RESEARCH COMMUNICATION

HLA-B*07 is a High Risk Allele for Familial Cervical Cancer

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Abstract

Aim: The purpose of this study was to investigate 50 women from eight families with familial cervical cancer in Wufeng County, Hubei Province, China, a region with a high incidence of cervical cancer. Eighty-nine healthy women, of similar age, location and ethnicity, were selected as a control group. Methods: Blood samples were collected from both groups, and HLA-A, HLA-B, and HLA-DRB1 genotypes were profiled with the Multi-Analyte Profiling system (xMAP) (Luminex HLA-SSO) using a WAKFlow HLA typing kit. Results were analyzed with Luminex HLA typing software and showed good stability, reproducibility and specificity. Results: We found several high risk alleles in women with familial cervical cancer, that associated with the highest risk being HLA-B*07 (OR = 8.7, 95% CI = 1.8-41.1). Conclusions: HLA-B*07 is a high risk allele for cervical cancer, and has strong potential for use as a molecular biomarker.

Keywords: Cervical cancer - familial risk - allele - haplotype - HLA

Asian Pacific J Cancer Prev, **12**, 2597-2600

Introduction

Thirty years ago, an interesting case of familial cervical cancer involving three sisters was reported (Bruinse etal., 1981). The sisters were diagnosed with familial cervical cancer one after the other and the author suspected that their cervical cancer was more likely caused by genetic factors rather than factors such as sexual transmitted disease or environmental risks, since the sisters were very conservative in their sexual behavior. However, no high HLA risk alleles were detected because of the absence of a control group.

In recent years, many studies have uncovered familial aggregation of cervical cancer, indirectly implying that genetic factors may play some roles in its genesis (Zoodsma etal., 2004; Hemminki etal., 1999; Fischer et al., 2001;), though it is likely that environmental risk factors and educational level may also be involved. Studies on twins have shown that genetic factors have some influence on cervical cancer (Ahlbom etal., 1997). Our (Tao etal., 2005; Qiu etal., 2007; 2010) and other previous studies (Wang etal., 2001; Bhattacharya etal., 2007) have shown that cervical cancer is related to HLA alleles. The incidence rate of cervical cancer in Wufeng County in Hubei province is extremely high (1073.3 per 100,000 (Liao 1994), making it a good location for investigating cervical cancer. The population is relatively stable as the county is located in a mountainous area, transportation is poor, and population mobility is limited. All these characteristics combine to make this a suitable population in which to investigate the genetic basis of cervical cancer genesis.

In order to study the effects of high risk HLA alleles in this population, we selected a high risk group of 50 women from eight families with a history of familial cervical cancer, and a control group of 89 healthy women. HLA -A, -B, and -DRB1 alleles and their haplotypes were studied in order to ascertain the causes of familial cervical cancer and identify a molecular biomarker for cervical cancer.

Materials and Methods

Study Population

With the approval of the Medical Ethics Committee of Wuhan University, we identified familial cervical cancer patients from eight families in Chengguan and Yuguan towns of Wufeng County, Hubei Province with the help of the local Maternity and Hygiene and Health Hospital. All members of the families gave informed consent, and received a questionnaire. We investigated eight generations of females within the family and produced a family tree. In six of the eight families, both the mother and one daughter had cervical cancer, while the other two families two sisters had cervical cancer.

The diagnosis of cervical cancer was confirmed for all patients at Wufeng Women and Child Health Hospital. The

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high risk group was composed of 50 women with familial cervical cancer, while the control group consisted of 89 unrelated healthy women of the same ethnicity and age from the same region. Both groups were from Wufeng County, Hubei Province and were of Tujia ethnicity. The age range of those investigated was between 20 and 62 years old.

Questionnaire

Each woman filled out a questionnaire including questions on various risk factors relating to cervical cancer. Trained inspectors examined the women and helped those who were unable to fill out the questionnaire due to their low education level.

Human Leukocyte Antigen genotyping

Genomic DNA was extracted from blood using a BDtract Genomic DNA Isolation Kit, according to the manufacturer's instructions. HLA-A, HLA-B, and HLA-DRB1 genotyping was performed on a Multi-Analyte Profiling system (xMAP) (Luminex HLA-SSO) using a WAKFlow HLA typing kit according to the manufacturer's instructions. Briefly,the highly polymorphic exons 2 and 3 of HLA-A and HLA-B genes, and exon 2 of HLA-DRB1 were amplified by PCR using the specific primer pairs included in the kit, giving products of 430 bp, 440 bp, and 288 bp for HLA-A, HLA-B and HLA-DRB1, respectively. The 5 prime end of up stream primers were labeled with biotin. Each PCR product was hybridized with probes (72 bp for HLA-A, 92 bp for HLA-B, and 68 bp for HLA-DRB1) which were complementary to the corresponding polymorphic sequences. Hybridized amplicons were labeled with streptavidin-R–phycoerythrin (SAPE), the specific fluorescent ligand of biotin. The beads were directed by the Luminex instrument through the scanning pathway. Beads emit red fluorescence and the reporter emits green fluorescence when beads are exposed under bi-laser. According to the intensity of red fluorescence to confirm the gene and the intensity of green to quantify the gene on the Luminex (Luminex, Austin, TX). Hybridization patterns were classified according to the manufacturer's instructions using the software provided.

