

HLA-DR association in papillary thyroid carcinoma

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Abstract. *Objective:* Papillary thyroid carcinoma (PTC) is the most frequent types of thyroid malignancies. Several genes may be involved in susceptibility of thyroid cancer including Human Leukocyte Antigens (HLA). The association of thyroid carcinoma with HLA alleles has been previously studied in other populations and certain HLA alleles were shown to be either predisposing or protective. The aim of this study was to determine the association between HLA-DR and papillary thyroid carcinoma in an Iranian population.

Design: HLA-DR antigen frequencies were determined in patients with papillary thyroid carcinoma (N = 70) and non-related healthy controls (N = 180) using PCR -SSP.

Main Outcome: We found that HLA-DRB1*04 frequency was significantly higher in our patients compared to the controls [$P = 0.02$, OR; 1.9, 95% CI (1.04–3.57)].

Conclusions: Our results revealed HLA-DRB1*04 as predisposing factor in papillary thyroid carcinoma in Iranian population. This confirms the previous findings for associations between HLA-DRB1 and differentiated carcinomas in other populations.

Keywords: Genetics, HLA, Papillary carcinoma

1. Introduction

Thyroid carcinoma is the most common malignancy of the endocrine system and papillary thyroid carcinoma (PTC) is the most frequent type (80%) of thyroid cancer [1]. Epidemiologic data indicates a 4–10 fold increased risk of disease in the first generation relatives of papillary cancer patients [2]. The possibility of familial predisposition to papillary cancer has been estimated as 5% and pattern of inheritance is suggested to occur in autosomal dominant manner with partial pen-

etrance. However the genetic factors responsible have not been identified yet [3].

Frequent coalition of autoimmune thyroid diseases (AITD) such as Graves' disease and lymphocytic thyroiditis (LT) with papillary thyroid carcinoma is observed and has been suggested to influence the prognosis of such tumors [4].

It is speculated that the contribution of human leukocyte antigen (HLA) molecules in immunological recognition of tumor cells is responsible for both tumor development and immunity [5].

Our knowledge about the molecular basis of thyroid carcinoma is rapidly increasing. Therefore an approach for better understanding of the mechanism of immune system response in thyroid carcinoma may be helpful in finding better strategies and new treatment options [6].

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The association of the human lymphocyte antigen (HLA) system with papillary thyroid carcinoma is controversial. Several reports support the association between different alleles of HLA and thyroid carcinoma in different populations [7–17]. However different results have also been reported [12,14,15]. Most studies on HLA Class II molecules antigens, have shown association with well differentiated thyroid carcinoma [7,9,11,13,16].

We have recently reported an association between HLACw*4 and HLACw*15 frequencies and papillary thyroid carcinoma in our patients [8]. An association between HLA-DR8-DQ4 haplotype and papillary thyroid carcinoma in Portugal has been reported previously [4].

The aim of the current study was to determine the association between HLA-DR frequencies and papillary thyroid carcinoma susceptibility in an Iranian population.

2. Material and methods

2.1. Patients' population

Cases group consisted of (N = 70) patients underwent surgery for differentiated thyroid carcinoma in Shariati Hospital and Amir-Aalam Hospital, Tehran, Iran. In histological study the patients were diagnosed as having papillary carcinomas which based on the nuclear feature of the cells on pathological examination all were sub-classified as classic papillary carcinoma. The mean age of patients was 44 ± 13 years, and females accounted for 76% and males 23% of the study population. Controls were comprised of (N = 180) unrelated healthy people. Controls were from the same area as cases and were age and sex matched. The study was approved by the Ethics committee of Tehran University. Informed consents were obtained from all of the patients attending the study.

3. HLA-DR molecular typing

DNA from cases and controls were extracted from anti-coagulated blood collected in EDTA using salting out method. HLA-DR typing was performed using Dynal AllSet^{+TM} SSP low resolution kit.

3.1. Statistical Analysis

HLA-DR frequencies were estimated by direct counts. To compare the differences between the allele frequencies in the control and carcinoma groups, a 2*2 contingency table analysis was performed using the Pearson chi-square tests, with Fisher exact test when the expected value for an HLA marker was < 0.05 .

