J. Endocrinol. Invest. *24:* 88-91, 2001

HLA DQA1*0501 and DRB1*0301 antigens do not independently convey susceptibility to Graves' disease

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ABSTRACT. Genes of, or closely associated to, the HLA complex are assumed to contribute to the genetic predisposition of Graves' disease. The aim of this study was to investigate the presence of the HLA DQA1*0501 and DRB1*0301 antigens in Greek patients with Graves' disease. In addition, we tried to establish if there is any association between these antigens and any of the clinical manifestations of the disease. We examined 117 patients with Graves' disease and 104 healthy controls. DNA was extracted from peripheral lymphocytes and the HLA DQA1*0501 and DRB1*0301 genomic regions were amplified by PCR and characterized by hybridization with sequence specific oligonucleotides (SSO). Two of the patients had a positive family history for Graves' disease and 46 had clinical thyroid eye disease (TED). The frequencies of both DQA1*0501 and DRB1*0301 antigens were significantly increased in patients compared to controls (relative risk [RR] 4.2 and 4.5 for each antigen respectively). Neither of these two antigens was an independent risk factor for Graves' disease. However, the combination of both these HLA antigens resulted in a striking increase in the RR for development of Graves' disease especially in females (RR/F=27, RR/M=8.4). No association was found between these antigens and positive family history or the presence of TED. These data suggest that HLA DQA1*0501 and DRB1*0301 antigens are not independent risk factors for the development of Graves' disease. On the contrary, the presence of both these alleles results in a significant increase in the RR for the development of Graves' disease in the Greek population, particularly in females.
(J. Endocrinol. Invest. *24:* 88-91, 2001)

INTRODUCTION

Graves' disease is common in the general population; the frequency of the disease is 2.7% in females and 0.3% in males (1). A significant proportion of patients have a positive family history of the disease. Its pathogenesis remains largely unknown, however it is believed to be a multifactorial disease, since a combination of environmental and genetic factors appear to contribute to its manifestation. It has long been recognized that both Graves' disease as well as thyroid eye disease (TED) are associated with specific HLA antigens. HLA-DR3 and its polymorphisms have been consistently associated with the disease in Caucasians (2-4). More recently, an association with the antigen DQA1*0501 has been reported which is probably in linkage with DR3 (5). However, there are conflicting results in the literature as to whether this represents an independent risk factor or even more whether it is associated with the sex of patients (6-8). The aim of the present study was to examine the frequency of DQA1*0501 and of one of the polymorphisms of the previously known as DR3 antigen (DRB1*0301) in Greek patients with Graves' disease. We also examined if the DQA1*0501 is an independent risk factor for the disease and whether it is associated with gender, TED and positive family history for the disease.

MATERIALS AND METHODS

A total of 117 patients with Graves' disease were examined (93 F and 24 M). Diagnosis was based on the presence of hyperthyroid syndrome associated with elevated T3 and T4 levels and suppressed TSH levels. One of the following criteria was used to confirm the autoimmune nature of the hyperthyroidism: diffuse thyroid enlargement with diffuse radionucleotide up-

Key-words: Graves' disease, HLA, autoimmunity, thyroid eye disease.

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Accepted 24 July, 2000.

Table 1 - *HLA DQA1*0501 and DRBI*0301 antigens in Graves' disease patients and controls.*

	Graves' disease		Normal controls				
	N	DQAI*0501 (+)	N	DQAI*0501 (+)	X^2	*p*	RR
Male	24	19 (79)	68	30 (44)	8.7	<0.01	4.8
Female	93	74 (80)	36	20 (55)	7.6	<0.01	3.1
Total	117	93 (79.5)	104	50 (48.1)	23.8	<0.001	4.2
	DRB1*0301 (+)		DRBI*0301 (+)				
Male	24	15 (62)	68	24 (35)	5.3	<0.05	3.1
Female	93	63 (68)	36	8 (22)	21.7	<0.001	7.3
Total	117	78 (67)	104	32 (30)	28.4	<0.001	4.5

Percentage in parenthesis. RR: relative risk; (+) positive.

take in the thyroid scan, presence of TED, or elevated thyrotropin receptor antibodies (TRAb). The TRAb antibodies were determined by a radioreceptor assay (Biocode biotechnology, Belgium). Our results were compared to those in 104 random healthy blood donors (36 F/68 M) who did not have a positive family history for autoimmune thyroiditis and did not have antithyroid autoantibodies in their serum.

Genomic DNA was extracted from peripheral lymphocytes and the second exon of the DQA1*0501 gene was amplified by PCR using the sequence specific primers F: GGTGTAAACTTGTACCAG and R: GGTAGCAGCGGTAGAGTTG (5). The following PCR conditions were used, 30 cycles of 1 min denaturation at 94 C, 1 min annealing at 58 C and 1 min extension at 72 C. One microlitre of PCR reaction was dot-blotted on nylon membranes and was hybridized to the oligonucleotide probe TCAGACAATTTAGATTTG (9). Hybridization conditions were according to Yanagawa *et al.*, 1993. For DRB1*0301 genotyping the second exon was amplified by PCR using the following primers F: CCCCACAGCACGTTTCTTG and R: CCGCTGCACTGTGAAGCTCT. The PCR conditions were the same as described above for DQA1* 0501. The probe used for hybridization was GGCCGGGTGGACAACTAC (5). Results were obtained after an overnight exposure to autoradiography film.

The results were analyzed using 2x2 contingency tables and the Fisher exact test for calculating the chi-square values and odds ratio and the corresponding confidence intervals (C.I.) for evaluation of the relative risk (RR).

