*Brief Genetics Report*

# The *HLA-DPB1*–Associated Component of the *IDDM1* and Its Relationship to the Major Loci *HLA-DQB1*, *-DQA1*, and *-DRB1*

**Francesco Cucca,[1] Frank Dudbridge,[3] Miriam Loddo,[1,2] Anna P. Mulargia,[1,2] Rosannna Lampis,[1] Efisio Angius,[2] Stefano De Virgiliis,[1] Bobby P.C. Koeleman,[3] Stephen C. Bain,[4] Anthony H. Barnett,[4] Frances Gilchrist,[5] Heather Cordell,[2] Ken Welsh,[6] and John A. Todd[3]**

**The major histocompatibility complex (MHC) HLA region on chromosome 6p21 contains the major locus of type 1 diabetes (*IDDM1*). Common allelic variants at the class II *HLA-DRB1*, *-DQA1*, and *-DQB1* loci account for the major part of *IDDM1*. Previous studies suggested that other MHC loci are likely to contribute to *IDDM1*, but determination of their relative contributions and identities is difficult because of strong linkage disequilibrium between MHC loci. One prime candidate is the polymorphic *HLA-DPB1* locus, which (with the *DPA1* locus) encodes the third class II antigen–presenting molecule. However, the results obtained in previous studies appear to be contradictory. Therefore, we have analyzed 408 white European families (200 from Sardinia and 208 from the U.K.) using a combination of association tests designed to directly compare the effect of *DPB1* variation on the relative predisposition of DR-DQ haplotypes, taking into account linkage disequilibrium between *DPB1* and the *DRB1*, *DQA1*, and *DQB1* loci. In these populations, the overall contribution of *DPB1* to *IDDM1* is small. The main component of the *DPB1* contribution to *IDDM1* in these populations appears to be the protection associated with DPB1*0402 on DR4-negative haplotypes. We suggest that the HLA-DP molecule itself contributes to *IDDM1*. *Diabetes* 50: 1200–1205, 2001**

From the [1]Department of Biomedical Science and Biotechnology, University of Cagliari; the [2]Pediatric Diabetes Unit, G. Brotzu Hospital, Via Peretti, Cagliari, Sardinia, Italy; the [3]Wellcome Trust Center for Molecular Mechanisms in Disease, University of Cambridge, Addenbrooks Hospital, Cambridge; the [4]Department of Medicine, University of Birmingham, Birmingham Heartlands Hospital, Birmingham; the [5]Royal Brompton Hospital, Fulham Road, London; and the [6]Transplant Immunology, Oxford Transplant Centre, Churchill Hospital, Oxford, U.K.

Address correspondence and reprint requests to the following authors:

Francesco Cucca, Dipartimento di Scienze Biomediche e Biotecnologie, University of Cagliari, Via Jenner, Cagliari 09121, Italy. E-mail: fcucca@mcweb.unica.it.

John Todd, Wellcome Trust Centre for Molecular Mechanisms in Disease, Wellcome Trust/MRC Building, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2XY, U.K. E-mail: john.todd@cimr.cam.ac.uk.

Received for publication 30 June 2000 and accepted in revised form

AFBAC, affected family-based control subject; ETDT, extended transmission/disequilibrium test; HM, haplotype method; MHC, major histocompatibility complex; ORT, odds ratio for transmission; PCR, polymerase chain reaction; PW, pairwise; SSP, sequence-specific primer.

A positive association of the DPB1*0301 allele with type 1 diabetes has previously been reported in a study analyzing 42 Mexican-American type 1 diabetic families and ethnically matched control subjects. Analysis of the linkage disequilibrium patterns in Mexican-Americans indicated that this association was not explained by the linkage disequilibrium of DPB1*0301 with high-risk DR-DQ haplotypes (1). In a subsequent study of 180 white European-derived nuclear families largely from the U.S., Noble et al. (2) found that after stratifying a number of DR-DQ haplotypes according to *DPB1* type, the DPB1*0301 allele was more frequent in type 1 diabetic patients than in affected family-based control subjects (AFBACs). Another allele, DPB1*0402, was decreased in the DR3 haplotypes of the type 1 diabetic patients compared with those of the family-based control subjects (2). Importantly, the frequency of DPB1*0402 was also decreased in the Mexican-American families (1). These positive results were not replicated in a case-control study of Norwegians. Comparing the frequencies of *DPB1* alleles in 237 patients and 287 control subjects matched for the same high-risk DR3/DR4 and DR4/DR4 genotypes, no significant independent association of *DPB1* alleles was found (3). Most recently, Noble et al. (4) extended their initial observations by analyzing an additional 89 type 1 diabetic families (total $n = 269$ families). Their analyses suggested that DPB1*0301 and DPB1*0202 appeared to be primarily predisposing, whereas DPB1*0402 and DPB1*0401 showed possible protective effects. They suggested that *DPB1* might primarily contribute susceptibility to, rather than protection from, type 1 diabetes.

