

Comparison between HLA-DRB and DQ DNA sequences and classic serological markers as Type 1 (insulin-dependent) diabetes mellitus predictive risk markers in the Spanish population

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Summary. The question of HLA susceptibility to Type 1 (insulin-dependent) diabetes mellitus remains unresolved. In the present study, 127 diabetic patients and 177 unrelated control subjects have been analysed for their class I and class II serological antigens, class II (DR, DQ) DNA restriction fragment length polymorphisms and DQA1 and B1 exon-2 nucleotide sequences and their corresponding amino acid residues. By using the aetiologic fraction (δ) as an almost absolute measure of the strongest linkage disequilibrium of an HLA marker to the putative Type 1 diabetes susceptibility locus, it has been found that the strength of association of the HLA markers may be quantified as follows: DR4 < DR3 < DR3 or DR4 < non-Aspartate 57 β DQ and Arginine 52 α DQ < Arginine 52 α DQ. Thus, molecular HLA-DQ markers appear to be more accurate as susceptibility markers than the classic serologically defined ones (DR3 and DR4); however, any effect of DQ markers disappears when non-DR3/DR4 individuals are considered, suggesting that DR factors (or others in between DQ and DR) are also important. In addition, a dominant non-Aspartate 57 β DQ susceptibility theory does not hold (but a re-

cessive one does) in our diabetic population (probably due to the high frequency of the protective DR7-non-Aspartate 57 β DQ haplotypes); Arginine 52 α DQ is the best single HLA marker found in our population, both as a recessive or as a dominant one. Also there are 13 patients in our sample who bear neither Arginine 52 α DQ nor non-Aspartate 57 β DQ susceptibility factors. On the other hand, a predominant Type 1 diabetes association of Spanish patients to the B18-DR3-Dw25 haplotype (and not to B8-DR3-Dw24) has been found; this distinctive association has also been recorded in adult systemic lupus patients and may reflect the existence of common pathogenetic HLA factors for both diseases present only in the B18-DR3-Dw25 haplotype in the Spanish population. These factors are probably placed at the non-coding regions which are different from the B8-DR3-Dw24 haplotype.

Key words: HLA-DR3, HLA-DR4, HLA-DQ, aetiologic fraction, attributable risk, human leucocyte antigens, Type 1 (insulin-dependent) diabetes mellitus.

Type 1 (insulin-dependent) diabetes mellitus is inherited together with HLA antigens but the particular HLA locus/loci and alleles have not been unequivocally identified, and it is uncertain whether DR and/or DQ (or other) HLA genes are those involved in disease susceptibility. DR3 and DR4 have been found to be increased in patients of Caucasoid populations [1], including the Spanish population [2]. However, other HLA antigens have also been reported to be linked to Type 1 diabetes in other disease populations: DR7-DQw9 in American Blacks [3], DRw8 in Japanese [4] and DR9 in Chinese subjects [5]. On the other hand, a negative correlation exists with the DR2 [5],

DR5 and DR7 antigens [2], which suggests a 'protective' role for these particular antigens.

Recently, a Type 1 diabetes HLA-susceptibility unifying theory has been put forward to explain these apparently puzzling findings and to designate the particular susceptible locus/loci and allele/s: any HLA haplotype bearing a non-Aspartate (non-Asp) residue 57 at the DQ β chain would be a susceptible haplotype [6]. However, exceptions to this theory have been found in DR7-associated β DQ chains, in some diabetic patients [7] and in Japanese populations [8]; to circumvent these objections, an Arginine (Arg) 52 α DQ residue has been postulated to interact with a non-Asp 57 β DQ residue in susceptible haplotypes [9]. Moreover, data from the NOD mouse [7] and from other human diabetic subjects [10], also implicate the DR locus in HLA susceptibility to

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Type 1 diabetes. However, it is difficult to dissect the specific HLA susceptibility locus (if it exists) and it would be necessary to study unusual haplotypes in which Type 1 diabetes-associated alleles occur separated from their neighbours in the standard HLA haplotypes in individuals or in certain populations [11].

