

## DRB1\*04 and DQB1\*03 Alleles are Very Prevalent in Bahraini Families with Both T2DM and T1DM

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### Abstract

**Objectives:** Several investigations in families with both T1DM and T2DM have been reported to elucidate the genetic interaction between T1DM and T2DM and the clinical consequences for both diseases. The frequent occurrence of T1DM in relatives of patients with T2DM has also been previously observed. This study investigated whether the T2DM parent/grandparents share a specific HLA class II-DRB1 and DQB1 alleles and their haplotype combination with the T1DM child.

**Methods:** Twenty four Bahraini families with a T1DM child and either parents or grandparents with T2DM and with no family history of T1DM were selected. HLA class II-DRB1 and DQB1 were examined by SSP-PCR method and the distribution was analyzed.

**Results:** In relation to DRB1\*, the most common shared allele was DRB1\*04:01:01 83% (n=20), while the most common shared DQB1\* allele was DQB1\*03:02:01 83% (n=20). In addition, the most common shared haplotype was DRB1\*04:01:01-DQB1\*03:02:01 83% (n=20).

**Conclusions:** The current study showed that DR4 and DQ3 alleles and its haplotype combination are the highest prevalence in the selected Bahraini families with mixed T1DM and T2DM patients. T2DM parents possessing this haplotype are more likely to have a child with T1DM, especially in families with no history of T1DM. The excess transmission of DR4-linked haplotypes from parents with T2DM to offspring with T1DM has been clearly observed in the present study.

**Keywords** Bahrain; Bahraini families; HLA; T1DM; T2DM

### Introduction

Several investigations in families with both type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) have been reported to elucidate the genetic interaction between T1DM and T2DM and the clinical consequences for both diseases [1]. The evidence for an increased frequency of T2DM in families with T1DM has been observed [2-7]. In Sweden, 32% of patients with T1DM reported a family history of T2DM compared with 12.5% in a non-diabetic reference group [2]. The true prevalence is difficult to ascertain, because most patients are diagnosed with T1DM at an age when their parents, or grandparents, might still be too young to have developed T2DM. In addition, reliable age-adjusted prevalence data for T2DM in the general population is rarely available.

Meanwhile, evidence for frequent occurrence of T1DM in relatives of patients with T2DM has also been observed [8-11], with a 2-fold increased prevalence of T1DM in families with T2DM in Finnish

population compared to the prevalence in the population [12]. Thus, a total of 14% of Finnish families with more than one T2DM patient also included T1DM patients, and 5% of the T2DM pro-bands had a first-degree relative with T1DM [11]. It was postulated that the genetic interaction between T1DM and T2DM could be mediated by the IDDM1 locus in the Human leukocyte antigen (HLA) class II region on chromosome 6p21 which is strongly linked to T1DM [13,14] and explains the 42% of the familial risk for T1DM [13].

Similar to Finland, Bahrain has a small population with a high prevalence of Diabetes mainly T2DM [15]. In relation to the association of HLA and diabetes risk in Bahrain, Previous studies on the association of HLA class II alleles and molecular level with Bahraini T1DM were previously published [16,17]. The results obtained from these initial studies clearly demonstrated that for T1DM in Bahraini the genetic risk for disease pathogenesis is strongly linked to HLA class II loci, as select HLA-DRB1 and -DQB1 alleles and haplotypes conferred disease susceptibility or resistance, respectively to T1DM [16,17]. These data are consistent with most reports from other Caucasian populations. In addition to a study conducted on

Bahrainis with T2DM the findings show that there is a significant association with both HLA-DRB1 and -DQB1 genotypes, with some alleles and haplotypes appearing to confer susceptibility and others playing a protective role [18].

Therefore, the aim of this study was to investigate any inherited genetic interaction between T1DM and T2DM with reference to HLA alleles by studying couples of T2DM and T1DM in the same family.

