RESEARCH ARTICLE

# Negative Association of the HLA-DQB1*02 Allele with Breast Cancer Development among Jordanians

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## Abstract

**Background: In the literature, data concerning the relationship between breast cancer and HLA class II gene polymorphisms are limited, so the aim of this study was to determine if HLA-DQB1 and HLA-DRB1 MHC class-II alleles may confer susceptibility or resistance to the disease among Jordanian females. Materials and Methods: This case control study enrolled 56 Royal Hospital breast cancer patients and 60 age matched healthy controls, all of whom provided blood samples (2011-2013). A questionnaire was filled after signing a consent form and DNA was extracted, nucleic acids being amplified for assessment of HLA-DQB1 and HLA-DRB1 alleles by muliplex INNO-LiPA and allele typing carried out by reverse hybridization. Comparison of HLA-DQB1 and HLA-DRB1 allele distributions was carried out with paired t-test and chi-square statistics. Risk factors were assessed by odd ratios with 95% confidence intervals. Results: A significant negative correlation was observed between HLADQB1* 02 alleles and breast cancers (p=0.013). No significant associations were observed among HLADQB1* 03, 04, 05 and 06 or among HLA-DRB1* 01, 03, 04, 07, 08, 10, 11, 13, 14 and 15. Conclusions: HLADQB1* 02 alleles may provide positive protection against breast tumor risk among Jordanians, but not HLADQB1* 03, 04, 05 and 06 or HLA-DRB1* 01, 03, 04, 07, 08, 10, 11, 13, 14 and 15 alleles.**

**Keywords:** HLA-DQB1 - HLA-DRB1 - alleles - breast cancer - susceptibility

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## Introduction

Cancer becomes one of the most wide-spreading diseases nowadays (Atoum et al., 2010), breast cancer is the most common female malignancy worldwide, and the second leading cause of cancer mortality among women (Downs-Holmes and Silverman, 2011) accounting for 23% of all cancers (Liang et al., 2008) with more than one million new patients are diagnosed annually (Watanabe et al., 2010). Globally it accounts for 18% of all female cancers (Cantu De Leon et al., 2009). In Jordan, breast cancer is the most common malignancy afflicts women, accounting for 36.7% of all female cancers, and is the leading cause of cancer deaths among Jordanian women (www.khcc.jo/National_cancer_statistics.aspx, 2012). Age, hormonal disturbances, genetics, environmental factors, infectious agents and even MHC system could play a role in breast cancer development (Mahmoodi et al., 2012).

HLA class II (MHC class II) is a highly variable genetic system (Chaudhuri et al., 2000) that spans 800kb of DNA (Ragoussis et al., 1989). It comprises the classical class II genes (HLA-DP, -DQ, -DR and pseudogenes) and the non-classical class II genes (HLA-DM and -DO). The classical class II genes and the non-classical class II genes located on the short arm of chromosome 6 p21.3 (Levine et al., 1985). DRB1 and DQB1 loci are separated by 85kb on the chromosome (Harrath et al., 2006), and exhibit high levels of linkage disequilibrium since no recombination have been reported between them (Huang et al., 1995).

HLA class II alleles play an important role in immunity (Chen et al., 2007) and tumor surveillance since the function of the products of the highly polymorphic MHC loci is to present peptide fragments including tumor antigens to the cells of immune system. MHC class II regulate the immune response against tumors (Redondo et al., 2003), by guiding the activation of CD4+ T helper lymphocytes (Alfonso et al., 2000; Scanlan et al., 2001; Goa et al., 2003; Horton et al., 2004). MHC class II (Gun et al., 2012) allelic variation affects T-lymphocyte toxicity and humeral immunity against tumors, and these variation may involved in cancer predisposition such as malignant melanoma (Zaloudik et al., 1988), gastric (Lee et al., 1996), ovarian (Kubler et al., 2006), (Jiang et al., 2013), colorectal cancer (Wolkersdorfer et al., 2011) and breast cancer (Yang et al., 2011).

In literature, the data bout the relationship between breast cancer and HLA class II gene polymorphism is limited, so molecular analysis of HLA DQB1 and DRB1 alleles among breast cancer females compared to

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ethnically matched controls might provide information on the potential existence of alleles that could confer susceptibility or resistance to this breast cancer.