In the present work, our aim was to test several HLA susceptibility theories in the Spanish Type 1 diabetic population by comparing the different HLA susceptibility loci and alleles using DR and DQ allele serotypes and their DNA subtypes and a novel approach which applies the population attributable risk or aetiologic fraction (δ) of the different markers. Our main conclusion is that several HLA factors (and not exclusively DQ) may be involved in disease susceptibility and that a group of DQ alleles (defined by Arg 52 α DQ residues) has the strongest HLA association to Type 1 diabetes.

Subjects, materials and methods

Patients

The study groups consisted of 127 Type 1 diabetic unrelated patients (as defined according to the criteria of the National Diabetes Data Group, [12]) and 177 healthy unrelated individuals. The age range of the diabetic patients was between 9 and 39 years. Control subjects were blood donors aged 19 to 56 years.

HLA Class I, II typing

HLA class I (-A, -B, -C) and II (-DR, -DQ) typing was done by a two-step microlymphocytotoxicity technique on T or B lymphocytes [13], respectively.

HLA DRB1, DQA1 and DQB1 allogenotyping

DNA extraction, digestion with Taq I restriction endonuclease (Boehringer Mannheim, Mannheim, FRG), electrophoresis, transfer of the DNA fragments, hybridization and washing were performed as previously described [14, 15]. A 517 base pair (bp) Pst I fragment of the exon-specific DRB cDNA clone pRTV1 [16], a 797 bp Pst I fragment DQA, pDCH1 [17] and a 620 bp Ava I fragment DQB, pII- β -1 [18] were used as probes.

HLA DQA1 and DQB1 oligotyping for direct determination of exon-2 DNA sequences

Amplification of genomic DNA. Exon-2 DQA1 and DQB1 DNA was amplified by polymerase chain reaction (PCR) using Taq polymerase (Cetus, Emeryville, Calif., USA) as previously described [19] and the primers DQAAMP-A/DQAAMP-B and DQBAMP-A/DQBAMP-B, respectively; they were synthesized in a DNA-SM automated synthesizer (Beckman, Palo Alto, Calif., USA), from information given at the 11th International Histocompatibility Workshop (Table 1).

Dot blot hybridization. 5% of the amplified DNA was denatured in 0.4 mol/l NaOH for 10 min, neutralized in 1 mol/l ammonium acetate and transferred to a Hybond-N membrane (Amersham, Amersham, Bucks, UK). The filters were pre-hybridized for 1 h in a solution containing 50 mmol/l Tris-HCl (pH = 8.0), 3 mol/l tetramethylammonium chloride, 2 mmol/l EDTA (pH = 8.0), 5 \times Denhart solution (2% bovine serum albumin, 2% polyvinyl pyrrolidone 40, 2% ficoll 400) and 0.1 mg/ml salmon sperm DNA. Then the

radiolabelled oligonucleotide was added and hybridized at 54°C for 3 h. The filters were washed twice in 2 \times SSPE (30 \times SSPE: 4.5 mol/l NaCl, 0.3 mol/l NaH₂PO₄, 30 mmol/l EDTA, pH = 7.7), 0.1% SDS at room temperature, for 10 min, once in TMAC solution (50 mmol/l Tris-HCl (pH = 8.0), 3 mol/l tetramethylammonium chloride, 2 mmol/l EDTA, 0.1% SDS) at room temperature, for 10 min, and twice in TMAC solution at 58°C, for 10 min. The dots were visualized after 8 h exposure to Kodak XAR5 film at -80°C with intensifying screens.

Oligonucleotide probes. The information of the sequences and specificities of the DQA1 and DQB1 oligonucleotides used is from 11th International Histocompatibility Workshop (Table 1). The oligonucleotide synthesis was made using the cyanoethyl phosphoramidite technique in a Beckman DNA-SM automated DNA synthesizer following the manufacturer's protocol. The oligonucleotides were labelled and purified according to the methods of Nepom et al. [20].