## Materials and Methods

### Subjects

The study group included twenty four unrelated Bahraini families with a T1DM child and either parents or grandparents with T2DM and with no family history of T1DM. They were selected from the diabetes clinics at Salmaniya medical center (SMC). All participants were asked to sign a consent form according to the study protocol and The Arabian Gulf University Ethics Committee approved the study. All T2DM patients were diagnosed based on 1998 World Health Organization (WHO) diagnostic and classification criteria [19]. None of the T2DM patients ever had ketoacidosis and the treatment for diabetes included diet and/or oral anti-diabetic drugs and/or insulin (to achieve glycemic control). All subjects commenced on insulin therapy had been treated with oral drugs for at least 2 years. Patients with T1DM were diagnosed according to clinical and laboratory findings and all patients with T1DM were receiving insulin to control hyperglycemia.

### HLA genotyping

Genomic DNA was extracted by the Qiagen mini-spin column technique as per manufacturers (Qiagen; Hilden, Germany)

specifications, and was used for PCR analysis. The HLA-DRB1 and DQB1 gene alleles were analyzed with the Polymerase chain reaction-sequence specific priming (PCR-SSP) technique, with the SSP2L HLA class II (DRB/DQB) genotyping kit according to the manufacturer's specifications (One Lambda, Thousand Oaks, Calif.). PCR products were analyzed on 2.5% (wt/vol) agarose gels stained with ethidium bromide (0.5 µg/ml-1).

### Data analysis

HLA class II alleles were deduced from the electrophoresis pattern, using the HLA worksheet, and analyzed on the specific One Lambda genotyping software. The alleles and haplotypes distributions were determined by direct counting.

## Results

### HLA class II - distribution among families with mixed T1DM and T2DM patients (n=24)

Table 1 compares HLA class II-DRB1\* and DQB1\* of T1DM and T2DM patients in 24 Bahraini families by examining whether the T2DM parents/relatives had shared HLA class II-DRB1 and DQB1 alleles and their haplotype combination with T1DM child. Of the 24 families with T1DM and T2DM patients, 45.8% (n=11) of T1DM children had mothers with T2DM, 37% (n=9) had fathers with T2DM, 12.5% (n=3) had grandfathers with T2DM, and 4.2% (n=1) had grandmothers with T2DM (Table 1).

	Children T1DM	Parent/ Relative T2DM	Parent/Relative Relation	Shared haplotypes
1	DRB1*03:01:01:01, DRB1*04:01:01 DQB1*02:01:01, DQB1*03:02:01	DRB1*04:01:01, DRB1*04:01:01 DQB1*03:02:01, DQB1*03:02:01	Father	DRB1*04:01:01- DQB1*03:02:01
2	DRB1*03:01:01:01, DRB1*04:01:01 DQB1*02:01:01, DQB1*03:02:01	DRB1*04:01:01, DRB1*04:01:01 DQB1*03:02:01, DQB1*03:02:01	Father	DRB1*04:01:01- DQB1*03:02:01
3	DRB1*04:01:01, DRB1*11:01:01 DQB1*03:02:01, DQB1*03:02:01	DRB1*04:01:01, DRB1*04:01:01 DQB1*03:02:01, DQB1*03:02:01	Father	DRB1*04:01:01- DQB1*03:02:01
4	DRB1*03:01:01:01, DRB1*04:01:01 DQB1*02:01:01, DQB1*03:02:01	DRB1*04:01:01, DRB1*04:01:01 DQB1*03:02:01, DQB1*03:02:01	Father	DRB1*04:01:01- DQB1*03:02:01
5	DRB1*04:01:01, DRB1*11:01:01 DQB1*03:01:01, DQB1*03:02:01	DRB1*04:01:01, DRB1*04:01:01 DQB1*03:02:01, DQB1*03:02:01	Father	DRB1*04:01:01- DQB1*03:02:01
6	DRB1*03:01:01:01, DRB1*04:01:01 DQB1*02:01:01, DQB1*03:02:01	DRB1*04:01:01, DRB1*11:01:01 DQB1*03:01:01, DQB1*03:02:01	Mother	DRB1*04:01:01- DQB1*03:02:01
7	DRB1*03:01:01:01, DRB1*04:01:01 DQB1*02:01:01, DQB1*03:02:01	DRB1*04:01:01, DRB1*04:01:01 DQB1*03:02:01, DQB1*03:02:01	Father	DRB1*04:01:01- DQB1*03:02:01
8	DRB1*03:01:01:01, DRB1*04:01:01 DQB1*02:01:01, DQB1*03:02:01	DRB1*04:01:01, DRB1*04:01:01 DQB1*03:02:01, DQB1*03:02:01	Mother	DRB1*04:01:01- DQB1*03:02:01
9	DRB1*04:01:01, DRB1*04:01:01 DQB1*02:01:01, DQB1*03:02:01	DRB1*04:01:01, DRB1*04:01:01 DQB1*03:02:01, DQB1*05:01:01	Mother	DRB1*04:01:01- DQB1*03:02:01
10	DRB1*04:01:01, DRB1*05:01:01 DQB1*03:02:01, DQB1*03:02:01	DRB1*04:01:01, DRB1*04:01:01 DQB1*03:02:01, DQB1*03:02:01	Father	DRB1*04:01:01- DQB1*03:02:01

