RESEARCH COMMUNICATION

HLA Class II Variants in Chinese Breast Cancer Patients

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Abstract

Alterations of human leukocyte antigen (HLA) class II molecules are relevant to the development of breast cancer and metastatic progression. However, the role of HLA class II polymorphisms in the pathogenesis and progression of breast cancer is unclear. This study aimed to investigate the association between HLA class II variants and breast cancer susceptibility and prognosis in a Chinese population. Sixteen variants in HLA class II were detected with the Sequenom MassArray® iPLEX System in 216 breast cancer patients and 216 healthy controls. An association analysis based on unconditional logistic regression was carried out to determine the odds ratio (OR) and 95% confidence interval (95% CI) for each SNP. Stratified analysis by oestrogen receptor (ER) and progesterone receptor (PR) status was also performed. Among 16 variants, only seven conformed to Hardy–Weinberg proportions in the controls. None of these seven variants showed statistically significant differences between the case and control groups in this Han Chinese population. However, chr6_32737733, a variant in HLA-DQB1, showed significant associations with both ER-negative and PR-negative breast cancer in the best fit to the dominant model. Furthermore, another significant correlation was seen between chr6_32606112, a variant in HLA-DRB5, and PR positivity. These results indicate that although no breast cancer risk variants in HLA class II were found in this Chinese population, HLA-DQB1 chr6_32737733 may to be involved in determining a poor prognosis, whereas HLA-DRB5 chr6_32606112 may relate to a good prognosis.

Keywords: Breast cancer - HLA class II - HLA-DQB1 - HLA-DRB5 - susceptibility - prognosis

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Introduction

Breast cancer is one of the most common cancers among women; according to a 2011 investigation, the incidence of breast cancer has been ranked first among cancers in women (Jemal et al., 2011). Compared with Western countries, China has a lower incidence of breast cancer (http://globocan.iarc.fr/factsheets/cancers/breast.asp). With the development of industry and changes in lifestyle, however, the incidence of breast cancer in China is increasing. In 2008, 169,000 new cases were diagnosed, and an estimated 44,000 deaths were attributed to breast cancer in China (http://globocan.iarc.fr/factsheets/cancers/breast.asp). Moreover, the incidence of breast cancer among Chinese women is expected to reach 85.3/100,000 in 2021 (Ziegler et al., 2008). Therefore, taking effective measures to control the incidence of breast cancer is becoming increasingly important in China.

The concept of tumour immunology is based on the assumption that the immune system is able to discriminate between normal and malignant tissues and to protect the host from tumour development by recognition and subsequent elimination of aberrant cells (Dunn et al., 2002). Tumours are believed to emerge only when immune surveillance fails (Bhutia et al., 2010). The major histocompatibility complex (MHC) and more specifically, the human leukocyte antigen (HLA) system, plays important roles in antitumour immune response and immune surveillance by mounting and recruiting cytotoxic T lymphocytes against tumour antigens (Hamaï et al., 2010). HLA, located on the short arm of chromosome 6, is a highly variable region. The HLA encoding region can be subdivided into three physical regions encoding class I, class II, and class III molecules. Among them, class II HLA molecules present tumour antigenic peptides on the cell surface that are recognised by T lymphocytes. Alterations of HLA class II molecules are relevant to the development of breast cancer and metastatic progression (Concha et al., 1995; Feinmesser et al., 2000; Lazzaro et al., 2001; Redondo et al., 2003). Therefore, certain individuals who inherit specific alleles of the highly polymorphic HLA class II, DPA, DPB, DQA, DQB, DRA, or DRB genes might have alterations in the risk of breast cancer and metastatic progression.