In the present study, we analyzed the association of the *HLA-DPB1* alleles in a collection of 408 type 1 diabetic white European families, of which 200 were from Sardinia and 208 were from the U.K. These families did not overlap with those considered in previous studies (1–4). First, we compared the overall association of the *DPB1* locus with the *DRB1* and *DQB1* loci in these two sets of families in a single-point analysis using the extended transmission/disequilibrium test (ETDT) (5). Without taking into account linkage disequilibrium between the three class II loci, the overall association of *HLA-DPB1* with type 1

TABLE 1
Transmission of *DPB1* alleles in a combined data set of 408 type 1 diabetic families from Sardinia and the U.K.

| | Sardinia | | U.K. | | Total | | Percent- | |
|---|---|---|---|---|---|---|---|---|
| *DPB1* | T | NT | T | NT | T | NT | age T | $P < 0.05$ |
| 0202 | 1 | 0 | 14 | 1 | 15 | 1 | 93.8 | $4 \times 10^{-4}$ |
| 0301 | 129 | 53 | 65 | 54 | 194 | 107 | 64.5 | $1.1 \times 10^{-6}$ |
| 1501 | 1 | 0 | 7 | 5 | 8 | 5 | 61.5 | — |
| 0601 | 0 | 0 | 11 | 7 | 11 | 7 | 61.1 | — |
| 0101 | 4 | 1 | 40 | 28 | 44 | 29 | 60.3 | — |
| 0501 | 2 | 1 | 12 | 10 | 14 | 11 | 56.0 | — |
| 1001 | 10 | 11 | 9 | 8 | 19 | 19 | 50.0 | — |
| 1101 | 2 | 0 | 5 | 7 | 7 | 7 | 50.0 | — |
| 0401 | 65 | 75 | 105 | 120 | 170 | 195 | 46.6 | — |
| 0201 | 60 | 89 | 49 | 42 | 109 | 131 | 45.4 | — |
| 0901 | 10 | 14 | 2 | 4 | 12 | 18 | 40.0 | — |
| 1301 | 3 | 12 | 9 | 9 | 12 | 21 | 36.4 | — |
| 1401 | 2 | 4 | 2 | 6 | 4 | 10 | 28.6 | — |
| 0402 | 3 | 31 | 21 | 46 | 24 | 77 | 23.8 | $1 \times 10^{-7}$ |
| Others | 2 | 3 | 3 | 7 | 5 | 10 | — | — |

Data are $n$ or %. Only alleles present in at least 10 heterozygous parents in the total data set were included. T, transmitted; NT, not transmitted.

diabetes in these families was strong ($P = 5.3 \times 10^{-10}$). However, this association did not approach the level of significance of the association of *HLA-DRB1* and *-DQB1* ($P = 2.1 \times 10^{-90}$ and $8.5 \times 10^{-83}$, respectively). These associations did not vary significantly between the two populations (data not shown). However, the vast majority of the overall *DPB1* association was due to linkage disequilibrium with the *DQB1* and *DRB1* loci. Using a modified version of the ETDT—the conditional ETDT (6)—we found that after taking into account linkage disequilibrium with the HLA-*DQB1* and *–DRB1* loci, the association of *DPB1* was only weakly significant ($P = 1.0 \times 10^{-2}$ and $1.9 \times 10^{-2}$, respectively). Conversely, after taking *DPB1* into account, the overall associations of *DRB1* and *DQB1* yielded *P* values of $7.9 \times 10^{-41}$ and $1.5 \times 10^{-39}$, respectively. Although these results suggest that *DPB1* might contribute to the association of the HLA region to type 1 diabetes, they also indicate that its overall genetic effect is considerably smaller than that of *DRB1* and *DQB1* in these two populations.

