the apparent absence of linkage to the MHC region in this family (Fig. 2). However, the substantive evidence for linkage disequilibrium between diabetes and HLA alleles suggests that the whole-genome candidate gene association approach proposed as the solution to the difficulties of identifying linkage in affected sib pairs (27) will be especially effective in these families. Another disadvantage of studying remarkable multiplex families is that the relevance of a particular susceptibility gene may be restricted to one or a few families. Even so, the identification of such genes provides an important basis for exploring specific pathogenetic pathways or families of genes having wider import, as exemplified by the subtypes of maturity onset of diabetes in the young (MODY). One of these subtypes (MODY3) is caused by mutations in the gene for hepatocyte nuclear factor-1-α on chromosome 12 (28), whereas another subtype (MODY1) is caused by mutations in the gene for hepatocyte nuclear factor-4-α on chromosome 20 (29). Importantly, the map position of MODY1 was determined by genetic linkage mapping in a single large family (30).

Bedouin Arabs living in Israel show a remarkably low overall frequency of diabetes compared with Israeli Jews, yet Bedouin Arab tribes with multiple affected members are not uncommon. This is consistent with the high frequency of consanguinity (40–50%) and endogamy ($\sim$ 100%) among Bedouin Arabs, most of whom strongly adhere to the conviction of a genetically pure tribal lineage. As a result, the susceptibility allele(s) inherited by affected relatives in the same tribe is likely to be identical by descent from either a recent or historical common ancestor. Further, given the evidence that many different genes are involved in type 1 diabetes, the loci and/or alleles involved are likely to differ in different multiplex families. Thus, studies of a set of remarkable multiplex families may facilitate the genetic dissection of type 1 diabetes and other complex diseases.

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References

1. Thomson, G., W.P. Robinson, M.K. Kuhner, S. Joe, M.J. MacDonald, J.L. Gottschall, J. Barbosa, S.S. Rich, J. Bertrams, M.P. Baur, et al. 1988. Genetic heterogeneity, modes of inheritance, and risk estimates for a joint study of Caucasians with insulin-dependent diabetes mellitus. *Am. J. Hum. Genet.* 43: 799–816.

2. Noble, J.A., A.M. Valdes, M. Cook, W. Klitz, G. Thomson, and H.A. Erlich. 1996. The role of HLA class II genes in insulin-dependent diabetes mellitus: molecular analysis of 180 Caucasian, multiplex families. *Am. J. Hum. Genet.* 59:1134–1148.

3. Todd, J.A., and M. Farrall. 1996. Panning for gold: genome-wide scanning for linkage in type I diabetes. *Hum. Mol. Genet.* 5:1443–1448.

4. Todd, J.A., T.J. Aitman, R.J. Cornall, S. Ghosh, J.R. Hall, C.M. Hearne, A.M. Knight, J.M. Love, M.A. McAleer, J.B. Prins, et al. 1991. Genetic analysis of autoimmune type I diabetes mellitus in mice. *Nature.* 351:542–547.

5. Ghosh, S., S.M. Palmer, N.R. Rodrigues, H.J. Cordell, C.M. Hearne, R.J. Cornall, J.-B., Prins, P. McShane, G.M. Lathrop, L.B. Peterson, et al. 1993. Polygenic control of autoimmune diabetes in nonobese diabetic mice. *Nat. Genet.* 4:404–409.

6. Wicker, L.S., J.A. Todd, and L.B. Peterson. 1995. Genetic control of autoimmune diabetes in the NOD mouse. *Ann. Rev. Immunol.* 13:179–200.

7. Colle, E., R.D. Guttmann, T.A. Seemayer, and F. Michel. 1983. Spontaneous diabetes mellitus syndrome in the rat. IV. Immunogenetic interactions of MHC and non-MHC components of the syndrome. *Metabolism.* 32(Suppl. 1): 54–61.

8. Jacob, H.J., A. Pettersson, D. Wilson, Y. Mao, Å. Lernmark, and E.S. Lander. 1992. Genetic dissection of autoimmune type I diabetes in the BB rat. *Nat. Genet.* 2:56–60.

9. Kloting, I., L. Vogt, and T. Serikawa. 1997. Locus on chromosome 18 cosegregates with diabetes in the BB/OK rat subline. *Diabetes Metab.* 21:338–344.

10. Yokoi, N., M. Kanazawa, K. Kitada, A. Tanaka, Y. Kanazawa, S. Suda, H. Ito, T. Serikawa, and K. Komeda. 1997. A non-MHC locus essential for autoimmune type I diabetes in the Komeda diabetes-prone rat. *J. Clin. Invest.* 100: 2015–2021.

11. Nagamine, K., P. Peterson, H.S. Scott, J. Kudoh, S. Minoshima, M. Heino, K.J.E. Krohn, M.D. Lalioti, P.E. Mullis, S.E. Antonarakis, et al. 1997. Positional cloning of the APECED gene. *Nat. Genet.* 17:393–398.

12. Aaltonen, J., P. Björses, J. Perheentupa, N. Horelli-Kuitunen, A. Palotie, L. Peltonen, Y.S. Lee, F. Francis, S. Hennig, C. Thiel, et al. 1997. An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. *Nat. Genet.* 17:399–403.