## Materials and Methods

This case control study investigates the genetic association between some HLA class II alleles and breast cancer among Jordanian females, by comparing genotype and allele frequencies between breast cancer patients and control. Ethical approval was received from the Institutional Review Board Committee at the Hashemite University and from the ethical approval committee at the Royal Medical Service.

Fifty six Jordanian females diagnosed with breast cancer and sixty randomly selected age matched volunteers without any family history of breast cancer were enrolled in this study, from Al-Hussein Medical City- Royal Medical Services at the breast cancer clinic (2011-2013). Diagnosis was carried out by specialized pathologist according to clinical signs, mammography, fine needle aspiration and histopathological findings.

Data including age, clinical and family history of breast cancer were filled after participant signed a consent form and answered the questionnaire about age, family history and diagnosis. Blood samples were collected from subjects using Ethylene-di-amine-tetra-acetic acid (EDTA) anticoagulant tubes (AFCO, Jordan) and DNA was extracted using Wizard® Genomic DNA Purification kit (Promega Corporation, USA). The second and the third exons of the HLA-DQB1 locus and the second exon of the HLA-DRB1 locus were amplified according to the manufacturer's recommendation using HLA-DQB1 and HLA-DRB1 INNO-LiPA multiplex kit (Innogenetics, Fujirebio Group, Belgium) for nucleic acid amplification. The amplification reaction carried out inside the iCycler 96×0.2ml reaction Module, BioRad (Bio-Rad Laboratories, USA) after preheating the blocks and before samples insertion, at 96.3°C. The cycler then programmed for denaturation, primer annealing, primer extension and finally elongation according to manufacturer's recommendation for HLA-DQB1 and HLA-DRB1 (Innogenetics, Fujirebio Group, Belgium).

HLA-DQB1 and HLA-DRB1 genotyping was carried out by INNO-LiPA HLA-DQB1 updated kit (Innogenetics, Fujirebio Group, Belgium) for the molecular typing of HLA-DQB1 and HLA-DRB1 alleles, based on reverse hybridization principle on membrane-based strips. According to the manufacturer's recommendations, the genotyping was carried out using the manual test procedure and after hybridization colour development steps were carried out at 20-25ºC on an orbital shaker (Edmund Bühler TiMix 2, Germany), strips then dried completely and stored in the dark before reading using LiRAS™ software for LiPA HLA.

Statistical analysis was carried out using SPSS Statistics 17.0 (IBM Corporation, USA). HLA-DQB1, -DRB1 alleles were determined by direct counting. Allelic frequency percentage was calculated as follows: allelic frequency percentage=(n/N)*100%, where n represents number of individuals carrying this allele, and N for total number of subjects. Comparison of the distribution of HLA-DQB1, -DRB1 alleles in two groups was evaluated by *Paired student t test*, and *chi-square* was used to test the relation of level of two categorical variables. Evaluation or the risk ratio was expressed by odds ratios (ORs) with 95% confidence intervals (CI). The p≤0.05 was considered to be statistically significant.

**Table 1. HLA-DQB1 and HLA-DRB1 Allele Frequencies among Breast Cancer Patients and Control**

| | Case group N=56 | Control group N=60 | OR (95%CI) | p value |
|---|---|---|---|---|
| HLA-DQB1 | | | | |
| DQ1*02 | 10 (17.9%) | 30 (50.0%) | 0.212 (0.065-0.724) | 0.013[a] |
| DQ1*03 | 20 (35.7%) | 30 (50.0%) | 0.582 (0.194-1.593) | 0.266 |
| DQ1*04 | 2 (3.6%) | 6 (10.0%) | 0.364 (0.033-3.410) | 0.356 |
| DQ1*05 | 6 (10.7%) | 18 (30.0%) | 0.271 (0.067-1.170) | 0.071 |
| DQ1*06 | 8 (14.3%) | 18 (30.0%) | 0.369 (0.104-1.449) | 0.17 |
| HLA-DRB1 | | | | |
| DR1*01 | 2 (4.2%) | 6 (10.0%) | 0.341 (0.038-4.024) | 0.441 |
| DR1*03 | 2 (4.2%) | 0 (0.0%) | Uncalculated | 0.588 |
| DR1*04 | 12 (25.0%) | 6 (10.0%) | 3.021 (0.664-13.564) | 0.234 |
| DR1*07 | 4 (8.3%) | 6 (10.0%) | 0.714 (0.125-5.339) | 0.678 |
| DR1*08 | 0 (0.0%) | 6 (10.0%) | Uncalculated | 0.41 |
| DR1*10 | 2 (4.2%) | 0 (0.0%) | Uncalculated | 0.795 |
| DR1*11 | 12 (25.0%) | 0 (0.0%) | Uncalculated | 0.056 |
| DR1*13 | 6 (12.5%) | 12 (20.0%) | 0.564 (0.127-2.573) | 0.345 |
| DR1*14 | 2 (4.2%) | 6 (10.0%) | 0.332 (0.038-4.024) | 0.456 |
| DR1*15 | 2 (4.2%) | 6 (10.0%) | 0.345 (0.038-4.024) | 0.567 |