Next, we considered the single-point association of the different *DPB1* alleles using the transmission/disequilibrium test (TDT) (7) (Table 1). The most significant results were the increased transmission frequencies of the putative predisposing DPB1*0202 and DPB1*0301 alleles ($P = 4 \times 10^{-4}$ and $1.1 \times 10^{-6}$, respectively) and the decreased transmission of the putative protective allele DPB1*0402 ($P = 1 \times 10^{-7}$). Based on these results and on previous findings (1,2,4), we evaluated the relative transmission or predisposition of the DPB1*0301, DPB1*0202, and DPB1*0402 alleles according to which *DRB1-DQA1-DQB1* haplotypes they were on. To carry out these analyses, we used the haplotype method (HM) (8), which we modified by incorporating TDT into the test (HM-TDT). This modified test evaluates the association of specific alleles of *DPB1*, taking into account linkage disequilibrium with alleles of the *DRB1* and *DQB1* loci as well as the association of an allele of any locus in linkage disequilibrium with alleles at another locus (see RESEARCH DESIGN AND METHODS). DPB1*0301 was significantly and consistently more frequently transmitted than DPB1*0402 on DR3 (DRB1*0301-DQA1*0501-DQB1*0201) ($P = 2.3 \times 10^{-2}$) (Table 2) and DR1 (DRB1*01-DQA1*0101-DQB1*0501) haplotypes ($P = 2.1 \times 10^{-2}$) (Table 3). A similar finding, albeit not significant at the 5% level, was found for DR16 (DRB1*1601-DQA1*0102-DQB1*0502) haplotypes ($P = 0.13$) (Table 4). For example, the DPB1*0301 allele is associated with a 5.4-fold greater disease risk than the DPB1*0402 allele on DR3 haplotypes, as estimated using the odds ratio for transmission (ORT) (Table 2). Also, DPB1*0202 was significantly more frequently transmitted than DPB1*0402 on DR3 haplotypes ($P = 2.0 \times 10^{-3}$) (Table 2). In addition, DPB1*0202 was also significantly more frequently transmitted on DR3 haplotypes than were DPB1*0401 and DPB1*0201 ($P = 3.1 \times 10^{-2}$ and $4.4 \times 10^{-2}$, respectively) (data not shown), two other *DPB1* alleles. The heterogeneity in transmission of DPB1*0401 and DPB1*0201 was not observed on other DR-DQ haplotypes (data not shown). No significant heterogeneity was detected at the *DPB1* locus between the transmitted and nontransmitted chromosomes of the DR4 haplotypes (data not shown). There was consistency in the trends shown by the DPB1*0301 and DPB1*0402 alleles between the U.K. and Sardinian populations. The DPB1*0202 allele was virtually absent in the Sardinian families, and thus the putative permissive effect in disease susceptibility of this allele on DR3 haplotypes could not be evaluated in this population.

The relative contributions of *DPB1* alleles to *IDDM1* were delineated by studying an even larger data set—which included 176 U.S. families that have been previously studied for DP (2) (total $n = 582$)—and by determining the effect of each *DPB1* allele on the relative association of *DQB1-DQA1-DRB1* haplotypes (see RESEARCH DESIGN AND METHODS) (Table 5). Overall, the most important component of the *DPB1* association with type 1 diabetes in this mixed sample set seemed to be the "protective" effect of the DPB1*0402 allele. For instance, the ORT drops from 1.2 for the DR1 haplotype with DPB1*0301 to 0.1 for DR1 with DPB1*0402 ($P = 5.1 \times 10^{-3}$ in a pairwise comparison of

TABLE 2
The relative predisposition of *DPB1* alleles on DR3 (DRB1*301-DQA1*0501-DQB1*0201) haplotypes in 408 Sardinian and U.K. families

| | | | | Sardinia | | U.K. | | Total | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *DRB1* | *DQA1* | *DQB1* | *DPB1* | T | NT | T | NT | T | NT | ORT | 95% CI | *P* HM-TDT |
| 0301 | 0501 | 0201 | 0202 | 1 | 0 | 13 | 0 | 14 | 0 | 16.9 | (1.8–158.1) | $2.0 \times 10^{-3}$ |
| 0301 | 0501 | 0201 | 0301 | 105 | 21 | 20 | 7 | 125 | 28 | 5.4 | (1.7–17.2) | $2.3 \times 10^{-2}$ |
| 0301 | 0501 | 0201 | 0402 | 3 | 3 | 4 | 5 | 7 | 8 | 1 | — | — |

Data are $n$. The HM-TDT *P* values as well as the ORT were calculated with pairwise comparisons using BRB1*0301-DQA1*0501-DQB1*0201-DPB1*0402 as the reference haplotype. T, transmitted; NT, not transmitted.

TABLE 3
The relative predisposition of *DPB1* alleles on DR1 (DRB1*01-DQA1*0101-DQB1*0501) haplotypes in 408 Sardinian and U.K. families

| | | | | Sardinia | | U.K. | | Total | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *DRB1* | *DQA1* | *DQB1* | *DPB1* | T | NT | T | NT | T | NT | ORT | 95% CI | *P* HM-TDT |
| 01 | 0101 | 0501 | 0301 | 6 | 7 | 2 | 7 | 8 | 14 | 10.9 | (1.2–97.1) | $2.1 \times 10^{-2}$ |
| 01 | 0101 | 0501 | 0402 | 0 | 2 | 1 | 17 | 1 | 19 | 1 | — | — |

Data are *n*. The HM-TDT *P* values as well as the ORT were calculated with pairwise comparisons using DRB1*01-DQA1*0101-DQB1*0501-DPB1*0402 as the reference haplotype. T, transmitted; NT, not transmitted.