At present, there are few reports of HLA system alleles in Chinese female populations with breast cancer. Therefore, it is necessary to investigate whether HLA class II variants are associated with breast cancer susceptibility or prognosis in the Chinese population. In the present study, 16 variants in HLA class II were genotyped in

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Table 1. Distribution of 7 SNPs in Breast Cancer and Control Groups

Nearest Gene	SNP_ID	ID	Model	Genotype	Case(n)	Control(n)	OR(95%CI)	P
HLA-DPA1	chr6_33149553	snp5470	Codominant	G/G	198 (93%)	195(90.7%)	1 (referent)	0.68
				G/A	14 (6.6%)	19 (8.8%)	1.38 (0.67-2.83)	
				A/A	1 (0.5%)	1 (0.5%)	1.02 (0.06-16.4)	
HLA-DPB1	chr6_33151612	snp5495	Codominant	T/T	198(92.5%)	194(91.1%)	1 (referent)	0.42
				T/C	15 (7%)	18 (8.4%)	1.22 (0.60-2.50)	
				C/C	1 (0.5%)	1 (0.5%)	1.02 (0.06-16.4)	
	chr6_33151645	snp5496	Codominant	C/C	198(92.5%)	195(90.7%)	1 (referent)	0.78
				C/T	15 (7%)	19 (8.8%)	1.29 (0.64-2.60)	
				T/T	1 (0.5%)	1 (0.5%)	1.02 (0.06-16.4)	
HLA-DQA1	chr6_32717724	rs9272744	---	C/C	197(92.1%)	203(95.3%)	1 (referent)	0.17
				C/T	17 (7.9%)	10 (4.7%)	0.57 (0.26-1.28)	
HLA-DQB1	chr6_32737733	snp4845	Codominant	G/G	113(52.3%)	105(50.2%)	1(referent)	0.86
				G/A	88 (40.7%)	87 (41.6%)	1.06 (0.71-1.58)	
				A/A	15 (6.9%)	17 (8.1%)	1.22 (0.58-2.57)	
HLA-DRA	chr6_32520787	rs1041885	Codominant	T/T	176(81.9%)	172(81.5%)	1 (referent)	0.49
				A/T	39 (18.1%)	38 (18%)	1.00 (0.61-1.63)	
				A/A	0 (0%)	1 (0.5%)	NA (0.00-NA)	
HLA-DRB5	chr6_32606112	snp2935	Codominant	A/A	138(65.7%)	144(67.3%)	1 (referent)	0.53
				C/A	63 (30%)	65 (30.4%)	0.99(0.65-1.50)	
				C/C	9 (4.3%)	5 (2.3%)	0.53(0.17-1.63)	

The P value is counted by the web-based tool SNPstats, the corresponding OR is counted after age adjustment

a case–control study. Although no variants showed statistically significant associations with breast cancer risk, HLA-DQB1 chr6_32737733 variants and HLA-DRB5 chr6_32606112 were found to be associated with good prognoses for breast cancer patients in this Chinese population.

Materials and Methods

Study population

Following pathology-based diagnoses, 216 patients with breast cancer were enrolled at the Nanfang Hospital, Southern Medical University, Guangzhou, Guangdong Province, China. The control group consisted of 216 subjects with no cancer of any type. All of the subjects were female. The mean ages of the patients and the control subjects were 47.62 and 47.46 years, respectively.

Additionally, the presence of oestrogen and progesterone receptors in the tumour tissue was examined. The hormonal receptors of these tumours were distributed as follows: positive oestrogenic receptors in 53 cases; negative oestrogenic receptors in 136 cases; positive progesterone receptors in 93 cases; negative progesterone receptors in 96 cases. In 27 cases, it was not possible to determine the oestrogenic receptors and the progesterone receptors.

The study was approved by the NanFang Hospital Ethics Committee, and written informed consent was obtained from all participants.