This methodology was used in 108 control subjects and 61 diabetic patients. The specific allelic DQA1 and DQB1 sequences for which each patient or control DNA was tested, are listed in Table 1. The corresponding DQA1 allele sequences thus obtained were classified as Arg 52 positive (susceptible) or Arg 52 negative (non-susceptible); DQB1 sequences were also classified as non-Asp 57 positive (susceptible) or Asp 57 positive (non-susceptible).

Assignment of exon-2 DQA1 and DQB1 sequences by DNA-RFLP analysis (allogenotyping)

DNA-RFLPs (restriction fragment length polymorphisms) of DQA1 genes have been associated with Dw typing [21-23] which, in turn, have been associated with exon-2 DQA1 and DQB1 sequences [24]; therefore, it has been possible to associate DQA1 and DQB1 allogenotypes with their corresponding exon-2 sequences, and, thereafter, to record the presence or absence of the Asp 57 in the β DQ chain and Arg 52 in the α DQ chain. The DNA of 66 diabetic patients and 69 control subjects was analysed with this technique.

In addition, the DNA of 108 control individuals was processed by both direct DNA sequence determination by oligotyping and this RFLP sequence assignment technique; there was a full coincidence of direct oligotyping DNA sequence assignment and indirect RFLP-DNA sequence assignment.

Statistical analysis

The distribution of each DNA-allogenotype, of non-Asp 57 β DQ homozygotes and of individuals with at least one non-Asp 57 in the β DQ chain and at least one Arg 52 in the α DQ chain in patients and control subjects was compared calculating: chi-square (with Yates correction), δ (aetiologic fraction) and relative risk (RR) values and their corresponding levels of significance (*p*).

δ value comparisons can determine which of the different HLA markers has the strongest association with Type 1 diabetes. δ is calculated as follows: $\delta = d-p/1-p$, where *d* is the frequency of the antigen among the individuals with the disease and *p* is the frequency of the antigen in the general population. This calculation has advantages over RR values when the association is due to linkage disequilibrium between a genetic marker and the true 'diseased' genetic marker, both markers being very close at the genomic level. Since the true susceptibility marker is unknown, this type of analysis will tend to assign higher δ values to the genetic marker placed closer to the true susceptibility markers or loci [25, 26]. Also, 95% confidence intervals for δ (*I*₂) have been calculated as described [26].

Results

Type 1 diabetic patients show an increase of HLA-DR3 ($p = 8 \times 10^{-11}$) and DR4 ($p = 4 \times 10^{-7}$) antigen frequency, and a decrease in HLA-DR2 ($p = 2 \times 10^{-5}$) and DR7

Table 1. Primers and oligonucleotides used for determination of exon-2 DQ alleles sequences

