HLA-DQB1*05 Association with Hashimoto's Thyroiditis in Children of Northern Greek Origin

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Abstract

*In order to investigate HLA-DRB1 and HLA-DQB1 gene polymorphisms in Northern Greek pediatric population with Hashimoto's thyroiditis (HT), we analyzed the distribution of these alleles in 17 patients and in 181 healthy subjects using polymerase chain reaction. No significant association was detected between HT and alleles analyzed. However, HLA-DQB1*05 was significantly increased in patients with age of diagnosis >10 years (87.5%) compared to those with age of diagnosis ≤10 years (33.3%) (P=0.05). These results question the role of sexual maturity in combination with HLA-DQB1*05 as predisposing factor for the onset of HT in Northern Greek children and adolescents.*

Key words: *Children, Hashimoto's thyroiditis, HLA-DRB1 / HLA-DQB1 alleles.*

Introduction

Hashimoto's thyroiditis (HT) is referred to be associated with the major histocompatibility complex (MHC). Association studies of human leucocyte antigens (HLA) with HT have revealed inconsistent results(1). Several studies associated HT with HLA-DR3(2-4) and HLA-DR4(4). Badenhoop, *et al*.(5) found association with HLA-DR4, HLA-DR5, HLA-DQw3 and HLA-DQw7 in Caucasians from Canada and England. HT has been also associated with HLA-DQw2 (2) and HLA-DQB1*0301(6, 7). However, other studies failed to demonstrate an association between HT and HLA-DRB1 and HLA-DQB1(4,8-10). All the above mentioned studies concern adults. Data on HT in children and adolescents are rare(11,12). In this study we analyzed the distribution of HLA-DRB1 and HLA-DQB1 alleles in Northern Greek children and adolescents with HT. This is the first study of its kind in this specific ethnic and age group.

Methods

We studied 17 non-related Northern Greek children and adolescents with hypothyroidism due to HT. The diagnosis of HT was based on the following criteria: goiter, subclinical or clinical hypothyroidism (thyroid stimulating hormone, TSH >5μIU/mL), response to treatment with L-thyroxine and elevated titers of anti-thyroid peroxidase (anti-TPO >35 IU/mL) and/or anti-thyroglobulin autoantibodies (anti-TG >40 IU/mL)(1). Demographic and clinical data were recorded. The control group consisted of 181 randomly chosen, Northern Greek, healthy, euthyroid individuals with negative antithyroid antibodies and no family history of thyroid or other auto-immune diseases(13). Patients, their parents and controls provided informed consent. The study was approved by the ethical committee of Medical School of Aristotle University of Thessaloniki.

Serum free thyroid hormones (fT_3, fT_4), TSH, anti-TPO and anti-TG were determined by

chemiluminescent immunoassay system (Immulite® 2000, Diagnostic Products Corporation, Los Angeles, CA, USA). DNA-obtained from peripheral blood sample was extracted using the Pel Freez DNA isolation kit (Dynal Biotech, USA). HLA-DRB1 and HLA-DQB1 alleles were genotyped using polymerase chain reaction and sequence specific primers (PCR-SSP)(14) and specifically the median-resolution PCR-SSP system PROTRANS (GmbH, Germany) and Pel Freez (Dynal Biotech, USA).

Data were analyzed using SPSS for Windows (version 12.0). Differences of frequencies for HLA alleles between patients and controls and between the two patient groups were tested by the chi-square test. The relative risk (RR) was evaluated for HLA alleles differing significantly and calculated with Woolf's formula. Statistical significance was defined at $P<0.05$.

RESULTS

Clinical characteristics and biochemical measurements of patients are shown in ***Table* I**. The distribution of HLA-DRB1 and HLA-DQB1 alleles in patients and controls is shown in ***Table* II**. HLA-DRB1*16 was more frequent in patients (7/17, 41.2%) than in controls (35/181, 19.3%) but the difference was not statistical significant. HLA-DQB1 alleles did not differ significantly between patients and controls. Patients were divided into two groups according to age at the time of diagnosis. Group A included patients whose age at diagnosis was ≤10 years and Group B patients whose age at diagnosis was >10 years. The distribution of HLA-DRB1 and HLA-DQB1 alleles in Group A and B is shown in ***Table* II**. HLA-DRB1 alleles were not significantly different between the two groups. HLA-DQB1*05 was more frequent in Group B (7/8, 87.5%) than in Group A (3/9, 33.3%) ($P<0.05$).