the two haplotypes) (Table 5). When the DR3 haplotype with DPB1*0301 was compared with the DR3 haplotype with DPB1*0402, the ORT values were 5.8 and 1.5, respectively ($P = 1.4 \times 10^{-2}$ in a pairwise comparison of the two haplotypes) (Table 5). The negative association of DPB1*0402 was further illustrated by evaluating the net effect of allelic variation at *DPB1* on the transmissions of *DRB1-DQA1-DQB1* haplotypes to affected children (Fig. 1). Most strikingly, DPB1*0402-positivity converted neutral DR-DQ haplotypes, such as DR1 and DR16, into protective haplotypes. The percentage transmission of the grouped DR1 + DR16 haplotypes was 36.6% when *DPB1* alleles were not taken into account; this decreased to 6.1% when DPB1*0402 was considered. Only a small increase toward a positive association of DR1 + DR16 was observed in the presence of DPB1*0301 (43.1% transmission to affected children). The protection associated with DPB1*0402 was even able to reduce, but not to completely override, the predisposition conferred by DR3 haplotypes; in the presence of DPB1*0402, the DR3 haplotypes had a neutral association (47.4% transmission to affected children) instead of the highly positive type 1 diabetes association (78.8%) normally seen. In contrast, the inclusion of DPB1*0301 only marginally increased the transmission of DR3 haplotypes (80.9%) (Fig. 1).

As shown in Table 5, the DPB1*0202-DR3 haplotype may be more predisposing than the DPB1*0301-DR3 haplotype (ORT = 31.3 and 5.8, respectively), but the difference in transmission between the two DR3 haplotypes was not significant ($P = 0.14$ in a pairwise comparison of the two haplotypes). Furthermore, in northern European populations, DPB1*0202 very frequently occurs on the extended and predisposing DR3-B18 haplotype. This makes it difficult to define its individual predisposing effect within this extended haplotype. Further studies from other populations in which DPB1*0202 is included within different extended haplotypes might clarify whether DPB1*0202 is independently predisposing to type 1 diabetes.

Finally, allelic variation at the *DPB1* locus did not affect the transmission of the highly predisposing DRB1*0405/*0401-DQA1*0301-DQB1*0302 haplotypes (Table 5). Because Lie et al. (3) only analyzed DR4-positive individuals, this is likely to be the explanation for their failure to detect an effect of DP in their Norwegian data set. Contrast these data with the protection against type 1 diabetes provided by the DR15 (DRB1*1501-DQA1*0102-DQB1*0602), DR14 (DRB1*1401-DQA1*0101-DQB1*0503), or DR7 (DRB1*0701-DQA1*0201-DQB1*0303) haplotypes, which are independent of allelic variation at *DPB1* (F.C. and J.A.T., unpublished data).

Taken together, our results provide consistent and significant evidence that haplotypes that are identical at the *DRB1*, *DQA1*, and *DQB1* loci but different at the *DPB1* locus have different associations with type 1 diabetes. These conclusions are in agreement with those of Erlich and colleagues (1,2,4). We cannot conclude, however, that the *DPB1* effects described here or elsewhere are directly attributable to polymorphisms in the *DPB1* locus itself. Evidence that DR and DQ are primary etiological determinants of *IDDM1* and not just in linkage disequilibrium with another locus includes the correlation of polymorphic amino acids in the peptide-binding active site of the molecules with susceptibility and resistance to disease (9,10) as well as data from biochemical (11), structural (12), transgenic (13), and mechanistic studies (14). Hence, we compared the exon 2–encoded amino acid sequences of the positively associated DPB1*0301 and *0202 alleles with that of the protective DPB1*0402 allele. No simple amino acid residue disease-risk correlation was evident (data not shown). The *DPB1* effect might result from a complex interaction owing to the joint action of multiple residues at different peptide-binding pockets, including P9, P4, and P1. Alternatively, the association of *DPB1* alleles may not be caused by residue variation in the second exon of this locus at all but instead may result from other non–DP-DR-DQ polymorphism(s) in strong linkage disequilibrium with it. However, given its function in antigen presentation and its homology to DR and DQ, we favor a model in which *DPB1* contributes in a primary way to type 1 diabetes predisposition/resistance. That the association of specific *DPB1* alleles was consistently observed on different *DRB1-DQA1-DQB1* haplotypes and even in distantly related populations is consistent with a primary role for the products of the *DPB1* locus. Nevertheless, the contribution of *DPB1* to

TABLE 4
The relative predisposition of *DBP1* alleles on DR16 (DRB1*1601-DQA1*0102-DQB1*0502) haplotypes in the Sardinian and U.K. family sets

| | | | | Sardinia | | U.K. | | Total | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *DRB1* | *DQA1* | *DQB1* | *DPB1* | T | NT | T | NT | T | NT | ORT | 95% C.I. | *P* HM-TDT |
| 1601 | 0102 | 0502 | 0301 | 11 | 16 | 0 | 0 | 11 | 16 | 6.4 | (0.7–57.0) | 0.13 |
| 1601 | 0102 | 0502 | 0402 | 0 | 8 | 0 | 0 | 0 | 8 | 1 | — | — |

Data are *n*. The HM-TDT *P* values as well as the ORT were calculated with pairwise comparisons using the DRB1*1601-DQA1*0102-DQB1*0502-DPB1*0402 haplotype as the reference. T, transmitted; NT, not transmitted.

