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Type 1 diabetes risk for HLA-DR3 haplotypes depends on genotypic context: Association of DPB1 and HLA class I loci among DR3 and DR4 matched Italian patients and controls

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Abstract

Patients with high-risk HLA-DR-DQ genotypes for type 1 diabetes (T1D) were compared to HLA-matched controls to evaluate T1D risk for other HLA loci, including HLA-A, -B, -Cw, and DPB1. Patients (n=133) with high-risk genotypes (DR3/DR3, DR3/DR4, DR4/DR4) were selected from the Lazio (Rome) region of Italy. Screening of more than 9000 subjects from the Lazio region and northern Italy yielded 162 controls with high- T1D-risk haplotypes. Although the overall distributions were not significantly different, allele frequency differences were discovered between the controls from Lazio and those from Northern Italy for some alleles previously shown to affect T1D risk, such as A*3002, DPB1*0301, and DPB1*0402. Therefore, Lazio patient data were compared both to the Lazio subset of controls (n=53) and to the entire group of controls for association analyses. Significant allele frequency differences between patients and DR-DQ-matched controls were found for specific alleles at all loci. Data for the DR3/DR3 subset of patients and controls showed an increase of Cw*0702 in patients. Reduced patient, compared to control, frequencies were seen for several alleles, including A*0101, B*0801, and Cw*0701, all found on the highly-conserved, extended DR3 haplotype known as 8.1 in DR3/DR3, but not DR3/DR4, subgroup. DPB1*0101, often found on 8.1 haplotypes, was also less frequent in DR3/DR3 patients than controls. Analysis of family-based data from the HBDI repository was consistent with the observed results from the Italian subjects, suggesting the presence of a T1D-protective locus at or near A*0101 and a second T1D-protective locus at or near DPB1*0101. These data suggest that T1D risk conferred by the 8.1 haplotype is genotype dependent.

Keywords

HLA Class I; DPB1; Type 1 Diabetes; DR3 haplotype; Disease Association

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INTRODUCTION

The association of specific alleles at the DRB1, DQA1, and DQB1 loci with type 1 diabetes has been well established in many different populations. Multiple DR-DQ haplotypes confer increased risk and several DR-DQ haplotypes confer protection. The DR-DQ haplotypes conferring the greatest risk in Caucasian populations are DRB1*0301-DQA1*0501-DQB1*0201 and various DRB1*04 haplotypes, excluding DR4 haplotypes with DQB1*0301, an allele considered protective for type 1 diabetes [1–3]. A growing number of reports shows that additional HLA-region genes contribute to type 1 diabetes risk [4–8]. One difficulty in demonstrating a type 1 diabetes association for non-DR- or DQ-encoding loci within the HLA region is the very high linkage disequilibrium (LD) among many of the loci in this gene-dense region. This unusually high LD requires data adjustment for LD of any nominally type 1 diabetes-associated allele with DR-DQ haplotypes. Multiple approaches can be utilized to account for the LD. Disease association studies may be performed in many different populations with differing patterns of LD to search for associations that are consistent among populations, or LD patterns can be estimated in a given data set and frequency data adjusted mathematically to account for the observed LD of the allele in question to DR-DQ haplotypes [9]. The latter method has led to the identification of type 1 diabetes-associated DPB1 alleles, HLA-A alleles, HLA-B alleles, and non-HLA loci within this region [6,7,10,11]. A third approach is to focus on individual haplotypes, e.g., the high-risk DR3 and DR4 haplotypes, to examine potential type 1 diabetes association of non-DR- and non-DQ-encoding HLA region genetic markers (microsatellites, SNPs, or other HLA loci). This method can help elucidate novel type 1 diabetes-associated loci in the HLA region as well as reveal type 1 diabetes-risk heterogeneity of DR3 and DR4 haplotypes [4]. The approach of focusing specifically on DR3 and DR4 haplotypes was selected for this study, which reports a comparison of HLA-A, -B, -C, and -DPB1 allele frequencies in DR-DQ matched type 1 diabetes cases and controls from Italy. Only patients and controls with DR3/DR3, DR3/DR4, or DR4/DR4 genotypes are included in the study. The cases were drawn from the Lazio region of Italy. Because of the very low frequency of these genotypes in the control population (generally less than 1% for each genotype), HLA-matched controls were initially selected both from Lazio and from Northern Italy (Genoa and Milan). However, comparing the allele frequency distributions for the control samples from the Lazio to those from the Northern Italian regions showed that, even though the allele distributions were similar, frequencies of some alleles, including alleles that were previously reported as type 1 diabetes-associated, differed significantly between the two regions. Consequently, in addition to comparing Lazio patients to the whole control group, disease association analyses were performed comparing Lazio patients to only the Lazio controls. Given the modest number of geographically-matched controls in each of the three genotype groups and the multiplicity of tests, novel associations that do not appear in more than one genotype category in this study may be spurious (type 1 error). This report focuses primarily on those associations that are previously-reported (e.g., the susceptible effect of DPB1*0301) [4,5,8,10]. and on heterogeneity of risk for both DR4 subtypes and for DR3 haplotypes. The risk heterogeneity results of this study for the extended, conserved haplotype known as 8.1 were replicated in a second, larger cohort and illustrate the importance of genotypic context in searching for disease-associated alleles.