13. American Diabetes Association. 1997. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care.* 20: 1183–1197.

14. Grubin, C.E., T. Daniels, B. Toivola, M. Landin-Olsson, W.A. Hagopian, L. Li, A.E. Karlsen, E. Boel, B. Michelsen, and Å. Lernmark. 1994. A

novel radioligand binding assay to determine diagnostic accuracy of isoform-specific glutamic acid decarboxylase antibodies in childhood IDDM. *Diabetologia.* 37:344–350.

15. Vardi, P., S.A. Dib, M. Tuttleman, J.E. Connelly, M. Grinbergs, A. Rabizadeh, W.J. Riley, N.K. Maclaren, G.S. Eisenbarth, and J.S. Soeldner. 1987. Competitive insulin autoantibody assay. Prospective evaluation of subjects at high risk for development of type I diabetes mellitus. *Diabetes.* 36:1286–1291.

16. Bugawan, T.L., and H.A. Erlich. 1991. Rapid typing of HLA-DQB1 DNA polymorphism using nonradioactive oligonucleotide probes and amplified DNA. *Immunogenetics.* 163–170.

17. Scharf, S.F., R.L. Griffith, and H.A. Erlich. 1991. Rapid typing of DNA sequence polymorphism at the HLA-DRB1 locus using the polymerase chain reaction and nonradioactive oligonucleotide probes. *Hum. Immunol.* 30:190–201.

18. Dauset, J., H. Cann, D. Cohen, M. Lathrop, J.-M. Lalouel, and R. White. 1990. Collaborative genetic mapping of the human genome. *Genomics.* 6:575–578.

19. Kruglyak, L., M.J. Daly, M.P. Reeve-Daly, and E.S. Lander. 1996. Parametric and nonparametric linkage analysis: a unified multipoint approach. *Am. J. Hum. Genet.* 58:1347–1363.

20. Green, P., K. Falls, and S. Crooks. 1990. Documentation for CRI-MAP, version 2.4.

21. Davis, S., M. Schroeder, L. Goldin, and D.E. Weeks. 1996. Nonparametric simulation-based statistics for detecting linkage in general pedigrees. *Am. J. Hum. Genet.* 58:867–880.

22. Spielman, R.S., R.E. McGinnis, and W.J. Ewens. 1993. Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). *Am. J. Hum. Genet.* 52:506–516.

23. Cucca, F., F. Muntoni, R. Lampis, F. Frau, L. Argiolas, M. Silvetti, E. Angius, A. Cao, S. De Virgiliis, and M. Congia. 1993. Combinations of specific DRB1, DQA1, DQB1 haplotypes are associated with insulin-dependent diabetes mellitus in Sardinia. *Hum. Immunol.* 37:85–94.

24. Barakat, H. 1993. The Arab World. University of California Press, Berkeley. 348 pp.

25. Luo, D., R. Buzzetti, J.I. Rotter, N.K. Maclaren, L.J. Raffel, L. Nistico, C. Giovannini, P. Pozzilli, G. Thomson, and J. She. 1996. Confirmation of three susceptibility genes to insulin-dependent diabetes mellitus: IDDM4, IDDM5, and IDDM8. *Hum. Mol. Genet.* 5:693–698.

26. Lander, E., and L. Kruglyak. 1995. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat. Genet.* 11:241–247.

27. Risch, N., and K. Merikangas. 1996. The future of genetic studies of complex human diseases. *Science.* 273:1516–1517.

28. Yamagata, K., N. Oda, P.J. Kalsaki, S. Menzel, H. Furuta, M. Vaxillaire, L. Southam, R.D. Cox, G.M. Lathrop, V.V. Borhaj, et al. 1996. Mutations in the hepatocyte nuclease factor-1-alpha gene in maturity-onset diabetes of the young (MODY). *Nature.* 384:455–457.

29. Yamagata, K., H. Furuta, N. Oda, P.J. Kaisaki, S. Menzel, N.J. Cox, S.S. Fajans, S. Signorini, M. Stoffel, and G.I. Bell. 1996. Mutations in the hepatocyte nuclear factor-4a gene in maturity-onset diabetes of the young (MODY1). *Nature.* 384:458–459.

30. Bell, G.I., K. Xiang, M.V. Newman, S. Wu, L.G. Wright, S.S. Fajans, R.S. Spielman, and N.J. Cox. 1991. Gene for non-insulin-dependent diabetes mellitus (maturity-onset diabetes of the young subtype) is linked to DNA polymorphism on human chromosome 20q. *Proc. Natl. Acad. Sci. USA.* 88:1484–1488.

31. Chumakov, I.M., P. Rigault, I. Le Gall, C. Bellanne-Chantelot, A. Billault, S. Guillou, P. Soularue, G. Guasconi, S. Beaufils, H. Bui, et al. 1995. A YAC contig map of the human genome. *Nature.* 377:175–197.

32. Hudson, T.J., L.D. Stein, S.S. Gerety, J. Ma, A.B. Castle, J. Silva, D.K. Slonim, R. Baptista, L. Kruglyak, S.-H. Xu, et al. 1995. An STS-based map of the human genome. *Science.* 270:1945–1954.

33. James, M.R., C.W. Richard III, J.-J. Schott, C. Yousry, K. Clark, J. Bell, J.D. Terwillger, J. Hasan, C. Dubay, A. Vignal, et al. 1994. A radiation hybrid map of 506 STS markers spanning human chromosome 11. *Nat. Genet.* 8:70–76.