TABLE 5
The relative transmission of *DRB1-DQA1-DQB1-DPB1* haplotypes in a combined data set of 584 Sardinia, U.K., and U.S. families

| *DRB1* | *DQA1* | *DQB1* | *DPBI* | T | NT | Percentage T | *P* TDT <0.05 | ORT | 95% CI | *P* PW-TDT <0.05 |
|---|---|---|---|---|---|---|---|---|---|---|
| 0301 | 0501 | 0201 | 0202 | 22 | 1 | 95.7 | $1.2 \times 10^{-5}$ | 31.3 | (4.0–245.8) | $3.8 \times 10^{-5}$ |
| 0405 | 03 | 0302 | 0201 | 18 | 1 | 94.7 | $9.6 \times 10^{-5}$ | 29.4 | (3.7–231) | $3.2 \times 10^{-5}$ |
| 0401 | 03 | 0302 | 0301 | 26 | 3 | 89.7 | $1.9 \times 10^{-5}$ | 13.3 | (3.7–48.0) | $8.2 \times 10^{-5}$ |
| 0401 | 03 | 0302 | 0402 | 17 | 2 | 89.5 | $5.7 \times 10^{-4}$ | 13.9 | (3.0–64.4) | $1.1 \times 10^{-4}$ |
| 0401 | 03 | 0302 | 0201 | 19 | 4 | 82.6 | $1.8 \times 10^{-3}$ | 7.8 | (2.4–25.0) | $8.7 \times 10^{-4}$ |
| 0401 | 03 | 0302 | 0401 | 103 | 25 | 80.5 | $5.4 \times 10^{-12}$ | 6.2 | (3.2–11.8) | $8.1 \times 10^{-8}$ |
| 0405 | 03 | 0302 | 0301 | 33 | 9 | 78.6 | $2.1 \times 10^{-4}$ | 6.6 | (2.7–16.3) | $2.4 \times 10^{-4}$ |
| 0301 | 0501 | 0201 | 0301 | 136 | 32 | 81.0 | $9.1 \times 10^{-15}$ | 5.8 | (3.2–10.9) | $4.5 \times 10^{-7}$ |
| 0404 | 03 | 0302 | 0301 | 13 | 4 | 76.5 | $3.9 \times 10^{-2}$ | 4.8 | (1.4–16.3) | $1.7 \times 10^{-2}$ |
| 0301 | 0501 | 0201 | 0201 | 53 | 25 | 67.9 | $1.5 \times 10^{-3}$ | 3.8 | (1.9–7.4) | $3.8 \times 10^{-3}$ |
| 0301 | 0501 | 0201 | 0401 | 122 | 61 | 66.7 | $8.1 \times 10^{-6}$ | 3.6 | (2.0–6.4) | $1.1 \times 10^{-4}$ |
| 0301 | 0501 | 0201 | 0101 | 61 | 28 | 68.5 | $5.4 \times 10^{-4}$ | 3.1 | (1.6–6.1) | $9.9 \times 10^{-2}$ |
| 0404 | 03 | 0302 | 0601 | 9 | 6 | 60.0 | — | 3.0 | (0.9–10) | — |
| 0402 | 03 | 0302 | 0401 | 9 | 6 | 60.0 | — | 2.5 | (0.8–7.6) | — |
| 0404 | 03 | 0302 | 0401 | 13 | 8 | 61.9 | — | 2.2 | (0.8–6.0) | — |
| 0301 | 0501 | 0201 | 0402 | 9 | 10 | 47.4 | — | 1.5 | (0.5–4.0) | — |
| 01 | 01 | 0501 | 0301 | 14 | 17 | 45.2 | — | 1.2 | (0.5–2.9) | — |
| 01 | 01 | 0501 | 0201 | 11 | 14 | 44.0 | — | 1.1 | (0.5–2.9) | — |
| 1601 | 0102 | 0502 | 0401 | 10 | 13 | 43.5 | — | 1.1 | (0.4–2.9) | — |
| 1601 | 0102 | 0502 | 0301 | 11 | 16 | 40.7 | — | 1.0 | (0.4–2.5) | — |
| 01 | 01 | 0501 | 0401 | 30 | 49 | 38.0 | $3.2 \times 10^{-2}$ | 1.0 | — | 1.0 |
| 0701 | 0201 | 0201 | 0401 | 9 | 15 | 37.5 | — | 1.0 | (0.4–2.5) | — |
| 1601 | 0102 | 0502 | 0201 | 22 | 36 | 37.9 | — | 0.9 | (0.5–1.9) | — |
| 0401 | 03 | 0301 | 0401 | 8 | 23 | 25.8 | $7.0 \times 10^{-7}$ | 0.5 | (0.2–1.4) | — |
| 0701 | 0201 | 0201 | 0201 | 2 | 13 | 13.3 | $4.5 \times 10^{-3}$ | 0.3 | (0.1–1.3) | — |
| 11-12 | 0501 | 0301 | 0301 | 2 | 14 | 12.5 | $2.7 \times 10^{-3}$ | 0.2 | (0.0–1.1) | $5.1 \times 10^{-2}$ |
| 11-12 | 0501 | 0301 | 0401 | 5 | 40 | 11.1 | $5.7 \times 10^{-7}$ | 0.2 | (0.1–0.7) | $13 \times 10^{-2}$ |
| 11-12 | 0501 | 0301 | 0201 | 3 | 27 | 10.0 | $1.2 \times 10^{-5}$ | 0.2 | (0.1–0.7) | $6.4 \times 10^{-3}$ |
| 01 | 01 | 0501 | 0402 | 2 | 23 | 8.0 | $2.7 \times 10^{-5}$ | 0.1 | (0.0–0.6) | $4.2 \times 10^{-3}$ |
| 11-12 | 0501 | 0301 | 0402 | 1 | 29 | 3.3 | $3.2 \times 10^{-7}$ | 0.1 | (0.0–0.4) | $3.7 \times 10^{-4}$ |
| 1501 | 0102 | 0602 | 0401 | 2 | 64 | 3.0 | $2.3 \times 10^{-14}$ | 0.1 | (0.0–0.2) | $3.1 \times 10^{-6}$ |