RESEARCH DESIGN AND METHODS

Control subjects

Control subjects for this study were identified through the PREVEFIN and DIABFIN studies [12]. These multicenter projects, aimed to identify subjects at risk for type 1 diabetes in the general population by genetic screening, have been carried out in Italy since year 2000. The studies were approved by the ethical committees of University La Sapienza in Rome and

Gaslini Institute. After informed consent, consecutive newborn Caucasians from different centers in continental Italy were recruited. Only newborns with two Caucasian parents were included. From the 4855 infants screened in the DIABFIN study, and the 4850 infants screened for PREVEFIN, a total of 162 control subjects were found to fit our inclusion criteria, which included DR3/DR4 (not DQB1*0301) heterozygotes, DR3/DR3 homozygotes, and DR4/DR4 (not DQB1*0301) homozygotes. Because DIABFIN and PREVEFIN are newborn screening studies, the possibility exists that the high-type 1 diabetes risk individuals identified in the screening could include future patients. However, due to the low prevalence of type 1 diabetes in the Italian population, the number of future patients is expected to be extremely low and is not expected to have a significant effect on the statistical analyses. Control subjects were taken from three different regions of Italy: Lazio (Rome) (n=53), Lombardy (Milan) (n=20), and Liguria (Genoa) (n=89). DQB1 genotypes were confirmed, DRB1 alleles were subtyped for DR4 haplotypes, and samples were genotyped for HLA-DPB1, HLA-A, and HLA-C. HLA-B genotyping was performed on Lazio, but not Northern Italian, controls.

Patients

Type 1 diabetes patients (n = 245) from the Lazio region of Italy were collected by the IMDIAB study group [13]. Clinical criteria for inclusion in the IMDIAB study were based on American Diabetes Association guidelines[14]. The IMDIAB study was approved by the ethical committees of “Campus Miomedico” and “Sapienza” Universities. A total of 133 IMDIAB patients fit our genetic selection criteria, and were subsequently genotyped for HLA-DPB1, HLA-A, HLA-B, and HLA-C. DQB1 genotypes were confirmed from the initial screening process, and DRB1*04 alleles were subtyped.

Genotyping methods—Molecular HLA typing data were generated with PCR/Sequence-Specific Oligonucleotide Probe (SSOP) linear array technology, similar to previously-described methodology for genotyping the HLA-A locus [6]. Briefly, unlabeled oligonucleotide probes corresponding to polymorphic sequence motifs in the loci of interest were immobilized in a linear array onto a backed nylon membrane. Regions of interest were amplified with biotinylated amplification primers. Biotinylated PCR products were hybridized to the linear array, and probe binding was assessed with a colorimetric assay. Probe-binding patterns were utilized to determine the genotype of the sample.

Statistical Methods

Allele frequencies between control groups, between type 1 diabetes patients and controls within DR-DQ genotype categories and for specific subsets of genotypes or haplotypes were compared using a Fisher's exact test where the overall sample size was less than 50, or using a Pearson's chi-square statistic. A Yates continuity correction to compute the chi-squared value was applied where observed counts for either cases or controls were 0 [15].

RESULTS

Heterogeneity in Control Populations

Based on a pilot study of 121 controls from the Lazio region, high-risk genotypes (DR3/DR3, DR3/DR4, and DR4/DR4) were anticipated to be present at about 4% total in the control population. The actual proportion of high-risk genotypes observed was approximately 2%. The original group of 162 control samples represents screening of more than 9000 individuals. Because of the low frequency of the high-type 1 diabetes-risk genotypes, controls were identified from Lazio (n=53), Genoa (n=89), and Milan (n=20) with the assumption that control frequencies of HLA alleles would not differ significantly among these geographically proximal regions. However, comparison of the HLA-A, HLA-C, and HLA-DPB1 data for the Lazio controls to data from Northern Italian controls, including Lombardy (Milan) and Liguria

(Genoa), revealed that some allele frequencies were significantly, or nearly significantly ($p < 0.1$), different between groups. Notable results from this comparison include A*3002 ($p = 0.056$) in the DR3/DR3 subset, DPB1*0301 ($p = 0.038$) in the DR4/DR4 subset, and DPB1*0402 ($p = 0.079$) in the DR4/DR4 subset. Complete data from this comparison can be found in Supplemental Table 1, online (*need the URL for online table here*).