Primers		DQAAMP-B	DQBAMP-B
DQAAMP-A	5'-ATGGTGTAACCTGTACCAGT	5'-TTGGTAGCAGCGGTAGAGTTG	
DQBAMP-A	5'-CATGTGCTACTTCACCAACGG	5'-CTGGTAGTTGTGTCTGCACAC	
Oligonucleotide probes			
	Codons	Sequences	Specificities
DQA1			
2501	22–28	5'-TGGCCAGTACACCCATGA	0101, 0102, 0401, 0501
2502	22–28	5'-TGGCCAGTTCACCCATGA	0103, 0201, 0601
2503	22–28	5'-TGGGCAGTACAGCCATGA	03011, 03012, 0302
3401	31–37	5'-GAGATGAGGAGTTCTACG	0101
3402	31–37	5'-GAGATGAGCAGTTCTACG	0102, 0103, 0501
3403	31–37	5'-GAGACGAGCAGTTCTACG	0401, 0601
4102	38–44	5'-ACCTGGAGAAGAAGGAGA	0103
5501N	51–57	5'-TCAGCAAATTTGGAGGTT	0101, 0102, 0103
5502N	51–57	5'-TCCACAGACTTAGATTTG	0201
5504	51–57	5'-TCAGACAATTAGAAGAT	0401, 0501, 0601
6902	66–71	5'-ATCGCTGTGCTAAAACAT	0201, 03011, 0302
6903	66–71	5'-ATCGCTGTCTAAAACAT	0501, 03012
7502	72–78	5'-CTTGAACATCCTGATTAA	0201, 0401, 0601
7504N	72–78	5'-CTTGAACAGTCTGATTAA	0501
DQB1			
2301	28–34	5'-GACCGAGCTCGTGCGGGG	0401
2302N	28–34	5'-AACGGGACCGAGCGCGTG	03031, 0402
2601	33–38	5'-CGGGGTGTGACCAGACAC	0501, 0502, 05031, 05032
2602	33–38	5'-CGTTATGTGACCAGATAC	0601, 0301
2603	33–38	5'-CGTCTTGTGACCAGATAC	0602, 0302, 03031, 03032
2604	33–38	5'-CGTCTTGTAAACCAGACAC	0603, 0604
2606	33–38	5'-CGTCTTGTAAACCAGATAC	0605
3702N	35–41	5'-AGGAGGACGTGCGCTTCG	0601
4501	43–48	5'-GACGTGGAGGTGTACCGG	0301
4901	47–52	5'-GGTGTACCGGGCAGTGAC	0501
5701	54–60	5'-GCGGCCTGTTGCCGAGTA	0501, 0604, 0605
5702	54–60	5'-GCGGCCTAGCGCCGAGTA	0502, 0504
5703	54–60	5'-GGCGGCCTGACGCCGAGT	05031, 0601
5704	54–60	5'-GCGGCCTGATGCCGAGTA	05032, 0602, 0603
5705	54–60	5'-GGCTGCCTGCCGCCGAGT	0201
5706	54–60	5'-GGCCGCCTGACGCCGAGT	0301, 03031, 03032
5707	54–60	5'-GGCCGCCTGCCGCCGAGT	0302
5708	54–60	5'-GCGGCCTGACGCCGAGTA	0401, 0402
7003	69–75	5'-GAGGGGACCCGGGCGGAG	0602, 0603

The alleles DQA1*03011, 03012 and 0302 were all assigned as DQA1*03; DQB1*05031 and 05032 as DQB1*0503 and DQB1*03031 and 03032 as DQB1*0303. Also DQB1*0604 and 0605 were assigned

as DQB1*0604/5.

The 11th International Histocompatibility Workshop oligonucleotide probes nomenclature is used

($p = 2 \times 10^{-8}$) (Table 2). The extended haplotype B18-DR3 is significantly increased ($p = 2 \times 10^{-8}$) and is the most common in our Spanish diabetic population, in contrast to that found in other populations (B8-DR3) [5]. No specific DR4-bearing extended haplotype has been found to be increased, although a novel one, recently found in our diabetic population (B49-SC01-DR4, 27) is present at a low frequency. An increase in DQw2 ($p = 7 \times 10^{-5}$) and DQw8 ($p = 5 \times 10^{-9}$) and a decrease in DQw1 ($p = 7 \times 10^{-7}$) and DQw7 ($p = 8 \times 10^{-3}$) have been found (Table 2 and data not shown).

Positive Type 1 diabetes associations [21, 28]

DR3. Two different RFLP-Taq I patterns corresponding to the two DR3 splits (DRw17.1 and DRw17.2) [21], were found. The DRw17.2 allele (linked to B18), is significantly

increased in our diabetic sample ($p = 3 \times 10^{-8}$), whereas DRw17.1 (linked to B8) is not significantly increased in our diabetic patients (Table 3). This further confirms the predominance of B18-DR3 haplotype over the B8-DR3 in patients.

DQw2 is found increased in diabetic patients (Table 2). This is expected since it belongs to the same haplotype as B18-DR3 (17.2), the most frequent diabetogenic haplotype in the Spanish population and also to the B8-DR3 (17.1) haplotype.

DR4. Only the DR4-DQw8 (DQβ3a) haplotype is increased in our diabetic patients ($p = 1 \times 10^{-8}$); the other possible haplotype: DR4-DQw7, (DQβ3b) is not associated with the disease (Table 2).