Data are *n* or %. Only *DRB1-DQB1-DQA1-DPB1* haplotypes detected in at least 15 informative meioses were included. The PW-TDT *P* values as well as the ORT values were calculated with pairwise comparisons using the DRB1*01-DQA1*0501-DQB1*-0501-DPB1*0401 haplotype as reference. T, transmitted; NT, not transmitted.

*IDDM1* is small because DPB1*0402-positive DR3, DR1, and DR16 haplotypes have relatively low frequencies in these populations (4% in the total data set, according to the AFBAC frequencies of the different haplotypes). However, the *DPB1* locus could have a larger effect in populations in which these DPB1*0402-positive haplotypes are more frequent.

## RESEARCH DESIGN AND METHODS

The data set consisted of 200 Sardinian, 208 U.K., and 176 U.S. families (total affected children = 212 from Sardinia, 412 from the U.K., and 352 from the U.S.). The average age (means ± SD) at disease onset was 10.7 ± 7.2 years (females 10.1 ± 6.8, males 12.5 ± 8.6) in the U.K., 11.5 ± 7.9 years (females 10.2 ± 7.1, males 11.1 ± 7.3) in the U.S., and 8.4 ± 4.7 years (females 8.0 ± 4.0, males 8.6 ± 5.1) in the Sardinian patients. The U.K. families were part of the British Diabetic Association Warren Repository (15). The 176 U.S. multiplex families were from the Human Biological Data Interchange (16). The 200 Sardinian families were typed using polymerase chain reaction (PCR) amplification of the polymorphic second exon of the *HLA-DRB1*, *-DQB1*, and *-DPB1* genes and dot blot analysis of amplified DNA with sequence-specific oligonucleotide (2). The 208 U.K. families were typed for the *HLA-DRB1* and *-DQB1* loci using a combination of serological and PCR–sequence-specific primer (SSP) methods by the Transplant Unit in Oxford, U.K. (16). The *HLA-DPB1* locus was typed in these families using a PCR-based dot blot assay and PCR-SSP. Alleles at the *DQA1* locus in the Sardinian and U.K. sample sets were inferred based on their known patterns of linkage disequilibrium with the *DRB1-DQB1* haplotypes. Typing data for the U.S. families was obtained through the Human Biological Data Interchange, from which DNA samples from family members were purchased (17). The HLA data from the U.S. families reported in this article overlap with those previously reported (2).

Single-point analysis of the *HLA-DRB1*, *-DQB1*, and *-DPB1* loci was performed using the ETDT (5). This test takes into account the transmission or nontransmission of alleles of a marker relative to the alleles of the marker present on the other parental chromosome. The ETDT takes multiple alleles into account and obtains a global *P* value indicative of the degree of significance of the association with the disease at each individual locus. To distinguish primary associations from those due to linkage disequilibrium at the established disease predisposing loci, we used a variant of the ETDT called conditional ETDT (6). This test allows us to analyze the overall effect of one locus while taking into account the association of other linked loci. The conditional ETDT compares the transmission of haplotypes constructed from all the loci against the null hypothesis that all haplotypes identical at the conditioning loci have equal transmission weights. The single-point association of individual *DPB1* alleles was evaluated using the TDT (7). To study the transmission of specific *DPB1* alleles conditioned on alleles or haplotypes at other loci, we used an HM-TDT (8). The HM was originally designed as a test for homogeneity of relative allele frequencies at a test locus on haplotypes identical for alleles at another locus. In this study, we applied the same concepts contained in the original description of the HM (8) to test the null hypothesis of equality of transmission of marker haplotypes identical at one variant but different at another closely linked variant. If there is heterogeneity in the transmission of two marker haplotypes that are identical at a predisposing marker (variant A) but different at a putative predisposing marker at another site (variant B), then this is evidence that variant A does not entirely explain disease predisposition and that variant B itself or another marker in linkage disequilibrium with variant B is influencing the transmission of variant A and thus the disease susceptibility. Specifically, the transmission and nontransmission counts for the two haplotypes evaluated by TDT may be arranged in a 2 × 2 contingency table and tested by Fisher's exact test or Pearson's $\chi^2$ test. To maintain the independence of these data, we must exclude individual parents having both of the haplotypes being considered, but the transmission data for those parents may be analyzed by standard TDT, and the statistic may be added to that of the 2 × 2 table to give an overall $\chi^2$