Type 1 diabetes association

HLA genotyping for DPB1, HLA-A, and HLA-C was performed on 133 patients and 162 controls, and HLA-B genotyping was performed on patients and the subset of controls ($n = 53$) from Lazio (Rome). All samples had one of three high-T1D risk genotypes, including DR3/DR4, DR3/DR3, and DR4/DR4. All samples were genotyped for DQB1, and DRB1*04 alleles were subtyped. Complete data for association analyses for HLA-A, -B, -C, and -DPB1 in the three genotype groups in patients vs. Lazio controls for all class I loci and DPB1, and in patients vs. all controls for HLA-A, -C, and -DPB1, is shown in supplemental Table 2 online (*need the URL for online table here*). The subset of alleles that differed in frequency between patients and controls with a significance level of $p = 0.15$ or less are shown in Table 1, with p values of 0.05 or less shown in bold. As noted above, some of these observed associations may reflect type 1 error. However, several of the observed associations, replicate previously-reported associations and are likely to represent true susceptibility effects. For example, A*3002 and A*2402, which appeared significantly predisposing for type 1 diabetes in a family-based study of Caucasians [6], appear predisposing in these Italian data as well, particularly in the DR3/DR3 homozygous samples. DPB1*0301, which has appeared predisposing in multiple populations. [4, 5, 8, 10], also appears to be type 1 diabetes-associated in this study, although the effect is seen only in the DR4/DR4 subjects. The allele DPB1*0402 has been shown to be protective in several populations [4, 5, 10, 16]. Consistent with previous reports, DPB1*0402 is reduced in frequency among patients relative to controls in the DR3/DR4 and DR4/DR4 groups in this sample set; however, this difference does not reach statistical significance (18.4% in Lazio controls vs. 13.0% in patients for DR3/DR4 heterozygotes; 20.6% in Lazio controls vs. 12.5% in patients for DR4/DR4 homozygotes, data not shown.). Control and patient frequencies of DPB1*0402 in the DR3/DR3 subgroup are similar (5.9% vs. 6.9%, respectively), but the numbers are too small to make meaningful inferences (2 haplotypes in patients vs. 5 in controls). Several nominally significant associations that were not previously noted were observed and may provide hypotheses for further testing in other, larger data sets. In the DR3/DR3 subset of subjects, Cw*0702 is significantly increased in frequency in T1D patients vs. all controls (Table 1), and several alleles are reduced in frequency in patients relative to controls, most notably Cw*0701 and DPB1*0101, which are both seen on the conserved, extended haplotype 8.1, discussed below.

Conserved A1-B8-DR3 haplotype (8.1)

Italian data set—DR3 haplotypes (DRB1*0301-DQA1*0501-DQB1*0201) are predisposing for type 1 diabetes. A common, extended DR3 haplotype, A*0101-B*0801-Cw*0701-DRB1*0301-DQB1*0201 (referred to as 8.1), frequently also contains DPB1*0101. This haplotype is highly conserved among Caucasians and is commonly found in type 1 diabetes patients [17]. The data from this study led to the hypothesis that the 8.1 haplotype is less predisposing in the context of the DR3/DR3 homozygous genotype than it is in the context of a DR3/DR4 heterozygous genotype. Association data for all of the class II alleles in the 8.1 haplotype in Lazio patients vs. Lazio controls are presented in Table 2. Among the DR3/DR3 subjects, alleles usually found on the 8.1 haplotype are reduced in frequency in patients relative to controls. This reduction reaches statistical significance for Cw*0701 ($p = 0.037$ vs. Lazio controls) and DPB1*0101 ($p = 0.04$ and $p = 0.025$ vs. Lazio and all controls, respectively) (Table 2). A*0101 and B*0801 are also reduced in DR3/DR3 patients relative to controls, although not significantly (Table 2). In contrast, among the DR3/DR4 heterozygous subjects,

frequencies for all four alleles are increased in patients relative to controls, although not significantly (Table 2). The striking contrast between the DR3/DR3 subjects and the DR3/DR4 subjects is consistent with the notion that the putative protective effect for T1D of alleles on the 8.1 haplotype is overwhelmed by the high susceptibility of the DR3/DR4 genotype combination.

The case-control design of this study does not allow direct assignment of haplotypes based on transmission, however, the alleles on the 8.1 haplotype are known to be in extremely strong LD with each other, and the haplotype is very common. Thus, assigning A*0101, B*0801, Cw*0701, and DP*0101 to one of the two DR3 haplotypes in a DR3/DR3 individual or to the DR3, rather than the DR4, haplotype in a DR3/DR4 heterozygous individual represents a reasonable assumption. In addition, data from the HBDI families, a large, family-based study described below, support the hypothesis, (generated from this highly-selected, small, Italian case-control sample set) of a protective role for alleles on the the 8.1 haplotype in the context of the DR3/DR3 genotype. Looking at only DR3 haplotypes, the data from the Italian collection show a significant difference in the distributions of DR3 haplotypes carrying Cw*0701 (DR3-C*0701) vs. DR3 haplotypes carrying other HLA-C alleles (DR3-Cw*not0701) in the Lazio patients when the DR3/DR3 patient group was compared to the DR3/DR4 ($p=0.0196$, data not shown), suggesting that additional protective loci may be present on DR3 haplotypes carrying Cw*0701. These data are consistent with the idea that the 8.1 haplotype is less predisposing for type 1 diabetes than are other DR3 haplotypes (e.g., those containing Cw*0702) and support the hypothesis that the 8.1 haplotype lowers T1D risk for type 1 diabetes in the context of DR3/DR3 individuals. In contrast, distributions of DR3 haplotypes with and without B*0801 did not vary significantly between the two groups, suggesting that the B*0801 locus itself is not responsible for the observed protective effect. Of note, the B*0801 and Cw*0701 alleles are in nearly complete LD in the HBDI data set (see below), so the effects of these alleles cannot be examined separately in those data. This result underscores the value of comparing data from multiple studies of subjects with different LD patterns. In addition, the data illustrate the utility of small, focused studies in generating hypotheses that can be tested in larger studies.