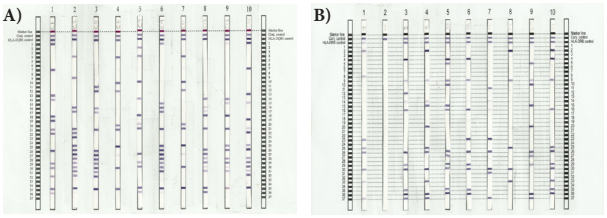


**Figure 1. A) HLA-DQB1 and B) HLA-DQR1 Dried Strips for Reading using LiRAS™ Software for LiPA HLA**

## Results

Fifty six breast cancer females and sixty controls were typed for HLA-DQB1 alleles. Prevalence of HLA-DQB1*02 allele was 17.9 % (10/56) among breast cancer whereas it was 50.0% (30/60) within the control group (Table 1). The results show statistical significant negative correlation between HLA-DQB1*02 allele and breast cancer development (p=0.013; OR=0.212; 95%CI: 0.065-0.724) (Table 1). Although HLA-DQ1*03 is the most frequent allele among both breast cancer females and control group (35.7% and 50%, respectively), it does not shows any statistically correlation with breast cancer.

Forty eight breast cancer females and sixty control were typed for HLA-DRB1 alleles. No statistical significance correlation between any of the HLA-DRB1 alleles and breast cancer development. The most frequent allele among breast cancer females was HLA-DR*04 and HLA-DR1*11 with 25% frequency for both alleles.

## Discussion

AImmunological surveillance is an important

mechanism in tumor genesis, and individuals who posses certain HLA class II alleles may be susceptible or resistant to tumor presentation (Yang et al., 2011). Inheritance of specific MHC class II genes may promote the generation of specific T cell that help the elimination of pathogens and thus, correlated with resistance to tumors, particularly those linked to viral etiologies (Chaudhuri et al., 2000). HLA class II is highly polymorphic and is directly associated with many cancers (Bustin et al., 2001; De Vreese et al., 2004; 2012) including breast cancer (Mahmoodi et al., 2012). However, HLA class II alleles differs according ethnicity (Amar et al., 1999) and the dominant pathogen among different geographical areas which affect the frequency of presentation of HLA alleles (Cantu De Leon et al., 2009).

Breast cancer ranked first among cancer in females, accounting for 36.7% of all female cancers, and is the leading cause of cancer deaths among Jordanian women (www.khcc.jo/National_cancer_statistics.aspx, 2012). The data about Jordanian HLA class II gene polymorphism is scare, in literature only one study examine the relation between HLA-DQA1*0501 and DQB1*0201 alleles in patients with coeliac disease by allele-specific DNA-based PCR-sequence-specific primer (El-Akawi et al., 2010) and non of the studies determine the association between MHC class II alleles and the risk of breast cancer among Jordanian population, so the highly variable HLA gene posses a challenge for the role of the genetic variation breast cancer tumor genesis.

HLA-DQB1 and HLA-DRB1 are the candidate genes in this study. The first association study worldwide carried out that shows the association between HLA-DQB1 and HLA-DRB and breast cancer by Chardhuri et al. (2000). They reported two significant negative associations of breast cancer development within HLA class II: DQB1*03 and DRB1*11 alleles. The results of our study also show negative significant association between HLA-DQB1*02 allele and breast cancer development (p=0.013; OR=0.212; 95%CI: 0.065-0.724). These result is consistent with previous studies; Harrath et al (2006) who revealed a negative association between HLA-DQB1*02 and breast cancer among Tunisian population. Also, Gun et al. (2012) showed a significant negative correlation between HLA-DQB1*02 alleles and breast cancer among Turkish women. The consistency with Harrath et al. (2006) and Gun et al. (2012) results may be due to a possible similarity in ethnicity between Jordanians, Tunisian and Turkish populations. Our results shows that HLA-DQB1*02 might be resistant to breast cancer development due to their drastic immune response that is more effective in presenting tumor antigens to T cells that or have special molecules in the antigen binding grooves (Gun et al., 2012), so these protective HLA-II alleles might have a potential in prophylaxis and treatment. On the other hand, poor prognosis other HLA-II alleles like HLA-DRB1*12 (Ghaderi et al., 2001) cause disturbance in the immune system defense against cancer.