The strength of association between HLA-DR and papillary thyroid carcinoma was estimated by odds ratios (OR) and 95% confidence intervals (95% CI) using the STATA 8 program. The *P* values were corrected by multiplying the number of alleles tested.

Only $P < 0.05$ was considered to be statistically significant.

PyPop (Python for Population genetics, <http://www.pypop.org>) was used for pairwise LD and estimated haplotype frequencies analyses between HLA-DR and HLA-Cw loci [19]. Two locus haplotype frequencies were estimated using the interactive expectation maximization (EM) algorithm [20,21]. Two measures of overall linkage disequilibrium are calculated. *D'* [22] weights the contribution to LD of specific allele pairs by the product of their allele frequencies and *W_n* [23] is a re-expression of the chi-square statistic for deviations between observed and expected haplotype frequencies. Both measures are normalized to lie between zero and one. For each locus pair the log-likelihood of obtaining the observed data given the inferred haplotype frequencies [$\ln(L_1)$], and the likelihood of the data under the null hypothesis of linkage equilibrium [$\ln(L_0)$] are given. The statistic *S* is defined as twice the difference between these likelihoods. The *p*-value is the fraction of permutations that results in values of *S* greater or equal to that observed. A *p*-value < 0.05 is indicative of overall significant LD.

4. Results

4.1. HLA-DR antigen frequencies in PTC patients

HLA-DR frequencies in PTC patients and controls are shown in Table 1. When we compared HLA-DR antigen frequencies in PTC patients with controls we observed that HLA-DRB1*04 was significantly increased in PTC patients compared to the controls group [*p* 0.02, OR: 1.9, 95%CI (1.04–3.57)]. There were no significant differences for other HLA-DR antigen frequencies between PTC patients and controls.

Table 1
HLA-DRB1 antigen frequencies in PTC patients and controls

HLA-DR	Controls (N = 180)	PTC (N = 70)
01	24 (6.6%)	9 (6.9%)
15 or 16	63 (17.5%)	22 (15.7%)
03	32 (8.8%)	11 (7.8%)
04	33 (9.2%)*	23 (16.4%)*
11 or 12	92 (25.5%)	31 (22.1%)
13	35 (9.7%)	14 (10%)
14	18 (5%)	6 (4.2%)
07	36 (10%)	17 (12%)
08	8 (2.2%)	4 (2.8%)
09	7 (1.9%)	1 (0.7%)
10	12 (3.3%)	2 (1.4%)
DRB3	177 (49.1%)	68 (48.5%)
DRB4	76 (21.1%)	38 (27.1%)
DRB5	50 (13.8%)	29 (20.7%)

HLA-DRB1*04 allele frequency was significantly increased in PTC patients vs controls, $P_{(uncorrected)}$ 0.02, OR: 1.9, 95%CI (1.04–3.57).

4.2. HLA-DR and HLA-Cw pairwise LD and haplotype analyses

Among 79 HLA-DR:HLA-Cw haplotypes predicted by the EM algorithm 13 haplotypes were found in three or more copies (Table 2). Pairwise LD measures for the strength of association was statistically significant between two loci $p = 0.0000$ (Table 3).

5. Discussion

Genetic factors have been implicated in susceptibility of several types of cancers [4]. The immune system has a complex effect on tumors behavior, limiting its progression or sometimes inducing further tumor growth [6]. In this study we have found an association between HLA-DRB1*04 and papillary thyroid carcinoma in an Iranian population. The difference for association between HLA-DRB1 found in this study with previous association reported on HLA-DQ4-DR8 and PTC in Portuguese population might be due to difference in populations however this is confirming a role for HLA-DRB1 in PTC in various populations [4].