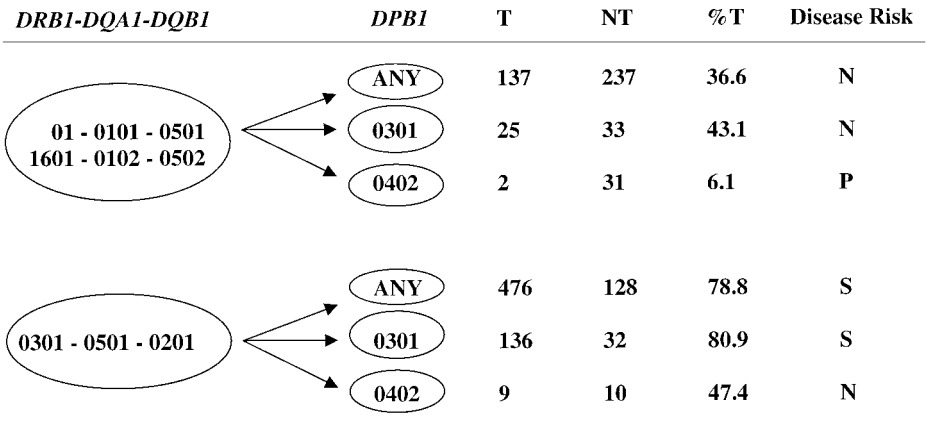


**FIG. 1. Effect of *DPB1* alleles on the transmission of *DRB1-DQA1-DQB1* haplotypes of interest in the Sardinian/U.K./U.S. data set. N, neutral; NT, not transmitted; P, protective; S, susceptible; T, transmitted.**

test with two df. The heterogeneity in transmission between the two haplotypes can be quantified by the ORT calculated from the 2 × 2 contingency table of TDT transmission counts. The transmission data from the individual parents carrying both of the haplotypes being compared have also been discarded in computing the ORTs. We applied the following formula: $[(a \times d)/(b \times c)]$, where $a$ is the number times a given haplotype is transmitted to affected children, $d$ is the number times another haplotype that is identical to the previous haplotype at a predisposing marker (variant A) but different at the test locus (variant B) is not transmitted to affected children, $b$ is the number times the first haplotype is not transmitted to affected children, and $c$ is the number times the second haplotype is transmitted to affected children. When one element of this equation was 0, we used the following formula: $[(2a + 1)\ (2\ \text{days} + 1)]/[(2b + 1)\ (2c + 1)]$.

Confidence intervals were calculated using the following formulas: variances were computed by taking the inverse of the sum of the inverses of $a$, $b$, $c$, and $d$. The standard deviation was calculated by taking the square root of the inverse of the variance. The standard deviation was then multiplied by 1.96, and this quantity was added to and subtracted from the mean to give a 95% confidence interval. We used the mathematical framework applied in the HM-TDT to rank the four locus *(DRB1-DQA1-DQB1-DPB1)* haplotypes around a "reference" haplotype; in this case we refer to the method as the pairwise (PW)-TDT.

Although it may be less powerful in comparison with the original HM (8), we applied the HM-TDT in this study because it has the advantage of not being sensitive to even recent population stratification and as such makes possible analysis and meta-analysis of mixed data sets. A requirement for the validity of HM-TDT is that the parental genotypes be in Hardy-Weinberg equilibrium. Measured directly by the exact test using the Markov-chain approach (18), the parental genotypes did not show any significant deviation from Hardy-Weinberg equilibrium (data not shown). To further exclude this possibility, in the Sardinian and U.K. sample sets, the *DPB1* alleles were also analyzed conditional on *DRB1-DQA1-DQB1* using another variant of the ETDT, the pairwise ETDT (PETDT), which is not sensitive to deviation from Hardy-Weinberg in the parental genotypes (6); the results obtained with the PETDT and the HM-TDT were fully consistent (data not shown). Both the HM-TDT and PETDT assume multiplicative allele effects for the genotype relative risks at the conditioning loci, as defined by Schaid (19), which is consistent with the genotype relative risks of the HLA region and implies that the haplotypes from both parents represent independent data points. The frequencies of alleles and haplotypes in the Sardinian, U.K., and U.S. populations were deduced from the AFBAC frequencies, calculated as described by Thomson (20). Haplotypes were established following the cosegregation of alleles within families and using computer programs written by F. Dudbridge. Only haplotypes certain from parental genotype data (and in the absence of intercrosses) were considered in the analyses shown in this report. Only the probands were evaluated in all the families with more than one affected sibling.