Genotyping

After informed consent was obtained, 5-ml peripheral blood samples were collected stored in the frozen state. Genomic DNA was extracted from a 200-μl peripheral blood sample using TiangenTM Genomic DNA Kit (Tiangen, Beijing, China) according to the manufacturer's instructions and stored at -70°C until use. Sixteen variants in the HLA class II coding region (Figure1) were

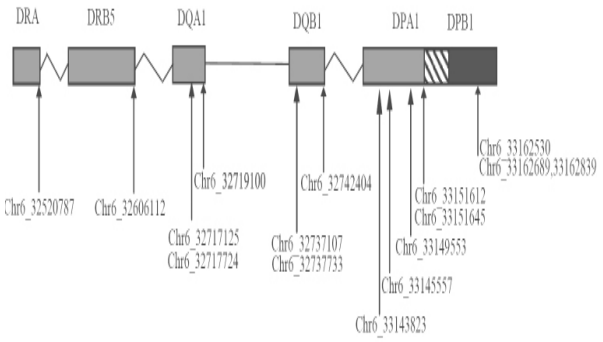

Figure 1. The Distribution of 16 Selected Variants in the HLA Class II Coding Region

selected based on data from the 1000 Genomes Project (http://www.1000genomes.org/). All of the SNPs were genotyped using the SEQUENOM MassARRAY matrix-assisted laser desorption ionisation–time of flight mass spectrometry platform (Sequenom, San Diego, California, USA). Primers were designed using a semiautomated method (Assay Design 3.1, Sequenom). Primer sequences are available on request. The call rate for each assay was set at >90%.

Statistical analysis

Hardy–Weinberg equilibrium (HWE) was examined using Haploview 4.1. Co-dominant and dominant genetic models of inheritance were chosen to evaluate the associations between each SNP and breast cancer. We further divided the cases into different groups according to oestrogen or progesterone receptor status. Association analysis based on unconditional logistic regression was carried out by calculating the odds ratio (OR) and 95% confidence interval (95% CI) for each SNP in co-dominant and dominant genetic models; the significance level was set at a P-value of 0.05. The statistical tests were implemented in the web-based tool SNPstats (http://bioinfo.iconcologia.net/SNPstats).

Table 2. Distribution of 7 SNPs in the ER-positive/negative Groups

Nearest Gene	SNP_ID	ID	Model	Genotype	Positive (n)	Negative (n)	OR(95%CI)	P
HLA-DPA1	chr6_33149553	snp5470	Codominant	G/G	69(89.6%)	102(93.6%)	1(referent)	0.28
				G/A	8 (10.4%)	6 (5.5%)	0.51 (0.17-1.53)	
				A/A	0 (0%)	1 (0.9%)	NA (0.00-NA)	
HLA-DPB1	chr6_33151612	snp5495	Codominant	T/T	67(88.2%)	104(93.7%)	1(referent)	0.18
				T/C	9 (11.8%)	6 (5.4%)	0.43 (0.15-1.26)	
				C/C	0 (0%)	1 (0.9%)	NA (0.00-NA)	
	chr6_33151645	snp5496	Codominant	C/C	69(88.5%)	102(93.6%)	1(referent)	0.2
				C/T	9 (11.5%)	6 (5.5%)	0.45 (0.15-1.32)	
				T/T	0 (0%)	1 (0.9%)	NA (0.00-NA)	
HLA-DQA1	chr6_32717724	rs9272744	---	C/C	72(94.7%)	101 (91%)	1(referent)	0.33
				C/T	4 (5.3%)	10 (9%)	1.78 (0.54-5.91)	
HLA-DQB1	chr6_32737733	snp4845	Codominant	G/G	31(39.7%)	67 (60.4%)	1(referent)	0.02
				G/A	40(51.3%)	38 (34.2%)	0.44 (0.24-0.81)	
				A/A	7 (9%)	6 (5.4%)	0.40 (0.12-1.28)	
			Dominant	G/G	31(39.7%)	67 (60.4%)	1(referent)	0.0051
				G/A-A/A	47(60.3%)	44 (39.6%)	0.43 (0.24-0.78)	
HLA-DRA	chr6_32520787	rs1041885	---	T/T	63(80.8%)	91 (82.7%)	1(referent)	
				A/T	15(19.2%)	19 (17.3%)	0.88 (0.41-1.86)	0.71
HLA-DRB5	chr6_32606112	snp2935	Codominant	A/A	55(71.4%)	66 (62.3%)	1(referent)	0.43
				C/A	19(24.7%)	35 (33%)	1.54 (0.79-2.98)	
				C/C	3 (3.9%)	5 (4.7%)	1.39 (0.32-6.07)	