11	DRB1*03:01:01:01, DRB1*04:01:01 DQB1*02:01:01, DQB1*03:02:01	DRB1*04:01:01, DRB1*04:01:01 DQB1*03:02:01, DQB1*03:02:01	Mother	DRB1*04:01:01- DQB1*03:02:01
12	DRB1*16:01:01, DRB1*04:01:01 DQB1*06:01:01, DQB1*03:02:01	DRB1*04:01:01, DRB1*04:01:01 DQB1*03:02:01, DQB1*03:02:01	Grandfather	DRB1*04:01:01- DQB1*03:02:01
13	DRB1*03:01:01:01, DRB1*04:01:01 DQB1*02:01:01, DQB1*03:02:01	DRB1*04:01:01, DRB1*04:01:01 DQB1*03:02:01, DQB1*03:02:01	Mother	DRB1*04:01:01- DQB1*03:02:01
14	DRB1*04:01:01, DRB1*07:01:01 DQB1*03:02:01, DQB1*03:02:01	DRB1*04:01:01, DRB1*04:01:01 DQB1*03:02:01, DQB1*03:02:01	Mother	DRB1*04:01:01- DQB1*03:02:01
15	DRB1*15:01:01:01 DRB1*04:01:01 DQB1*06:01:01, DQB1*03:02:01	DRB1*04:01:01, DRB1*04:01:01 DQB1*03:02:01, DQB1*03:02:01	Grandfather	DRB1*04:01:01- DQB1*03:02:01
16	DRB1*03:01:01:01, DRB1*04:01:01 DQB1*02:01:01, DQB1*03:02:01	DRB1*04:01:01, DRB1*04:01:01 DQB1*03:02:01, DQB1*03:02:01	Grandmother	DRB1*04:01:01- DQB1*03:02:01
17	DRB1*03:01:01:01, DRB1*04:01:01 DQB1*02:01:01, DQB1*03:02:01	DRB1*04:01:01, DRB1*04:01:01 DQB1*03:02:01, DQB1*03:02:01	Mother	DRB1*04:01:01- DQB1*03:02:01
18	DRB1*03:01:01:01, DRB1*04:01:01 DQB1*02:01:01, DQB1*03:02:01	DRB1*04:01:01, DRB1*04:01:01 DQB1*03:02:01, DQB1*03:02:01	Mother	DRB1*04:01:01- DQB1*03:02:01
19	DRB1*03:01:01:01, DRB1*04:01:01 DQB1*02:01:01, DQB1*03:02:01	DRB1*04:01:01, DRB1*04:01:01 DQB1*03:02:01, DQB1*03:02:01	Mother	DRB1*04:01:01- DQB1*03:02:01
20	DRB1*04:01:01, DRB1*10:01:01 DQB1*05:01:01, DQB1*03:02:01	DRB1*04:01:01, DRB1*04:01:01 DQB1*03:02:01, DQB1*03:02:01	Mother	DRB1*04:01:01- DQB1*03:02:01
21	DRB1*03:01:01:01, DRB1*13:01:01:01 DQB1*06:01:01, DQB1*02:01:01	DRB1*03:01:01:01, DRB1*10:01:01 DQB1*05:01:01, DQB1*02:01:01	Mother	DRB1*03:01:01:01- DQB1*02:01:01
22	DRB1*03:01:01:01, DRB1*03:01:01:01 DQB1*02:01:01, DQB1*02:01:01	DRB1*03:01:01:01, DRB1*16:01:01 DQB1*02:01:01, DQB1*05:01:01	Grandfather	DRB1*03:01:01:01- DQB1*02:01:01
23	DRB1*03:01:01:01, DRB1*07:01:01:01 DQB1*02:01:01, DQB1*03:01:01	DRB1*07:01:01:01, RB1*07:01:01:01 DQB1*03:01:01, DQB1*03:01:01	Father	DRB1*07:01:01:01- DQB1*03:01:01
24	DRB1*03:01:01:01, DRB1*07:01:01:01 DQB1*02:01:01, DQB1*02:01:01	DRB1*15:01:01:01, DRB1*07:01:01:01 DQB1*06:01:01, DQB1*02:01:01	Father	DRB1*07:01:01:01- DQB1*02:01:01