DQw8 is also increased in diabetic patients (Table 2). It is included in the DR4 haplotypes which bear diabetes susceptibility. Since no particular DR4 subtype has been found to be increased in Spanish diabetic patients, it has

Table 2. Significant DR and DQ serotypes associated with Type 1 (insulin-dependent) diabetes mellitus

	Control subjects (<i>n</i> = 177) (%)	Diabetic patients (<i>n</i> = 127) (%)
Susceptible		
DR3	27	71 ^a
B8-DR3	13	22
B18-DR3	9	36 ^b
DR4	23	51 ^c
Bw62-DR4	3	1
DR4-DQw7	6	2
DR4-DQw8	18	50 ^d
DQw2	54	77 ^e
DQw8	18	50 ^f
Protective		
DR2	24	5 ^g
DRw15	20	2 ^h
DRw16	3	2
DR7	38	9 ⁱ
DQw1	59	30 ^j

Aetiologic fraction (δ) values are only calculated in significant positive associations, together with their 95% confidence limits; Relative risk (RR) values are calculated in all significant associations ($p < 5 \times 10^{-2}$).

^a $\delta = 0.603$ (± 0.002), RR = 6.5 ($p < 1 \times 10^{-10}$); ^b $\delta = 0.297$ (± 0.002), RR = 5.7 ($p < 5 \times 10^{-8}$); ^c $\delta = 0.364$ (± 0.002), RR = 3.6 ($p < 5 \times 10^{-7}$); ^d $\delta = 0.390$ (± 0.002), RR = 4.6 ($p < 5 \times 10^{-8}$); ^e $\delta = 0.500$ (± 0.003), RR = 3.5 ($p < 1 \times 10^{-4}$); ^f $\delta = 0.390$ (± 0.002), RR = 4.6 ($p < 5 \times 10^{-8}$); ^g RR < 1, ($p < 5 \times 10^{-5}$); ^h RR < 1, ($p < 1 \times 10^{-5}$); ⁱ RR < 1, ($p < 5 \times 10^{-8}$); ^j RR < 1, ($p < 1 \times 10^{-6}$)

been suggested that susceptibility factors are closer to DQ than to DR [29].

DR3/DR4 individuals show the highest relative risk (Table 3), particularly the DRw17.2/DR4-DQw8 heterozygous individuals, as expected. The highest δ values appear when individuals DR3 or DR4 or, likewise, DRw17.2 or DR4-DQw8 are considered.

Negative Type 1 diabetes associations

DR2. Only the DRw15, DR2 subtype, is significantly decreased in our diabetic population ($p = 9 \times 10^{-6}$), while the subtype DRw16 is not (Table 2). DRw15 may generally be in linkage disequilibrium with either of the two Taq-I DQ alleles named DQ α 1b-DQ β 1b (Dw2) or DQ α 1c-DQ β x (Dw12), but only DRw15-DQ α 1b-DQ β 1b is decreased in our diabetic patients (patients, $n = 127$, 2%; control subjects, $n = 177$, 17%; $p = 4 \times 10^{-5}$, data not shown).

DQw1 is concordantly decreased ($p = 7 \times 10^{-7}$) (Table 2) because it is included in the protective DR2(DRw15-Dw2) haplotype.

DR7. Three DR7 subtypes are defined by RFLP analysis [21, 28]. Only the DR7.1 variant, included in the extended haplotype A29B44DR7, has been found to be significantly decreased in our diabetic population (patients, $n = 66$, 3%; control subjects, $n = 177$, 29%; $p = 2 \times 10^{-5}$, data not shown).

DQA1 and DQB1 sequences (Table 3)

Non-Asp 57 β DQ is not a dominant susceptibility marker. It has been possible to assign the DQB1 exon-2 sequences according to the method described in the Subjects, materials and methods section in the patients and control subjects not tested by DNA-oligotyping. No significant increase in the frequency of non-Asp 57 β DQ individuals has been found in our 127 diabetic patients (NA/A or NA in Table 3). These data found in a relatively high number of patients and control subjects favour the idea that HLA-DQ factors do not by themselves explain Type 1 diabetes susceptibility [30] and support the tendency reflected in our previous study [30]: the significance of β -DQ non-Asp 57 as a susceptibility factor disappears when a higher number of patients is studied. However 'a' recessive effect may be seen in 'homozygous' NA/NA subjects.