The P value is counted by the web-based tool SNPstats, the corresponding OR is counted after age adjustment

Table 3. Distribution of 7 SNPs in PR-positive/negative Groups

Nearest Gene	SNP_ID	ID	Model	Genotype	Positive (n)	Negative (n)	OR(95%CI)	P
HLA-DPA1	chr6_33149553	snp5470	Codominant	G/G	85(89.5%)	86 (94.5%)	1(referent)	0.29
				G/A	9 (9.5%)	5 (5.5%)	0.55 (0.18-1.71)	
				A/A	1 (1.1%)	0 (0%)	0.00 (0.00-NA)	
HLA-DPB1	chr6_33151612	snp5495	Codominant	T/T	83(88.3%)	88(94.6%)	1(referent)	0.14
				T/C	10(10.6%)	5 (5.4%)	0.41 (0.13-1.27)	
				C/C	1 (1.1%)	0 (0%)	0.00 (0.00-NA)	
	chr6_33151645	snp5496	Codominant	C/C	85(88.5%)	86(94.5%)	1(referent)	0.23
				C/T	10(10.4%)	5 (5.5%)	0.49 (0.16-1.51)	
				T/T	1 (1%)	0 (0%)	0.00 (0.00-NA)	
HLA-DQA1	chr6_32717724	rs9272744	---	C/C	89(94.7%)	84(90.3%)	1(referent)	0.41
				C/T	5 (5.3%)	9 (9.7%)	1.61 (0.51-5.11)	
HLA-DQB1	chr6_32737733	snp4845	Codominant	G/G	43(44.8%)	55(59.1%)	1(referent)	0.13
				G/A	46(47.9%)	32(34.4%)	0.54(0.30-0.99)	
				A/A	7 (7.3%)	6 (6.5%)	0.67(0.21-2.14)	
			Dominant	G/G	43 44.8%)	55(59.1%)	1(referent)	0.048
				G/A-A/A	53(55.2%)	38(40.9%)	0.56(0.31-1.00)	
HLA-DRA	chr6_32520787	rs1041885	---	T/T	75(78.1%)	79(85.9%)	1(referent)	0.17
				A/T	21(21.9%)	13(14.1%)	0.59(0.27-1.26)	
HLA-DRB5	chr6_32606112	snp2935	Codominant	A/A	70(73.7%)	50(56.8%)	1(referent)	0.045
				C/A	21(22.1%)	34(38.6%)	2.27 (1.18-4.36)	
				C/C	4 (4.2%)	4 (4.5%)	1.40 (0.33-5.87)	
			Dominant	A/A	70(73.7%)	50(56.8%)	1(referent)	0.016
				C/A-C/C	25(26.3%)	38(43.2%)	2.13(1.14-3.96)	

The P value is counted by the web-based tool SNPstats, the corresponding OR is counted after age adjustment

Results

Of 16 SNPs analysed in this case–control study, only seven (chr6_32606112, chr6_32717724, chr6_32737733, chr6_33149553, chr6_33151612, chr6_33151645, and chr6_32520787) conformed to the Hardy–Weinberg proportions in the controls ($P > 0.1$). Statistical analysis related to only these seven variants is outlined here. No statistically significant differences between the case and control groups were detected in this Han Chinese population.