**Table 1:** Distribution of shared HLA-DRB1 and DQB1 alleles and their haplotype combinations in 24 separate Bahraini families with T1DM and T2DM

### HLA Class II alleles/haplotypes among patients in families with mixed T1DM and T2DM patients

Table 2 summarizes the percentage of HLA class II -DRB1 and DQB1 alleles and their haplotype combinations shared between children of T1DM and their T2DM parent/relative in 24 Bahraini families. In relation to DRB1 alleles, the most common shared allele was DRB1\*04:01:01 83 % (n=20), while the most common shared DQB1\* allele was DQB1\*03:02:01 83% (n=20). In addition, the most common shared haplotype was DRB1\*04:01:01-DQB1\*03:02:01 83% (n=20). DRB1\*04:01:01 and DQB1\*03:02:01 alleles and (DRB1\*04:01:01-DQB1\*03:02:01) haplotype showed the strongest association with the selected Bahraini families with mixed T1DM and T2DM patients.

### Discussion

This study was conducted mainly to obtain further insight into the association of the HLA antigens in mixed families with T1DM and T2DM and their contribution to the pathogenesis and manifestation of DM. Earlier epidemiological data suggested that having a parent or relative with T2DM increased the risk for T1DM in children in mixed families of T1DM and T2DM [8-11,20-23]. Furthermore, it was

suggested that both HLA class I and class II may dependently or independently contribute to the susceptibility for T2DM, as was shown for families with mixed T1DM and T2DM patients [11].

Furthermore, a shared genetic background with a patient having T1DM predisposes T2DM patients to autoantibody positivity thereby leading to impaired insulin secretion and possible insulin insensitivity. However, T1DM is etiologically distinct from T2DM [1,24]. It is well established that T1DM is considered to be autoimmune and clearly associated with specific HLA antigens, and demonstrates linkage of a major susceptibility locus with the HLA complex [25]. On the other hand, T2DM is thought not to be autoimmune and the evidence for a genetic basis of the disease is stronger in T2DM compared to T1DM. The precise genetic mechanism is unclear except in some uncommon subtypes of T2DM who are associated with specific insulin receptor defects [26,27].

In the current study, twenty four Bahraini families with a T1DM child and either parents or grandparents with T2DM and with no family history of T1DM were selected. The analysis was based on whether the parent/grandparent with T2DM actually shared the susceptibility HLA class II -DR or -DQ alleles and haplotype with their T1DM children. The results clearly showed a high frequency of HLA

class II alleles (DRB1\*04:01:01, DQB1\*03:02:01) alleles and its corresponding haplotypes combination shared in T1DM children and their T2DM relatives.