Arg 57 α DQ as a susceptibility marker. A high frequency of Arg 52 α DQ individuals have been found in our 127 diabetic patients (AR/AR or NAR in Table 3) according to the hypothesis postulated by Khalil et al. [9]. If neither DR3 nor DR4 individuals are considered, the significant association disappears (patients, $n = 19$, 58%; control subjects, $n = 94$, 35%; $p > 5 \times 10^{-2}$). This marker, described as a diabetes susceptibility factor in the Spanish population [30], maintains its significance when the number of patients has been doubled, as for the present study, reaches the highest δ value of markers and confers susceptibility both as a dominant and as a recessive factor (Table 3).

Non-Asp 57 β DQ and Arg 52 α DQ susceptibility markers. When these two factors are considered, a significant high frequency is found in our whole diabetic population (patients, $n = 127$, 90%; control subjects, $n = 177$, 58%; $p = 3 \times 10^{-9}$, $\delta = 0.76$, RR = 6.4). However, 13 patients bear both 'protective' DQA1 and DQB1 genes (i.e.: Asp 57 β DQ and non-Arg 52 α DQ); these exceptions invalidate this susceptibility theory as universal. Also, if individuals who are neither DR3 nor DR4 are considered, the significant association disappears (patients, $n = 19$, 37%; control subjects, $n = 94$, 10%; $p > 5 \times 10^{-2}$).

δ (aetiologic fraction) values and different susceptibility markers (Table 3). δ values allow absolute comparisons among disease susceptibility markers when the same control and patient populations are used [25, 26].

Bearing DR3 or DR4 represents the most universal serological marker for Type 1 diabetes susceptibility ($\delta = 0.70$); this value parallels the one found by using RFLPs ($\delta = 0.63$) for the combined risk DRw17.2 or DR4-DQw8, which are the DR3 and DR4 subtypes increased in our diabetic population. DQw2 or DQw8 δ is not as high ($\delta = 0.65$) due to the existing low DQ polymorphism (so defined) in populations. The combination Arg at α DQ 52 residue with non-Asp at β DQ 57 gives a higher δ value ($\delta = 0.76$) than DR3 and/or DR4. Arg 52 at α DQ is by itself the best genetic marker ($\delta = 0.82$).

Discussion

Although the genetic model of Type 1 diabetes inheritance is uncertain and may be variable in each case [31], δ guarantees consistency in either an intermediate, recessive or dominant model of inheritance [25, 26], since a higher δ value represents a stronger association whatever the genetic cause might be; also, if two alleles, even from different loci, are associated with the same 'diseased' allele, the one with the strongest linkage disequilibrium will always give the highest δ value, irrespective of any difference in the frequencies of the two antigen alleles [25]. Moreover, apart from genetic HLA alleles defined by serology, α and β residues could be considered as 'alleles associated with Type 1 diabetes' because each of them represent, in this context, a group of true genetic alleles. Likewise RFLP alleles are considered as such because they generally represent splits of the classic serologically defined alleles. Within this framework, serological susceptibility markers (DR3 and/or DR4, $\delta = 0.70$) are associated with the hypothetical 'diseased' locus with approximately the same strength as RFLP alleles (DRw17.1 or DRw17.2 or DR4-DQw8, $\delta = 0.67$); however, δ values are 0.82 when Arg 52 α DQ is analysed and 0.76 when a combination of Arg 52 α DQ and non-Asp 57 β DQ is analysed. Although it is not mathematically possible to compare different δ values by using statistical significances [26], it is evident that over the last decade, HLA molecular genetic studies in Type 1 diabetes have aided in the understanding of both the susceptibility locus (possibly DQ) and pathogenetic mechanisms (differential autoantigen presentation according to different α - β DQ residues). However, the highest δ value in our population (α DQ residues) does not equal 1 (only 0.82) which means that some patients do not bear these postulated risk factors indicating that these markers are not in the 'diseased' locus. However, caution is warranted in interpreting this data as the particular patients found could not be classified as true Type 1 diabetic patients (although all are insulin-dependent) and the possible heterogeneity of the disease must be taken into account. Also, the existence of other class II genes or uncharacterized DQ factors (i.e. residue 56 at DQ, a mouse β 1A equivalent must be considered [32, 33].