Earlier studies in which serological typing for finding associations between HLA antigens and thyroid carcinoma were performed are also indicating the role of HLA antigens in thyroid carcinoma susceptibility. A highly significant association between HLA-DR1 and thyroid carcinoma in an Italian population has been reported [9]. Association between HLA-DR1 and papil-

Table 2
HLA two-locus haplotypes in 70 individuals with PTC

DR:C Haplotype	Frequency	copies
11:12	0.09357	9.9
07:06	0.08491	9.0
11:07	0.08491	9.0
13:04	0.04640	4.9
04:03	0.03774	4.0
04:15	0.03774	4.0
04:12	0.03774	4.0
15:12	0.02830	3.0
03:15	0.02830	3.0
15:02	0.02830	3.0
16:04	0.02830	3.0
04:16	0.02830	3.0
01:07	0.02830	3.0
13:12	0.01964	2.1
11:04	0.01964	2.1
04:02	0.01887	2.0
04:07	0.01887	2.0
01:06	0.01887	2.0
15:16	0.01887	2.0
07:12	0.01887	2.0
03:03	0.01887	2.0
12:15	0.01887	2.0
08:16	0.01887	2.0
14:06	0.01887	2.0
14:01	0.01887	2.0
07:02	0.01887	2.0
08:07	0.00943	1.0
11:14	0.00943	1.0
13:06	0.00943	1.0
13:01	0.00943	1.0
12:01	0.00943	1.0
07:08	0.00943	1.0
10:15	0.00943	1.0
11:15	0.00943	1.0
15:08	0.00943	1.0
15:05	0.00943	1.0
14:08	0.00943	1.0
15:04	0.00943	1.0
01:02	0.00943	1.0
16:15	0.00943	1.0
15:17	0.00943	1.0
08:14	0.00943	1.0
08:01	0.00943	1.0

lary thyroid carcinoma has also been reported in Hungarian population [6]. In another study in Spanish population an association has been reported between HLA-DR11 and papillary thyroid carcinoma [6]. The discrepancies in the previous findings on different HLA-DR associations and thyroid carcinoma are probably due the ethnic or geographic differences or in some cases might be due to the small sample size studied [15, 17]. Also the results obtained in our study must be confirmed on a population which cases and controls are fully age and sex matched.

Table 3
Pairwise global linkage disequilibrium (LD) estimates

Locus pair	D'	Wn	ln(L ₋₁)	ln(L ₋₀)	S	# permu	p-value
DRB1:C	0.67030	0.47599	-335.92	-421.35	170.86	999	0.0000*

D' values above 0 show a positive association between loci, and values above 0.5 indicate a very strong association. Wn is an alternative measurement of LD.

Studies on HLA-DR expression and lymphocyte infiltrations in papillary thyroid carcinoma imply the presence of an active immune response with increased HLA-DR expression and involvement of specific types of T cells in PTC etiopathogenesis [24].

The existence of an HLA-class II association with thyroid carcinoma has implications for our understanding of disease etiopathogenesis. It can be proposed that an association with class II molecule, such as HLA-DRB1*04 is due to its contribution to disease susceptibility by its role in presenting antigenic peptides derived from exogenous proteins to specific T cell receptors on CD4 positive cells. The HLA-DRB1*04 allele could exert disease susceptibility by exhibiting a particularly high affinity for an antigen involved in PTC. In addition the frequent co-presence of AITD with papillary thyroid carcinoma suggests the contribution of immune response in pathogenesis of PTC. The association between HLA-DR alleles and AITD in Iranian population is not clear yet. Weak association of some types of AITD and HLA-DR4 also has been reported in Caucasians population [25] which is in line with our finding. Recent studies also have found associations between HLA-C and Graves' disease, with HLA-C * 07 as predisposing and HLA-C * 03 and HLA-C*16 as protective alleles [26]. We have previously reported an association between HLACw*4 and HLACw*15 allele [18] and papillary thyroid carcinoma in our patients. In this study we have found that the Linkage disequilibrium is strong between HLA-DR and HLA-C loci in our population and HLA-DRB1*04 and HLA-Cw15 were predicted to form a haplotype in our population. Therefore the results observed in this study for association between HLA-DRB1*04 and PTC may be due to linkage disequilibrium between HLA-DRB1*04 and HLA-Cw alleles and HLA-DRB1*04 allele might be just a marker for a PTC susceptibility gene. Additional studies are required to further confirm the association of PTC susceptibility alleles encoded in the HLA region and confirm which locus is primarily associated with disease susceptibility.

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