HBDI data set—The hypothesis that haplotype 8.1 is protective in the context of the DR3/DR3 genotype was tested in our previously-published data from a set of 283 Caucasian, multiplex families from the HBDI repository (Philadelphia, PA) [3,6,7,10]. The HBDI data were consistent with the Italian data in that alleles from the 8.1 haplotype were reduced in frequency among DR3/DR3 compared to DR3/DR4 patients. Results for proportions of the alleles B*0801 and Cw*0701 in the DR3/DR3 and DR3/DR4 categories for patients in both the Italian and HBDI populations and for Italian controls are represented graphically in Figure 1. In the HBDI dataset, the HLA haplotypes were unambiguously determined based on family transmission patterns. Examination of DR3 haplotypes in the HBDI data set ($n=362$ transmitted, 38 controls) shows that the proportion of the 8.1 haplotype differs significantly depending on the genotype category (DR3/DR3 vs. DR3/DR4) (Table 3). Data in Table 3 are shown only for DR3 haplotypes and do not include any non-DR3 haplotypes. The top portion of Table 3 focuses on what could be called the “core” of the 8.1 haplotype (DRB1*0301-DQA1*0501-DQB1*0201-Cw*0701-B*0801, or “B8-DR3”). DR3 haplotypes including this core are significantly reduced, compared to other DR3 haplotypes, in the DR3/DR3 subjects relative to affected family-based controls (AFBAC) (O.R. 0.38, $p<0.0274$) but are not reduced in the DR3/DR4 heterozygous subjects relative to AFBAC. The difference between the proportion of B8-DR3 in DR3/DR3 genotypes, compared to DR3/DR4 genotypes, is significant ($p<0.0276$) and is suggestive of a genotype-specific protective effect. These data contrast sharply with data for DR3 haplotypes that do not include B*0801, which are significantly increased in the DR3/DR3 genotype group relative to AFBAC (O.R. 2.63 $p<0.046$). Both B8-DR3 and DR3-notB8 haplotypes do not differ significantly from AFBAC in the context of the DR3/DR4 genotype; however, in both cases, the proportions of the given

haplotype vary significantly when DR3/4 subjects are compared to DR3/DR3 subjects, underscoring the importance of genotype context in determining the risk of a DR3 haplotype. This result is consistent with the suggestion from the Italian data that the B*0801 locus itself is probably not responsible for the susceptibility effects of the B8-DR3 haplotype.

A*0101

The protective effect of the B8-DR3 haplotype appears to be dependent on the additional presence of the A*0101 allele. B8-DR3 haplotypes that include the allele A*0101 are significantly reduced in the DR3/DR3 patient group relative to controls (OR 0.37, $p=0.0374$, Table 3), but are not significantly reduced in the DR3/DR4 subjects. Proportions of DR3 haplotypes that are B8-DR3-A*0101 differ significantly between the DR3/DR3 group and the DR3/DR4 group ($p<0.0325$), suggesting that the effect of the haplotype is specific to its genotypic context. The proportion of B8-DR3 haplotypes not including A*0101 does not differ between the DR3/DR3 and DR3/DR4 patient groups. (19.0% vs. 19.3%, respectively, $p=ns$), suggesting that a locus important for type 1 diabetes protection lies telomeric of HLA-B (possibly, but not necessarily, the HLA-A* 0101 allele itself).

DPB1*0101

Having the allele DPB1*0101 on the B8-DR3 haplotype confers T1D protection in the context of DR3/DR3 genotypes (OR 0.09, $p<1.4 \times 10^{-5}$, Table 3). This protective effect appears to be very strong, since it is also seen in the context of the highly-predisposing DR3/DR4 heterozygous genotype (OR 0.26, $p=0.0003$). Even though the effect of including the DPB1*0101 allele appears protective in both the DR3/DR3 and DR3/DR4 patients groups relative to AFBAC, the effect is significantly stronger in the DR3/DR3 group compared to the DR3/DR4 ($p<0.0345$) B8-DR3 haplotypes that do not have the DPB1*0101 allele are predisposing in DR3/DR4 heterozygous genotypes (O.R. 2.89; $p<0.015$) and may also be predisposing in DR3/DR3 genotypes (O.R. 2.46; $p<0.0924$), although the latter result does not reach statistical significance. Unlike the B8-DR3-DPB1*0101 haplotype, the B8-DR3-DPB1*not0101 haplotype does not differ significantly between the DR3/DR3 and DR3/DR4 groups. Taken together, these data strongly suggest the presence of a T1D susceptibility locus on the DR3 haplotype near the DPB1*0101 allele. While the protective effect could be due to the DPB1*0101 allele itself, the more likely possibility is that the effect is due to an additional susceptibility locus, since DPB1*0101 itself does not appear predisposing or protective overall (i.e., when not limited to the context of DR3 haplotypes) in the HBDI families. [10] Whether the apparent protective effect of the DPB1*0101 allele on DR3 haplotypes is due to a protective allele at an undiscovered locus, or whether it is due to the lack of a predisposing allele that is present on other DR3 haplotypes but absent on DR3-DPB1*0101 haplotypes cannot be distinguished from these data and will be the subject of future studies.