In the ER positive/negative study, the chr6_32737733 on HLA-DQB1 genotype distribution in the ER-positive group was 39.7% GG, 51.3% GA, and 9.0% AA, which was significantly different from that in the ER-negative group (60.4% GG, 34.2% GA, and 5.4% AA) and was related to ER-negative breast cancer with OR values of 0.43 (95% CI, 0.24–0.78, $P = 0.0051$). In the PR positive/negative study, HLA-DQB1 chr6_32737733 variant genotype distribution in the PR-positive group was 44.8% GG, 47.9% GA, and 7.3% AA, which was significantly different from that in the PR-negative group (59.1% GG, 34.4% GA, and 6.5% AA) and also showed a strong and significant association with PR-negative breast cancer,

with an OR value of 0.56 (95% CI, 0.31–1.00, P = 0.048). Additionally, chr6_32606112 on HLA-DRB5 showed a statistically significant correlation with PR-positive breast cancer, with OR values of 2.27 (95% CI, 1.18–4.36, P = 0.045) and 1.40 (95% CI, 0.33–5.87, P = 0.016) for AC and CC, respectively.

Discussion

HLA, located on the short arm of chromosome 6, contains a large number of genes related to immune system function in humans. Polymorphisms of HLA are associated with many diseases, especially autoimmune disorders (Shiina et al., 2004). Some polymorphisms are directly involved in the promotion of cancer, especially in tumours with viral aetiology (Kübler et al., 2006; Kübler et al., 2008; Tse et al., 2009). However, this region is the most genetically variable coding locus in humans. The large extent of variability in HLA genes poses significant challenges in investigating the role of HLA genetic variations in diseases. Based on data from the 1000 Genomes Project (http://www.1000genomes.org/), we selected 16 SNPs on the HLA class II coding region. The present study also encountered problems caused by high variability in HLA genes; among 16 selected SNPs, only seven conformed to Hardy–Weinberg proportions.

Breast cancer is the most common malignancy among women worldwide, and attempts have been made to investigate the association between breast cancer and HLA. The first association study, carried out by Chardhuri, found that DQB3*02017/*0202 alleles were associated with breast cancer risk in Caucasians (Chaudhuri et al., 2000). Positive or negative associations between class II HLA and breast cancer have been successfully found in other studies (Ghaderi et al., 2001; Lavado et al., 2005; Baccar Harrath et al., 2006; Chen et al., 2007; Cantú de León et al., 2009; Gun et al., 2011; Mahmoodi et al., 2011). However, the results of these studies are inconsistent across geographic regions. In the present study, no significant association between HLA class II variants and breast cancer was detected in a Han Chinese population. These results were partly consistent with research in a Taiwanese population (Chen et al., 2007) but differ from other studies, which have shown significant associations between polymorphisms of HLA class II variants and breast cancer risk among Caucasian, Turkish, Iranian, Tunisian, and Spanish women (Chaudhuri et al., 2000; Ghaderi et al., 2001; Lavado et al., 2005; Baccar Harrath et al., 2006; Cantú de León et al., 2009; Gun et al., 2011; Mahmoodi et al., 2011).

Breast cancer patients with ER- and/or PR-negative tumours experience higher mortality after their diagnosis compared with women with ER-positive and/or PR-positive disease (Anderson et al., 2001; Dunnwald et al., 2007). It is thought that variants determined in patients negative for these receptors could be related to poor prognosis. Therefore, it is important to mention that chr6_32737733, which was significantly correlated with ER(-)/PR(-) breast cancer, may also be associated with poor prognosis. However, HLA-DRB5 chr6_32606112 may relate to good prognosis in breast cancer patients.

In conclusion, 16 variants on the HLA class II coding region of 216 breast cancer patients and 216 healthy controls were detected in a case–control study in a Chinese Han population. Although no significant associations between HLA class II variants and breast cancer risk were found, HLA-DQB1 chr6_32737733 variants were found to be associated with poor prognosis in breast cancer patients, whereas HLA-DRB5 chr6_32606112 may relate to good prognosis. Our findings are an important addition to previously published work on the association between HLA class II variants and breast cancer. Further validation with different geographic populations, meta-analyses, and functional studies of genetic variants are still necessary.

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