Common alleles shared	Total number	%
DRB1*	24	
DRB1*04:01:01	20	83%
DRB1*03:01:01:01	2	8.30%
DRB1*07:01:01:01	2	8.30%
DQB1*	24	
DQB1*03:02:01	20	83%
DQB1*02:01:01	3	12.50%
DQB1*03:01:01	1	4.16%
Common haplotypes shared	Total number	%
DRB1*-DQB1*	24	
DRB1*04 :01:01-DQB1*03:02:01	20	83%
DRB1*03:01:01:01 -DQB1*02:01:01	2	8.30%
DRB1*07 :01:01:01-DQB1*02:01:01	1	4.16%
DRB1*07:01:01:01 -DQB1*03:01:01	1	4.16%

**Table 2:** Percentage of the shared HLA-DRB1\* and DQB1\* alleles/haplotypes among patients in 24 families with T1DM and T2DM

To discuss that the HLA alleles from parents and grandparents who did NOT suffer from T2DM to demonstrate that the DRB1\*04:01 and or DQB1\*03:02 were not inherited from non-T2DM parents/grandparents, a previous study done on 50 Bahraini children with T1DM reported 28% versus 83% in the present study and 14% in the non-diabetic control group for the DRB1\*04:01 and DQB1\*03:02 HLA alleles [16]. Although parents and grandparents did NOT suffer from T2DM at the time of that study yet still they may carry the possibility to develop it later.

In addition, it was seen that (DRB1\*04:01:01, DQB1\*03:02:01) alleles and its corresponding haplotypes combination have the highest percentage in female parents/grandparents with T2DM. The Bahraini population is small with a high incidence of DM, in particular T2DM [15,16] with a rate of 20% of marriages between relatives [28].

In the view of a possible genetic interaction between T1DM and T2DM and the clinical consequences for T1DM in particular [11, 20, 21], the results obtained from the present study clearly supported this view and demonstrated that T2DM patients with HLA DR4-DQ8 haplotypes are carrying a risk of having a child with T1DM, especially in families where there is no family history of T1DM. This finding is in agreement with others who observed the frequent occurrence of T1DM in relatives of patients with T2DM [8-11].

The excess transmission of DR4-linked haplotypes from parents with T2DM to offspring with T1DM has been reported [22], thus supporting the same findings observed in the current study. DM in most cases is caused by a loss of the physical or functional  $\beta$ -cell mass, mostly due to an autoimmune process (type 1 etiological process), and/or increased need for insulin due to insulin resistance (type 2

process) [29]. Both of these major DM types are believed to include different stages of the disease, ranging from non-insulin-requiring, to insulin-requiring for control or survival [29]. Accordingly, it is quite possible that both processes would operate in a single patient and contribute to the phenotype of the patient [1,24,29]. Additionally, factors other than autoimmunity can lead to a defective insulin response to glucose. Moreover, both major DM types are considered multifactorial diseases with several predisposing genetic and environmental factors, some of which could be common to both types [1].

Predisposing environmental factors like a viral infection cannot be ignored. A previous study from Bahrain on T1DM reported interesting cases in the diabetic group. These include two HLA identical brothers both having the high risk HLA DR3-DR4, DQ2-DQ8, one 7 years and the other 15 years. Both developed T1DM at the same time. Although parents were non-diabetic yet the two brothers got DR3/DQ2 from one parent and DR4/DQ8 from the other and thus became having a much higher risk of developing T1DM. A viral infection that the two boys had made them develop T1DM on the same day. Another couple, two HLA identical twin sisters having both the high risk DR3/DQ2 haplotype one developed T1DM when she was 6 years and the other when she was 9 years. This could be explained by the need of an environmental factor like a viral infection to trigger T1DM in a genetically susceptible high risk subject [16]. In the current study such an environmental factor could not be traced.

In looking at the current study data in Table 1, more notable results were seen. First, the high frequency of DRB1\*04:01:01-DQB1\*03:02:01, DRB1\*03:01:01:01-DQB1\*02:01:01 (“DR3/DR4”) heterozygotes (12 of 24, or 50%) in the patients is even higher than might be expected. Although this is the highest risk genotype known for Caucasians, the frequency of 50% (12/24 patients) is particularly high. Most Caucasian reports range from 17% to 40%. A previous study done on 50 Bahraini children with T1DM reported 28% and 14% in the non-diabetic control group for DRB1\*04:01 and DQB1\*03:02 [16]. Although parents and grandparents did NOT suffer from T2DM at the time of study still they may carry the possibility to develop it later.