On the other hand, regarding the DQ susceptibility, it is found that α DQ Arg 52 ($\delta = 0.82$) and α DQ Arg 52/ β DQ non-Asp 57 ($\delta = 0.76$) are the strongest HLA and Type 1 diabetes associations found in our population. It is noteworthy that α DQ Arg 52 is by itself a susceptibility marker while β DQ non-Asp 57 is not, emphasizing the necessity of testing large numbers of patients and control subjects in different populations. Moreover, DR7 is a protective antigen, relatively frequent in our Spanish population (about twice that of other European populations, [34]). Its DQ β associated sequence is also non-Asp 57 which would also explain why the theory does not hold in our population. Also, α DQ Arg 52 and the combination of α DQ Arg 52 and non-Asp 57/ β DQ are more universal HLA markers in our diabetic patients than serological class II or class III markers [35]; indeed, it has been observed that both high frequency Spanish diabetic HLA haplotypes (B18-DR3(w17.2)-BF1C4A3BQ0) and 'low

Table 3. Aetiologic fraction (δ) comparisons among serological and genetic HLA class II Type 1 (insulin-dependent) diabetes susceptibility markers

DR Serology	Control subjects (n = 177) (%)	Diabetic patients (n = 127) (%)
Antigen(s)		
DR3	27	71 ^a
DR4	23	51 ^b
DR3 or DR4	49	85 ^c
DR3/DR4	3	37 ^d
DR Subtypes (allogentyping, sequences)	Control subjects (n = 177) (%)	Diabetic patients (n = 127) (%)
Allogentype(s)		
DRw17.1	14	26 ⁿ
DRw17.2	15	50 ^{e, n}
DRw17(1,2)	27	71 ^f
DR4-DQw7(DQ β 3b)	6	2
DR4-DQw8(DQ β 3a)	18	50 ^g
DRw17.2 or DR4-DQw8	37	77 ^{h, n}
DRw17.1 or 17.2 or 4-DQw8	47	83 ⁱ
DRw17.2/DR4-DQw8	1	27 ^{j, n}
α and β DQ chains (allogentyping, sequences)	Control subjects (n = 177) (%)	Diabetic patients (n = 127) (%)
Total		
NA/A or NA	90	96
NA/NA	53	77 ^k
AR/AR or NAR	66	94 ^l
AR/NA	58	90 ^m
AR/AR	17	65 ^r
AR or NA	98	100
	Control subjects (n = 94) (%)	Diabetic patients (n = 19) (%)
Neither DR3 nor DR4		
NA/A or NA	81	79
NA/NA	46	32
AR/AR or NAR	35	58
AR/NA	20	37
AR/AR	5	11
AR or NA	96	100

NA, Asp 57 β DQ negative; A, Asp 57 β DQ positive; AR, Arg 52 α DQ positive; NAR, Arg 52 α DQ negative.

The DRB1, DQA1 and DQB1 allogentyping nomenclature has been published previously [16, 21–23, 28]. The DQA1 and DQB1 sequence nomenclature has been published previously [24] and is from the 11th International Histocompatibility Workshop.

δ values are only calculated in significant positive associations, together with their 95% confidence limits; RR values are calculated in all significant associations ($p < 5 \times 10^{-2}$).