DR4 allele subtypes

Although Caucasian DR3 haplotypes are essentially monomorphic at DR- and DQ-encoding loci (DRB1*0301- DQA1*0501-DQB1*0201), DR4 haplotypes are quite heterogeneous. In Caucasians, DR4 haplotypes (haplotypes that have DRB1*04xx at the DRB1 locus) generally carry either DQB1*0301 or DQB1*0302, with a very small number carrying other alleles, such as DQB1*0201. DQB1*0302 is known to be highly-predisposing for type 1 diabetes, and DQB1*0301 is generally thought to be protective for type 1 diabetes. Therefore, DR4-DQB1*0301 haplotypes were excluded from this study. The DRB1 allele on a DR4 haplotype can vary; over 60 subtypes of DRB1*04 alleles have been reported to date (designations and sequences of all known alleles can be found at <http://www.anthonynolan.com/HIG/data.html>). For this study, initial selection criteria did not include DRB1*04 subtyping. Subsequent subtyping was performed, however, on all study

subjects. A hierarchy of risk has been observed for subtypes of DR4 alleles, and the data from this study are consistent with previous data [18]. Figure 2 shows the distribution of DRB1*04 alleles in the DR4 haplotypes in the patients and controls. As expected, DRB1*0403, considered protective, is more frequent in controls than in patients, while DRB1*0401, 0402, and 0405 are more frequent in patients than in controls.

DISCUSSION

The contribution of HLA-DR and DQ alleles to type 1 diabetes susceptibility is well established. To address the issue of linkage disequilibrium in the search for additional type 1 diabetes-associated genes within the HLA region, we compared allele frequencies at the HLA class I and DPB1 loci for DR3/DR3-, DR3/DR4-, and DR4/DR4- matched patients and controls. The final sample set presented in this report represents a selected subset of a very large number (>9000) of HLA-screened individuals. In our initial control sample set, the two regions from which the controls were drawn were not geographically distant; however, significantly different frequencies were observed for some type 1 diabetes-associated HLA alleles. This observation may reflect different origins of the populations in spite of their proximity. For example, the higher frequency of the type 1 diabetes-associated allele A*3002 in the Northern Italian population may reflect migration to that region from Sardinia, a population that has one of the highest incidences of type 1 diabetes in the world and has a high frequency of the allele A*3002. The observation that some HLA allele frequencies differed significantly between groups of control subjects taken from geographically proximal regions emphasizes the need for caution in all disease association studies. Care must be taken to ensure that case and control populations are well-matched for highly polymorphic loci, such as HLA.

Although the numbers of patient and control samples selected for full genotyping and reported on in this study are modest due to low frequencies of genotypes meeting the selection criteria in the Italian population, the strengths of this study for disease association include 1) the ability to replicate previous associations, 2) the ability to examine loci in the HLA region taking into account linkage disequilibrium and the contributions of the loci encoding DR and DQ, and 3) the detection of genotype-specific effects, apparent in this highly-focused study but previously unnoticed in studies including subjects unselected for HLA genotypes. Because of the high probability of type 1 error, we cannot conclude that novel apparent associations, e.g., that seen for A*3101, are real. However, because this association, like that seen for Cw*0702, is seen in DR3/DR3 homozygous individuals, where no confounding effect of LD should exist, this nominal association provides a hypothesis for testing in a larger population study.

In this study, all the alleles of the extended DR3 haplotype known as 8.1 (A*0101, B*0801, and Cw*0701), as well as DPB1*0101, are reduced in frequency among the DR3/DR3 patients but not among DR3/DR4 patients. Previous reports have indicated that this DR3 haplotype has a somewhat lower risk for type 1 diabetes than other DR3 haplotypes [19], although one study concluded that the type 1 diabetes risk for the 8.1 haplotype was not different than that of other DR3 haplotypes [20]. These data suggest that the 8.1 haplotype may contain protective alleles at loci near or identical to HLA-A*0101 as well as near or identical to DPB1*0101. This putative protective effect for HLA-A*0101 is not seen among DR3/DR4 genotypes and is greatly reduced in DR3/DR4 genotypes for DPB1*0101. Perhaps the susceptibility effects of the non-DR and DQ-encoding loci on DR3 haplotypes are less important in the context of a DR3/DR4 genotype, where the two trans-complementing DQA1 and DQB1 heterodimers (DQA1*0501 plus DQB1*0302 and DQA1*0301 plus DQB1*0201) may account for very high risk, than they are in the context of a DR3/DR3 genotype.

This study of Italian patients and controls matched for DR and DQ as well as for geographic origin provides evidence that additional loci within the HLA region influence type 1 diabetes

disease risk. These data support the previously-reported susceptible effects of DPB1*0301 and the protective effect of DPB1*0402, observed here in the DR4/DR4 group, as well as the risk heterogeneity of DR3 haplotypes. In general, this study illustrates the importance of evaluating disease risk in the context of particular DR-DQ genotypes as well as that of appropriate geographic matching for cases and controls.

Finally, the observations about the different susceptibility effects of the conserved 8.1 haplotype in two different genotype contexts, and its replication in a larger, previously-published data set where these effects were previously unnoticed, illustrate the added value of small, focused disease association studies to the many large and comprehensive studies currently underway in the diabetes research community.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

HLA, Human Leukocyte Antigen; T1D, Type 1 Diabetes; LD, Linkage Disequilibrium; DR3, an HLA haplotype containing DRB1*03; SNP, single nucleotide polymorphism.