Significant association between HLA-II variants and breast cancer risk was also reported among Caucasian (Chaudhuri et al., 2000), Iranian (Ghaderi et al., 2001), Tunisian (Harrath et al., 2006), Mexican (Cantu De Leonet al., 2009), Iranian ( Mahmoodi et al., 2012) and Turkish (Gun et al., 2012). Chaudhuri et al. (2000) used high-resolution PCR-sequence-specific oligonucleotide typing for HLA- DQB1 and -DRB1 alleles in 176 Caucasian women diagnosed with early-onset breast cancer and in 215 ethnically matched controls. Chaudhuri et al. (2000) reported two significant negative associations for the development of breast cancer at an early age, both of HLA class II: DQB1*03 and DRB1*11. Ghaderi et al. (2001) investigated the allele frequency of HLA-DRB1 among 36 breast cancer females from southern Iran by polymerase chain reaction using sequence specific primers. Their results indicated that the frequency of the HLA-DRB1*12 allele was significantly higher in patients with breast cancer compared to a control group and suggested that the HLA-DRB1*12 allele confers susceptibility to breast cancer among Iranians. Harrath et al. (2006) carried out a molecular typing of HLA-DRB1 and -DQB1 loci for 70 Tunisian female patients. Comparison of allele and haplotype distribution between patients and control subjects was carried out using Reverse Dot Blot (RDB) hybridization. The results revealed a significant negative association between HLADRB1*07 and HLA-DQB1*02 and the incidence of breast cancer in the Tunisian population. Cantu de Leon et al. (2009) study was enrolled 100 breast cancer Mexican mestizo patients and 99 matched healthy controls, using high resolution sequence-specific oligotyping after DNA amplification (PCR-SSOP). They found that HLA-DQB1*03 is protective allele against breast cancer. Mahmoodi et al. (2012) investigated the association between HLA class II alleles and breast cancer among Iranian women another time. One hundred patients with pathologically proven breast cancer were compared with a group of 80 healthy blood donor subjects. HLA-typing was performed using sequence-specific primer. HLA-DRB1*01, HLA-DRB1*03 and HLA-DRB1*13 alleles showed significant negative association with breast cancer risk. Gun et al. (2012) studied HLA-II polymorphism for 69 breast cancer patients and 45 healthy controls in Turkey by DNA typing for HLA-DRB1 and HLA-DQB1 using Sequence Specific Oligonucleotide Hybridization, significant negative correlations were observed between HLA-DRB1*03 and HLA-DQB1*02 alleles and breast cancer. Furthermore, there was a significant positive correlation between HLA-DRB1*13 and HLA-DQB1*06 alleles and PR positivity and a significant negative correlation between HLA-DQB1*03 allele and PR positivity among breast cancer females.

In contrast to other studies carried out among Taiwan (Chen et al., 2007) and Chinese (Yang et al., 2011). Chen et al. (2007) used PCR-SSP typing for HLA-DQB1 locus in order to compare the allele frequencies between breast cancer patients and healthy controls in southern Taiwan. They showed that HLA-DQB1*05 had higher frequencies but not statistically significant among control compared to the patient. Yang et al. (2011) also reported no significant association between HLA class II variants and breast cancer among a Han Chinese population. They studied sixteen HLA class II variants by the Sequenom MassArray® iPLEX System among 216 breast cancer

patients and 216 healthy controls. The inconsistency of these results could be explained by differences among different geographical zones. These variations are due the presentation of bacterial and viral antigens to T cells, which in turn triggers the necessary immune response to the pathogens (Lavado et al., 2005). Thus, the frequency of the HLA alleles is determined by the dominant pathogens in each particular region. This explains why alleles which are associated with susceptibility or protection for a disease in one geographical region do not confer the same susceptibility or protection in another region

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