^a $\delta = 0.603$ (± 0.002), RR = 6.5 ($p < 1 \times 10^{-10}$); ^b $\delta = 0.364$ (± 0.002), RR = 3.6 ($p < 5 \times 10^{-7}$); ^c $\delta = 0.706$ (± 0.002), RR = 6.4 ($p < 5 \times 10^{-10}$); ^d $\delta = 0.351$ (± 0.001), RR = 20.2 ($p < 1 \times 10^{-10}$); ^e $\delta = 0.412$ (± 0.002), RR = 5.8 ($p < 5 \times 10^{-8}$); ^f $\delta = 0.603$ (± 0.002), RR = 6.5 ($p < 1 \times 10^{-10}$); ^g $\delta = 0.390$ (± 0.002), RR = 4.6 ($p < 5 \times 10^{-8}$); ^h $\delta = 0.635$ (± 0.002), RR = 5.7 ($p < 1 \times 10^{-7}$); ⁱ $\delta = 0.679$ (± 0.002), RR = 5.7 ($p < 5 \times 10^{-10}$); ^j $\delta = 0.263$ (± 0.002), RR = 66 ($p < 5 \times 10^{-10}$); ^k $\delta = 0.511$ (± 0.003), RR = 2.9 ($p < 5 \times 10^{-5}$); ^l $\delta = 0.824$ (± 0.002), RR = 7.8 ($p < 5 \times 10^{-8}$); ^m $\delta = 0.762$ (± 0.002), RR = 6.4 ($p < 5 \times 10^{-9}$); ^r $\delta = 0.578$ (± 0.002) RR = 8.9 ($p < 1 \times 10^{-10}$); ⁿ Only 66 diabetic patients were studied

frequency' haplotypes (B8-DR3(w17.1)-BfS C4AQ0B1 and B49-DR4(DQw8)-BfS C4AQ0B1) are shorter (deleted or non-duplicated) at the class III level [27] than 'normal' length haplotypes. This intriguing phenomenon is common to other Mediterranean populations [36–38]. In addition, not all patients bear these extended haplotypes but fragments of them, always including the DR3-DQw2 and/or DR4-DQw8 region. Also, it may be further concluded that α DQ Arg 52 is the most common risk factor found within this DQ-region of the chromosome 6.

The involvement of DR in Type 1 diabetes susceptibility has also been suggested [7, 10, 39]; these data indicate continuation of the search for susceptible or resistant residues at the DR peptide chains. However, δ values show that none of the DR alleles defined either by serology or RFLPs, show a stronger association with the disease than the α - β DQ markers. Also, several results from studies in our and other populations suggest that regulatory non-coding DRB1 or DRB3 regions may have a role in Type 1 diabetes aetiopathogenesis: (1) A difference at the DRB1 gene non-coding region has been found between the B8-DR3(w17.1)-Dw24-DQw2 and the B18-DR3(w17.2)-Dw25-DQw2 haplotypes [40], in addition to other RFLPs differences found at the DRB3 and DQA level [41], (2) Type 1 diabetes is predominantly associated with B8-DR3 (w17.1)-Dw24 in North European/American subjects and B18-DR3 (w17.2)-Dw25 in Mediterranean subjects [2, 36–38], (3) Systemic lupus erythematosus is similarly associated to B8-DR3(w17.1)-Dw24 in North European/American subjects and B18-DR3(w17.2)-Dw25 in Spanish subjects [42] and (4) Neonatal lupus is associated with B8-DR3 (w17.1)-Dw24 in both North European/American and Spanish subjects [43]. Taken together, these data support that Type 1 diabetes or adult systemic lupus develop in B8-DR3-Dw24 or B18-DR3-Dw25 bearing individuals depending on the geographical location in which they live and this preference may be based on both an HLA (based on differences at non-coding regions of DR or at other neighbouring genes) or non-HLA genetical background and also on specific environmental factors. Regarding DR4 susceptibility haplotypes, our present and previous [29] studies show that DR4 splits are not by themselves the susceptibility genes, but the neighbouring DQw8 related factors.

Finally, before these data can be combined into a recessive, dominant or intermediate theory of Type 1 diabetes inheritance, it is necessary to detect the other genetic predisposing factors (of which there are at least six in the NOD mouse, [44]) and their respective penetrances. Taking into account our available HLA data, HLA factors included within DR3-DQw2 or DR4-DQw8 haplotypes would seem to act in a dominant and also over-dominant fashion.

Acknowledgements. This work was supported by the Fondo de investigaciones sanitarias de la seguridad social, Spain.

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Received: 4 September 1991
and in revised form: 3 January 1992

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