Even more striking is the extremely high frequency of DRB1\*04:01:01-DQB1\*03:02:01 in the T2DM relatives. Nineteen of 24 T2DM relatives are homozygous for this haplotype. To be homozygous for an HLA allele needs to be confirmed with different methods, yet this was not possibly in the current study. When the parent is homozygous, the child will necessarily inherit the genotype, whether or not he/she is affected by T1DM. In four cases, the T2DM relative did not have a DRB1\*04:01:01-DQB1\*03:02:01 haplotype. Again, when this is a parent, the child cannot inherit the DRB1\*04:01:01-DQB1\*03:02:01 haplotype. In only two cases was the T2DM relative was heterozygous for DRB1\*04:01:01-DQB1\*03:02:01 and another haplotype. In both cases, this haplotype was transmitted; however, this represents too few events to be considered significant. The haplotype frequency for DRB1\*04:01:01-DQB1\*03:02:01 in the T2DM relatives is 38 of 48 total haplotypes, or 0.79.

Similar to Bahrain, Finland has a small population with a high prevalence of DM, and a large proportion of patients with T2DM having inherited susceptibility genes for both types of DM. Also, the lifestyle changes leading to the T2DM epidemic around the world [30] may have an impact on the clinical picture of T1DM in the subjects at risk for T2DM as well. Indeed, obesity has been shown to be a risk

factor for childhood T1DM [31-33]. According to the "accelerator hypothesis", there are two accelerators which can precipitate diseases in all types of DM. They include the intrinsically high rate of  $\beta$ -cell apoptosis and insulin resistance resulting from weight gain and physical inactivity. In addition, a third accelerator is  $\beta$ -cell autoimmunity which would enhance the diabetic process in a subset [34].

Although the consequence of such genetic admixture of T1DM and T2DM is uncertain, from the existing data it was suggested that patients with mixed family history have an intermediate phenotype of DM. This is associated with insulin resistance and cardiovascular complications as in T2DM patients and lower BMI, less cardiovascular complication and lower C-peptide concentrations as in T1DM patients [1]. Meanwhile, patients with latent autoimmune diabetes of adulthood (LADA) have an increased frequency of the HLA alleles DQB1\*03:02:01 and 02, but the family history of T1DM could be a confounding factor [35,36]. As mentioned earlier, patients with LADA have family history of T1DM more often than other phenotypically T2DM patients [37-39].

Holoshitz has proposed an alternative theory for HLA-disease association involving allele-based, antigen presentation independent mechanism: MHC Cusp theory; whether this can explain the pathogenesis of inheriting an HLA allele as a risk factor for both types of diabetes needs to be explored. The theory proposes that the MHC codes for allele-specific ligands in the cusp region, which interact with non-MHC receptors and activate various pathways. Aberrations in those pathways could cause MHC associated diseases [40].

On the other hand, T2DM patients from the mixed families shared an increase in the moderate-risk HLA-DQB1\*03:02:01/Xgenotype with the adult-onset T1DM patients [11]. This was not unexpected because they were relatives of T1DM patients. However, similar sharing of the genotype conferring the highest risk (02/03:02:01) or absence of the genotype conferring protection [0602(3)/X] was not observed except for the GADA+ subgroup of patients from the mixed families. Thus, among the LADA patients, only those from T1DM families share the 02/03:02:01 and 0602 [31] association with T1DM patients, whereas all LADA patients share the 03:02:01/X association. This finding suggests that part of the observed heterogeneity among the LADA patients could be due to T1DM family history.

## Conclusion

In conclusion, the present results show that DRB1\*04:01:01 and DQB1\*03:02:01 alleles and (DRB1\*04:01:01-DQB1\*03:02:01) haplotype are very prevalent in the selected Bahraini families with mixed T1DM and T2DM patients. T2DM parents possessing this haplotype can produce a child with T1DM, especially in families with no history of T1DM. This study clearly demonstrated excess transmission of DR4-linked haplotypes from parent with T2DM to offspring with T1DM. Factors that may influence pancreatic  $\beta$ -cell viability and auto-antigens presented by DR4 and DQ3 in patients with T1DM are two points that may be investigated in future studies.

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