## ACKNOWLEDGMENTS

This study was supported in part by the Regione Autonoma Sardegna (L.R.11, 30–4-90), Diabetes U.K. (formerly the British Diabetic Association), the Juvenile Diabetes Foundation International, the Medical Research Council of U.K., Eli Lilly U.K., and the Wellcome Trust.

F.C. and J.A.T. are recipients of a Wellcome Trust Biomedical Research Collaboration Grant, J.A.T. was a Wellcome Trust Principal Research Fellow, and F.D. is a U.K. Medical Research Council Special Training Fellow in Bioinformatics.

We thank A. Cao, M. Silvetti, and Michael Whalen for continuous help and support; D. Clayton for statistical advice; M. Chessa, P. Frongia, and R. Ricciardi for DNA collection from Sardinian patients; the Human Biological Data Interchange for U.S. family DNA samples and HLA typing data; and the British Diabetic Association for provision of U.K. families.

## REFERENCES

1. Erlich HA, Rotter JI, Chang JD, Shaw SJ, Raffel LJ, Klitz W, Bugawan TL, Zeidler A: Association of HLA-DPB1*0301 with IDDM in Mexican-Americans. *Diabetes* 45:610–614, 1996
2. Noble JA, Valdes AM, Cook M, Klitz W, Thomson G, Erlich HA: 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, 1996
3. Lie BA, Akselsen HE, Joner G, Dahl-Jorgensen K, Ronningen KS, Thorsby E, Undlien DE: HLA associations in insulin-dependent diabetes mellitus: no independent association to particular DP genes. *Hum Immunol* 55: 170–175, 1997
4. Noble JA, Valdes AM, Thomson G, Erlich HA: The HLA class II locus DPB1 can influence susceptibility to type 1 diabetes. *Diabetes* 49:121–125, 2000
5. Sham PC, Curtis D: An extended transmission/disequilibrium test (TDT) for multi-allele marker loci. *Ann Hum Genet* 59:323–336, 1995
6. Koeleman BPC, Dudbridge F, Cordell HJ, Todd JA: Adaptation of the extended transmission/disequilibrium test to distinguish disease associations of multiple loci: the Conditional Extended Transmission/Disequilibrium Test. *Ann Hum Genet* 64:215–221, 2000
7. Spielman RS, McGinnis RE, Ewens WJ: Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). *Am J Hum Genet* 52:506–516, 1993
8. Valdes AM, Thomson G: Detecting disease-predisposing variants: the haplotype method. *Am J Hum Genet* 60:703–716, 1997
9. Todd JA, Bell JI, McDevitt HO: HLA-DQ β gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. *Nature* 329:599–604, 1987
10. Cucca F, Lampis R, Frau F, Macis D, Angius E, Masile P, Chessa M, Frongia P, Silvetti M, Cao A, De Virgiliis S, Congia M: The distribution of DR4 haplotypes in Sardinia suggests a primary association of type I diabetes with DRB1 and DQB1 loci. *Hum Immunol* 43:301–318, 1995
11. Nepom BS, Nepom GT, Coleman M, Kwok WW: Critical contribution of β chain residue 57 in peptide binding ability of both HLA-DR and -DQ molecules. *Proc Natl Acad Sci U S A* 93:7202–7206, 1996
12. Corper AL, Stratman T, Apostolopoulos V, Scott CA, Garcia KC, Kang AS, Wilson IA, Teyton L: A structural framework for deciphering the link between $I\text{-}A^{g7}$ and autoimmune diabetes. *Science* 288:505–511, 2000
13. Wen L, Wong FS, Tang J, Chen NY, Altieri M, David C, Flavell R, Sherwin R: In vivo evidence for the contribution of human histocompatibility leukocyte antigen (HLA)-DQ molecules to the development of diabetes. *J Exp Med* 191:97–104, 2000

14. Schmidt D, Verdaguer J, Averill N, Santamaria P: A mechanism for the major histocompatibility complex–linked resistance to autoimmunity. *J Exp Med* 186:1059–1075, 1997
15. Bain SC, Todd JA, Barnett AH: The British Diabetic Association—Warren Repository (Editorial). *Autoimmunity* 7:83–85, 1990
16. Lernmark A, Ducat L, Eisenbarth G, Ott J, Permutt MA, Rubenstein P, Spielman R: Family cell lines available for research. *Am J Hum Genet* 47:1028–1030, 1990
17. Bunce M, Barnardo MC, Welsh KI: The PCR-SSP Manager computer program: a tool for maintaining sequence alignments and automatically updating the specificities of PCR-SSP primers and primer mixes. *Tissue Antigens* 52:158–174, 1998
18. Guo SW, Thompson EA: Performing the exact test of Hardy-Weinberg for multiple alleles. *Biometrics* 48:361–372, 1992
19. Schaid DJ: Case-parents design for gene-environment interaction. *Genet Epidemiol* 16:261–273, 1999
20. Thomson G: Mapping disease genes: family-based association studies. *Am J Hum Genet* 57:487–498, 1995