RESULTS

Twenty-two patients had a positive family history of Graves' disease and 46 patients had signs of TED (class II-IV, Werner classification) (10). HLA DQA1* 0501 antigen was detected in 79.5% of patients *vs* 48.1% of controls (Table 1) (X^2=23.8, p<0.001, RR 4.2). The presence of the antigen was significantly increased both in male and female patients, the RR being higher in males (Table 1). An increased frequency in both male and female patients was also found for the HLA DRB1*0301 antigen (67% in patients *vs* 30% in controls) (X^2=28.4, p<0.001, RR 4.5) (Table 1). Neither of the two antigens was able to confer susceptibility to Graves' disease in the absence of the other, *i.e.* as an independent risk factor (Table 2). On the contrary, when both of these antigens were present, the relative risk was even higher (13.8). This

Table 2 - *Association of the presence of one of the two HLA haplotypes in the absence of the other with the development of Graves' disease.*

	Graves'	Controls	X^2	*p*
DQA1*0501 (+) DRB1*0301 (-)	25	38	1.3	NS
DQA1*0501 (-) DRB1*0301 (-)	14	34		
DRB1*0301 (+) DQA1*0501 (-)	10	20	0.1	NS
DQA1*0501 (-) DRB1*0301 (-)	14	34		

NS: not significant; (+) positive; (-) negative.

Table 3 - *Association of the presence of both DRB1*0301 and DQAI*0501 HLA antigens with the development of Graves' disease.*

	Graves'		Controls				
	DQA1*0501 (+) DRB1*0301 (+)	DQA1*0501 (-) DRB1*0301 (-)	DQA1*0501 (+) DRB1*0301 (+)	DQA1*0501 (-) DRB1*0301 (-)	X^2	*p*	RR
Male	14 (58)	4	10 (42)	24	11.1	<0.001	8.4
Female	54 (96)	10	2 (4)	10	24.0	<0.001	27.0
Total	68 (85)	14	12 (15)	34	40.6	<0.001	13.8

Percentage in parenthesis. RR: relative risk; (+) positive; (-) negative.

was particularly evident in female patients (RR=27 for females and 8.4 for males) (Table 3).
No associations were found between these two HLA polymorphisms and either TED or a family history of Graves' disease (Table 4).

DISCUSSION

This study in a Greek population confirms the association of HLA DRB1*0301, previously known as DR3, as well as of DQA1*0501 with Graves' disease. These associations have previously been observed in other populations as well (8, 11, 12). We also found, as previously reported, that the presence of the DRB1*0301 antigen confers a higher RR in women (2, 4, 11). In addition, we found that the presence of the DQA1*0501 antigen confers a relatively higher RR in male compared to female patients. This is in agreement with some (6) but not all of relevant previous studies (7, 8) which found a higher female RR. Our results, as well as those reported by Yanagawa *et al.*, (6) support the hypothesis that perhaps male patients, who are normally protected from autoimmune diseases, need a stronger genetic factor to manifest the disease (13, 14).
Neither of these two antigens, in the absence of the other, was able to confer susceptibility to Graves' disease. This finding is in agreement with the results of the recent study by Heward *et al.*, (15) who also found that all Graves' patients who were DR3 positive (albeit with a different polymorphism - DRB1*0304) were also DQA1*0501 positive. Interestingly, similar results can be obtained after analyzing the data in the study of Cuddihy *et al.*, (7) where again there was no patient who was DR positive but DQ negative. These results show the close linkage between these antigens in agreement with what has previously been suggested (16).
We found no association of these antigens with the presence of a family history of Graves' disease which suggests that the loci responsible for the predisposition to familial disease may be multiple and heterogeneous (16). Furthermore, there was no association of these two polymorphisms with the presence of TED, which suggests that other loci may be linked to the extrathyroidal manifestations of Graves' disease (17, 18).
An interesting result of our study was that the presence of both these antigens, rather than each individually, greatly increased the relative risk to develop Graves' disease, which in the female population was as high as 27, indicating that these two loci are probably part of a haplotype which does predispose to Graves' disease.
In recent years the genetic component is believed to be less important for the development of Graves' disease than previously considered (19). This notion is further supported by the recent observation that the discordance rate for the disease in monozygotic twins is higher than expected (20). Nevertheless, our results indicate that if the genetic component is of some importance for the development of Graves' disease, then the combination of the HLA DRB1*0304 and DQA1*0501 loci may be part of a combined haplotype which is important for the

Table 4 - *HLA DQAI*0501 and DRB1*0301 in Graves' disease according to family history and the presence of TED (FH=family history, TED=thyroid eye disease).*

	DQA1*0501 (+)	DQA1*0501 (-)	*p*	DRB1*0301 (+)	DRB1*0301 (-)	*p*
FH (+)	16 (85)	6 (15)	NS	13 (59)	9 (41)	NS
FH (-)	51 (84)	10 (16)		42 (69)	19 (31)	
TED (+)	39 (85)	7 (15)	NS	34 (74)	12 (26)	NS
TED (-)	39 (75)	13 (25)		31 (60)	21 (40)	

Percentage in parenthesis. (+) positive; (-) negative; NS: not significant.

development of this disease, possibly through linkage with an unknown locus.
In summary, this study demonstrates that the presence of neither of the DRB1*0304 and DQA1*0501 antigens are independent risk factors for Graves' disease. However, when both are present they may be of importance for the development of Graves' disease, probably as part of a combined haplotype, in some way predisposing to this autoimmune disease.

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