Figure 1. Proportions of B*0801 and C*0701 in Italian patients, Italian controls, and HBDI patients in DR3/DR3 homozygous subjects compared to DR3/DR4 heterozygous subjects.

Figure 2.
Distribution of DR4 subtypes in Lazio cases and controls.

Table 1

Table 1a: HLA-A allele frequencies in T1D patients, controls from the Lazio (Rome) region, and all controls.

Table 1b: HLA-B allele frequencies in T1D patients and controls from the Lazio (Rome) region.

Table 1c: HLA-Cw allele frequencies in T1D patients, controls from the Lazio (Rome) region, and all controls.

Table 1d: HLA-DPB1 allele frequencies in T1D patients, controls from the Lazio (Rome) region, and all controls..

Table 1a:

Table 1a.

HLA-A	DR3/DR3								DR3/DR4								DR4/DR4								
	T1D n=72	Lazio Controls n=34	All Controls n=132	P vs. Lazio*	P vs. Lazio**	P vs. All*	P vs. All**	O.R. (95% CI)	T1D n=162	Lazio Controls n=38	All Controls n=112	P vs. Lazio*	P vs. Lazio**	P vs. All*	P vs. All**	O.R. (95% CI)	T1D n=32	Lazio Controls n=34	All Controls n=80	P vs. Lazio*	P vs. Lazio**	P vs. All*	P vs. All**	O.R. (95% CI)	
0101	15.3%	26.5%	25.8%			0.128	0.128	0.52 (0.25-1.10)	19.8%	7.9%	13.4%	0.116	0.116			1.59 (0.82-3.10)	3.1%	11.8%	10.0%						0.29 (0.03-2.42)
0202	0.0%	2.9%	0.8%	0.146	0.364			0.00	0.0%	2.6%	0.9%	0.039	0.137			0.00	0.0%		1.3%						0.00
0207									0.0%	5.3%	1.8%	0.004	0.014	0.089	0.208	0.00									
0225																	3.1%	0.0%	0.0%			0.114	0.302		5.16 (0.17-157.79)
0301	6.9%	2.9%	4.5%					1.57 (0.46-5.33)	11.1%	2.6%	8.0%	0.127	0.127			1.43 (0.62-3.31)	21.9%	8.8%	7.5%			0.044	0.044		3.45 (1.06-11.25)
0302	0.0%	2.9%	2.3%	0.146	0.364			0.00	0.6%	0.0%	4.5%		0.034	0.034	0.13 (0.02-1.15)	0.00	2.9%	1.3%							0.00
1101	1.4%	0.0%	1.5%					0.92(0.08-10.27)	1.9%	10.5%	8.0%	0.010	0.010	0.016	0.016	0.22 (0.06-0.82)	6.3%	11.8%	7.5%						0.82 (0.16-4.31)
2301	1.4%	8.8%	3.8%	0.066	0.066			0.36 (0.04-3.12)	2.5%	2.6%	1.8%					1.39 (0.25-7.74)	6.3%	0.0%	5.0%	0.145	0.319				1.27 (0.22-7.28)
2402	12.5%	5.9%	4.5%			0.045	0.045	3.00 (1.02-8.80)	16.0%	15.8%	13.4%					1.24 (0.62-2.46)	12.5%	14.7%	13.8%						0.90 (0.26-3.05)
2501	4.2%	0.0%	2.3%					1.87 (0.37-9.51)	0.6%	5.3%	3.6%	0.035	0.035	0.075	0.075	0.17 (0.02-1.52)	3.1%	0.0%	0.0%			0.114	0.302		
2601	1.4%	8.8%	4.5%	0.066	0.066			0.30 (0.03-2.51)	1.9%	0.0%	1.8%					1.04 (0.17-6.31)	0.0%	5.9%	2.5%						0.00
2902	1.4%	5.9%	3.8%					0.36 (0.04-3.12)	1.2%	0.0%	0.9%					1.39 (0.12-15.49)	3.1%	0.0%	0.0%			0.114	0.302		
3001	6.9%	0.0%	2.3%	0.124	0.248	0.107	0.107	3.21 (0.74-13.84)	2.5%	2.6%	2.7%					0.92 (0.20-4.19)	3.1%	5.9%	3.8%						0.83 (0.00-8.27)
3002	9.7%	0.0%	7.6%	0.069	0.136			1.31 (0.48-3.61)	5.6%	2.6%	4.5%					1.26 (0.41-3.86)	1.3%		1.3%						0.00
3101	0.0%	2.9%	6.1%	0.146	0.364	0.037	0.073	0.00	1.9%	2.6%	0.9%					2.09 (0.22-20.40)	0.0%	2.9%	2.5%						0.00
3402									0.6%	0.0%	0.0%						3.1%	0.0%	0.0%			0.114	0.302		
6801	0.0%	2.9%	1.5%	0.146	0.364			0.00	0.6%	2.6%	1.8%					0.34 (0.03-3.81)	0.0%	2.9%	2.5%						0.00

Table 1b:

HLA-B	DR3/DR3					DR3/DR4					DR4/DR4				
	T1D n=72	Lazio Controls n=34	P vs. Lazio*	P _c vs. Lazio**	O.R.	T1D n=162	Lazio Controls n=38	P vs. Lazio*	P _c vs. Lazio**	O.R.	T1D n=32	Lazio Controls n=34	P vs. Lazio*	P _c vs. Lazio**	O.R.
0702	6.9%	2.9%			2.46 (0.28-21.94)	2.5%	0.0%				6.3%	0.0%	0.145	0.319	
3508	2.8%	2.9%			0.94 (0.08-10.77)	0.6%	5.3%	0.035	0.035	0.11 (0.01-1.27)	3.1%	5.9%			0.52 (0.04-5.99)
3801						1.2%	0.0%				6.3%	0.0%	0.145	0.319	
4001						1.9%	7.9%	0.053	0.053	0.11 (0.04-1.14)	0.0%	2.9%			0.00
4002						0.6%	5.3%	0.035	0.035	0.11 (0.01-1.27)	0.0%	2.9%			0.00
4403						3.7%	2.6%			1.42 (0.17-12.18)	0.0%	8.8%	0.093	0.200	0.00
4501	0.0%	2.9%	0.146	0.364	0.00	0.6%	0.0%				3.1%	0.0%			
5001	12.5%	0.0%	0.039	0.077		3.7%	7.9%			0.45 (0.11-1.88)	3.1%	5.9%			0.52 (0.04-5.99)
5501	2.8%	2.9%			0.94 (0.08-10.77)	2.5%	2.6%			0.94 (0.10-8.63)	0.0%	8.8%	0.093	0.200	0.00
7301						0.0%	2.6%	0.039	0.137	0.00					

Table 1c:

HLA-Cw	DR3/DR3								DR3/DR4								DR4/DR4							
	T1D n=72	Lazio Controls n=34	All Controls n=132	P vs. Lazio*	P _C vs. Lazio**	P vs. All*	P _C vs. All**	O.R. (95% CI)	T1D n=162	Lazio Controls n=38	All Controls n=112	P vs. Lazio*	P _C vs. Lazio**	P vs. All*	P _C vs. All**	O.R. (95% CI)	T1D n=32	Lazio Controls n=34	All Controls n=80	P vs. Lazio*	P _C vs. Lazio**	P vs. All*	P _C vs. All**	O.R. (95% CI)
0102	1.4%	2.9%	0.8%					1.85 (0.11-29.95)	1.2%	0.0%	2.7%					0.45 (0.07-2.76)	3.1%	0.0%	0.0%			0.114	0.302	
0202	0.0%	2.9%	3.0%	0.146	0.364	0.140	0.283	0.00	2.5%	5.3%	6.3%			0.125	0.125	0.38 (0.11-1.33)	6.3%	5.9%	3.8%					1.71 (0.27-10.76)
0303	1.4%	0.0%	1.5%					0.92 (0.08-10.27)	4.9%	5.3%	4.5%					1.11 (0.35-3.49)	0.0%	5.9%	10.0%			0.074	0.143	0.00
0401	9.7%	5.9%	8.3%					1.18 (0.44-3.20)	8.6%	18.4%	21.4%	0.094	0.094	0.005	0.005	0.35 (0.17-0.71)	15.6%	20.6%	18.8%					0.80 (0.27-2.43)
0602	12.5%	2.9%	9.8%	0.135	0.135			1.31 (0.53-3.23)	8.6%	7.9%	11.6%					0.72 (0.32-1.60)	6.3%	14.7%	11.3%					0.53 (0.11-2.58)
0701	29.2%	55.9%	39.4%	0.037	0.037			0.63 (0.34-1.17)	25.9%	18.4%	20.5%					1.35 (0.76-2.41)	15.6%	14.7%	7.5%					2.28 (0.64-8.10)
0702	19.4%	5.9%	3.8%	0.093	0.093	0.001	0.001	6.13 (2.11-17.83)	5.6%	5.3%	4.5%					1.26 (0.41-3.86)	3.1%	0.0%	0.0%			0.114	0.302	
0802	1.4%	0.0%	1.5%					0.92 (0.08-10.27)	3.1%	7.9%	3.6%					0.86 (0.23-3.28)	6.3%	0.0%	0.0%	0.145	0.319	0.025	0.072	
1202								5.70 (0.58-55.79)	1.9%	0.0%	0.0%			0.150	0.312	1.89 (0.49-7.28)	0.0%	2.9%	1.3%					0.00
1203	4.2%	0.0%	0.8%			0.097	0.097		4.9%	2.6%	2.7%					2.81 (0.31-25.48)	9.4%	2.9%	3.8%					2.66 (0.51-13.91)
1402	0.0%	2.9%	1.5%	0.146	0.364			0.00	2.5%	0.0%	0.9%					3.54 (0.41-30.68)								3.67 (0.77-17.42)
1502	0.0%		1.5%					0.00	3.1%	0.0%	0.9%					0.92 (0.08-10.27)	12.5%	2.9%	3.8%			0.094	0.094	0.00
1601	1.4%	0.0%	1.5%					1.85 (0.11-29.95)	0.0%	0.0%	1.8%			0.089	0.208	0.00 (0.14-3.46)	12.5%	2.9%	1.3%					0.00
1602	1.4%	0.0%	0.8%					0.69 (0.11-29.95)	1.9%	7.9%	2.7%	0.053	0.053				0.0%	5.9%	6.3%					0.00
1701	0.0%		1.5%					0.00	1.2%	0.0%	0.0%						0.0%		7.5%			0.121	0.239	0.00

Table 1d:

