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Type 1 diabetes risk for human leukocyte antigen (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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Summary Patients with high-risk human leukocyte antigen (HLA)-DR-DQ genotypes for type 1 diabetes (T1D) were compared with 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 patients from the Lazio region and northern Italy yielded 162 controls with high-T1D-risk haplotypes. Although the overall distributions did not differ significantly, allele frequency differences were discovered between the controls from Lazio and controls from northern Italy for some alleles previously determined to affect T1D risk, such as A*3002, DPB1*0301, and DPB1*0402. Therefore, Lazio patient data were compared both with the Lazio subset of controls ($n = 53$) and with the entire group of controls for association analyses. Significant allele frequency differences between patients and DR-DQ-matched controls existed for specific alleles at all loci. Data for the DR3/DR3 subset of patients and controls demonstrated an increase of Cw*0702 in patients. Compared with controls, reduced patient frequencies were seen for several alleles, including A*0101, B*0801, and Cw*0701, all on the highly conserved, extended DR3 haplotype known as 8.1 in DR3/DR3, but not DR3/DR4, subgroup. DPB1*0101, often reported 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 patients, indicating 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 indicate that T1D risk conferred by the 8.1 haplotype is genotype dependent.

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ABBREVIATIONS

DR3	an HLA haplotype containing DRB1*03
HLA	human leukocyte antigen
LD	linkage disequilibrium
T1D	type 1 diabetes

Introduction

The association of specific alleles at the DRB1, DQA1, and DQB1 loci with type 1 diabetes (T1D) 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 T1D [1–3]. A growing number of reports demonstrate that additional human leukocyte antigen (HLA)-region genes contribute to T1D risk [4–8]. One difficulty in demonstrating a T1D 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 T1D-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 led to the identification of T1D-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 the potential T1D association of non-DR- and non-DQ-encoding HLA-region genetic markers (microsatellites, single nucleotide polymorphisms, or other HLA loci). This method can help elucidate novel T1D-associated loci in the HLA region and reveal T1D-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 T1D 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 region with those from the northern Italian regions demonstrated that, although the allele distributions were similar, frequencies of some alleles, including alleles that were previously reported as T1D-associated, differed significantly between the two regions. Consequently, in addition to comparing Lazio patients with the whole control group, disease association analyses were performed comparing Lazio patients with only 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 the 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 patients

Control patients for this study were identified through the PREVEFIN and DIABFIN studies [12]. These multicenter projects, aimed to identify patients at risk for T1D in the general population by genetic screening, have been carried out in Italy since the 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. Of the 4855 infants screened in the DIABFIN study and the 4850 infants screened for PREVEFIN, a total of 162 control patients 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-T1D-risk individuals identified in the screening could include future patients. However, because of the low prevalence of T1D 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 patients 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

T1D 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 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