DISCUSSION

Association studies of HLA-DRB1 or HLA-DQB1 alleles with HT in children and adolescents are rare. A significant association of HLA-DRB1*1404 and HLA-DRB1*0301 was found in Indian and Italian

TABLE I CLINICAL CHARACTERISTICS AND BIOCHEMICAL MEASUREMENTS

Patient	Gender	Age* (years)	Age** (years)	Tanner stage	Family history	Anti-TPO (IU/mL)	Anti-TG (IU/mL)
1	F	13.2	11.5	I	yes	1000	70
2	F	11.4	5.8	I	yes	1000	76
3	F	14.5	3.8	I	yes	1000	781
4	F	14.4	14.4	IV	no	53	41
5	M	13.8	13.8	III	yes	69	negative
6	F	8.6	7.6	II	yes	80	3000
7	F	16.4	12.2	I	yes	1000	negative
8	M	12.9	11.6	I	unknown	975	46
9	F	9.7	9.7	I	no	1000	3000
10	M	10.5	9.9	I	yes	1000	negative
11	F	17.0	8.5	I	yes	424	225
12	F	14.8	10.5	III	yes	668	negative
13	F	12.9	11.7	III	no	1000	44
14	M	10.1	10.1	I	yes	1000	361
15	F	13.8	9.5	I	yes	1000	81
16	F	5.6	4.9	I	yes	negative	59
17	F	10.4	9.2	II	yes	1000	negative

* *Age at the time of participation in the study;* ** *Age at the time of diagnosis.*

TABLE II DISTRIBUTION OF HLA-DRB1 AND HLA-DQB1 ALLELES IN PATIENTS AND CONTROLS AND IN PATIENTS ACCORDING TO AGE AT THE TIME OF DIAGNOSIS

Allele	Patients (*N*=17)		Controls (*N*=181)		*P*value	Group A (*N*=9)		Group B (*N*=8)		*P* value
HLA-DRB1	N	PF (%)	N	PF (%)		N	PF (%)	N	PF (%)	
*01	2	11.8	18	9.9	NS	0	0	2	25.0	NS
*03	3	17.6	24	13.3	NS	2	22.2	1	12.5	NS
*04	5	29.4	38	21.0	NS	2	22.2	3	37.5	NS
*11	9	52.9	83	45.9	NS	6	66.7	3	37.5	NS
*14	2	11.8	26	14.4	NS	0	0	2	25.0	NS
*15	3	17.6	28	15.5	NS	2	22.2	1	12.5	NS
*16	7	41.2	35	19.3	0.057[1]	3	33.3	4	50.0	NS
HLA-DQB1										
*02	3	17.6	48	26.5	NS	2	22.2	1	12.5	NS
*03	11	64.7	140	77.3	NS	6	66.7	5	62.5	NS
*05	10	58.8	75	41.4	NS	3	33.3	7	87.5	0.050[2]
*06	3	17.6	46	25.4	NS	2	22.2	1	12.5	NS

PF phenotype frequency; NS non significant; Group A patients with age at the time of diagnosis ≤10 years; Group B patients with age at the time of diagnosis >10 years.

patients with juvenile autoimmune thyroiditis, respectively(11,12).

Genotyping of HLA-DRB1 and HLA-DQB1 alleles revealed no significant difference between patients and controls. Several studies have also failed to demonstrate an association between HT and an HLA-DRB1(4,8,9,) or an HLA-DQB1 allele(4,9, 10). Our findings may have been influenced by the small number of patients in this study. However, HLA-DRB1*16 was more frequent in patients than in controls although the difference did not reach statistical significance (*P*=0.06). Furthermore, the RR of patients positive for HLA-DRB1*16 was calculated to be 2.92, comparable to that found for alleles in studies including HT patients of different ethnic groups(2, 3, 5). Association of HT with HLA-DRB1*16 has not been mentioned before. HLA-DRB1*16 may predispose to earlier onset of HT.

To investigate the combined influence of HLA-DRB1 and HLA-DQB1 alleles on puberty, our patients were divided into two groups according to age at the time of diagnosis. The age limit of 10 years was chosen on the basis of the fact that the mean age at onset of puberty in Greek girls is 10.6 years(15) and normal sexual maturity occurs earlier in girls than in boys. As a result most patients of group A would be prepubertal and most patients of group B would be pubertal. HLA-DQB1*05 was found significantly increased in patients aged >10 years at diagnosis as compared to those with age at diagnosis ≤10 years (*P*=0.050). It is 14 times more probable for children positive for HLA-DQB1*05 to develop HT after the age of 10 years. It is possible that sexual maturity in combination with HLA-DQB1*05 may predispose to earlier onset of HT.

The results of this study concern children and adolescents and combine HLA-DRB1 and HLA-DQB1 alleles with age at onset of HT. The failure to detect significant association between HT and HLA-DRB1 as well as HLA-DQB1 alleles in children and adolescents of Northern Greek origin affected by HT is in agreement with several studies on other ethnic groups. However, the detection of a weak association between HT and HLA-DRB1*16 and the significantly increased frequency of HLA-DQB1*05 in patients aged >10 years at the time of HT diagnosis need further evaluation.

WHAT THIS STUDY ADDS?

- Sexual maturity in combination with HLA-DQB1*05 may predispose to the onset of Hashimoto's thyroiditis in children and adolescents of Northern Greek origin.

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