HLA-DPB1	DR3/DR3								DR3/DR4								DR4/DR4							
	T1D n=72	Lazio Controls n=34	All Controls n=132	P vs. Lazio*	P _C vs. Lazio**	P vs. All*	P _C vs. All**	O.R. (95% CI)	T1D n=162	Lazio Controls n=38	All Controls n=112	P vs. Lazio*	P _C vs. Lazio**	P vs. All*	P _C vs. All**	O.R. (95% CI)	T1D n=32	Lazio Controls n=34	All Controls n=80	P vs. Lazio*	P _C vs. Lazio**	P vs. All*	P _C vs. All**	O.R. (95% CI)
0101	8.3%	23.5%	22.0%	0.044	0.044	0.025	0.025	0.32 (0.13-0.82)	8.6%	2.6%	6.3%					1.42 (0.55-3.64)								2.33 (0.83-6.59)
0301	16.7%	23.5%	16.7%					1.00 (0.46-2.16)	16.7%	13.2%	16.1%					1.04 (0.54-2.00)	25.0%	2.9%	12.5%	0.015	0.015	0.136	0.136	
0501	1.4%	0.0%	0.0%						6.8%	2.6%	2.7%			0.139	0.139	2.65 (0.72-9.71)	3.1%	2.9%	1.3%					2.55 (0.15-42.03)
1101									0.0%	2.6%	0.9%	0.039	0.137			0.00 (0.11-29.95)	0.0%		1.3%					0.00
1301	1.4%	0.0%	0.8%					1.85 (0.11-29.95)	1.2%	7.9%	3.6%	0.019	0.019			0.34 (0.06-1.88)	6.3%	0.0%	0.0%	0.145	0.319	0.025	0.072	
1501	0.0%		0.8%					0.00	0.6%	0.0%	0.0%					0.69 (0.04-11.14)	6.3%	0.0%	1.3%	0.145	0.319	0.144	0.144	5.27 (0.46-60.25)
1701	0.0%	2.9%	3.0%	0.146	0.364	0.140	0.283	0.00	0.6%	0.0%	0.9%						6.3%	5.9%	5.0%					1.27 (0.22-7.28)
2001	1.4%	0.0%	0.0%						0.0%	2.6%	0.9%	0.039	0.137			0.00								

* P-values under p<0.15 are shown, statistically significant values are highlighted in bold typeface.

** P_C represents the P value after application of the Yates continuity correction.

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** P_C represents the P value after application of the Yates continuity correction.

Table 2

Association analysis for alleles on the 8.1 haplotype in Lazio patients vs. Lazio controls.

Allele	Control	DR3/DR3 T1D	Chi sq	P	P/C ratio
A*0101	26.5% (9)	15.3% (11)	1.53	0.22	0.58
C*0701	55.9% (19)	29.2% (21)	4.37	0.04	0.52
B*0801	47.1% (16)	30.6% (22)	1.75	0.19	0.65
DPB1*0101	23.5% (8)	8.3% (6)	4.04	0.04	0.35
DR3/DR4					
A*0101	7.9% (3)	19.8% (32)	2.47	0.12	2.50
C*0701	18.4% (7)	25.9% (42)	0.71	0.40	1.41
B*0801	15.8% (6)	20.4% (33)	0.33	0.56	1.29
DPB1*0101	2.6% (1)	8.6% (14)	1.48	0.22	3.28

Table 3

Frequency of HLA extended DR3 haplotypes among DR3/DR4 and DR3/DR3 type 1 diabetes cases and affected family based controls from 283 US multiplex families (HBDI).

	T1D				controls		is freq diff in DR3/4 than in DR3/3 in T1D?
	DR3/4 N=223	DR3/3 N=58	hap% (n) *	O.R.	hap% (n) *	(AFBAC) N=38	
HLA haplotype	any DP, any HLA-A A0101 *	p< [†] 0.41	64.1% (143)	0.73	48.3% (28)	71.1% (27)	p<0.0276
	other HLA-A	p<0.475	44.8% (100)	0.73	29.3% (17)	52.6% (20)	p<0.0325
B8+ Cw0701 ⁺		p<0.92	19.3% (43)	1.06	19.0% (11)	18.4% (7)	p<0.96
DR3	DPB10101 *	p<0.0003	20.6% (46)	0.26	8.6% (5)	50.0% (19)	p<0.0345
	other DPB1	p<0.0151	43.5% (97)	2.89	39.7% (23)	21.1% (8)	p<0.60
	DPB10101, A **	p<0.33	13.5% (30)	0.58	3.4% (2)	21.1% (8)	p<0.0326
B8+ Cw0701 ⁻	any DP, any HLA-A	N/A	2.2% (5)	N/A	0.0% (0)	0.0% (0)	N/A
B8-	any DP, any HLA-A	p<0.703	33.6% (75)	1.24	51.7% (30)	28.9% (11)	p<0.0112

* the haplotype frequencies refer only to DR3s, not to all haplotypes, e.g., 71% of DR3s in AFBACs are B8s, only 29% are not.

[†] the p-value refers only to DR3 haplotypes, not to all haplotypes, e.g., DR3-B8-A*0101 is protective relative to other DR3s, compared to AFBAC.

[‡] given the small number of haplotypes available, statistical tests were not carried out for this particular haplotype