Statistical Analysis

Allele frequency and haplotype were analyzed with Alrequin v3.5. Chi-square tests were carried out using SAS v8.0. The Holm procedure was used to decrease the type 1 error(Madeleine etal., 2008). Holm P values are presented for statistically significant ORs for each locus in Tables 1 - 3.

Results

The allele frequency of HLA -A, -B and -DRB1 in our investigation was similar to that in other studies of the Chinese population, indicating that the sample used in this study was representative. For example, the frequency of HLA-B*07 in our control group was 1.1%, while other studies reported a frequency of 2.4% in Guangdong, 1.5% in Beijing and 1.2% among Singaporean Chinese (HYPERLINK "http://www.allelefrequencies.net". The frequency of HLA-DRB1*4 and DRB1*11 in our control group was 10.1% and 7.9%, respectively, while the combined frequency of HLA-DRB1*040101, HLA-DRB1*040301, HLA-DRB1*040501 and HLA-DRB1*0406 was 9.1% in one study and that of HLA-DRB1*110101 and DRB1*110401 was 7.1% (Zhou etal., 2005). In another study, HLA-DRB1*4 and

Table 1. Risk of Cervical Cancer Associated with HLA -A and -B Alleles

	Control group (n=178),n(%)	High risk group (n=100),n(%)	OR(95%CI)	Holm P value
A*01	4 (2.2%)	1(1%)	0.4(0.0-4.0)	
A*02	70(39.3%)	25(25%)	0.5(0.3-0.9)	0.18
A*03	1 (0.6%)	2(2%)	3.6(0.3-40.3)	
A*11	49 (27.5%)	28(28%)	1.0(0.6-1.8)	
A*24	25(14%)	21(21%)	1.6(0.9-3.1)	
A*26	2(1.1%)	6(6%)	5.6(1.1-28.4)	0.3
A*30	2(1.1%)	6(6%)	5.6(1.1-28.4)	0.3
A*31	10(5.6%)	4(4%)	0.7(0.2-2.3)	
A*33	14 (7.9%)	7(7%)	0.9(0.3-2.3)	
A*68	1 (0.6%)		1.6(1.4-1.7)	
B*07	2(1.1%)	9(9%)	8.7(1.8-41.1)	0.04*
B*13	17(9.6%)	9(9%)	0.9(0.4-2.2)	
B*15	21(11.8%)	26(26%)	2.6(1.4-5.0)	0.08
B*27	1(0.6%)		1.6(1.4-1.7)	
B*35	3 (1.7%)		1.6(1.4-1.7)	
B*37	4 (2.2%)	1(1%)	0.4(0-4.0)	
B*38	3 (1.7%)		1.6(1.4-1.7)	
B*39	8 (4.5%)	1(1%)	0.2(0-1.7)	
B*40	41(23%)	28(28%)	1.3(0.7-2.3)	
B*44	5 (2.8%)		1.6(1.4-1.7)	
B*46	27(15.2%)	16(16%)	1.1(0.5-2.1)	
B*48	3 (1.7%)		1.6(1.4-1.7)	
B*50	2 (1.1%)		1.6(1.4-1.7)	
B*51	13 (7.3%)	1(1%)	0.1(0-1.0)	0.46
B*52	6 (3.4%)		1.6(1.4-1.7)	
B*53	1 (0.6%)		1.6(1.4-1.7)	
B*54	7 (3.9%)	5(5%)	1.3(0.4-4.2)	
B*55	3 (1.7%)	2(2%)	1.2(0.2-7.2)	
B*56	1 (0.6%)		1.6(1.4-1.7)	
B*58	9 (5.1%)	2(2%)	0.4(0.1-1.8)	
B*67	1(0.6%)		1.6(1.4-1.7)	

Bolding represents significant associations; The Holm P value corrects the Wald P value for multiple comparisons and was based on all alleles at a locus and is only shown for alleles with significant ORs.

Table 2. Risk of Cervical Cancer Associated with HLA -DRB1 Alleles

	Control group (n=178), n(%)	High risk group (n=100), n(%)	OR(95%CI)	Holm P value
DRB1*01	5 (2.8%)		1.6(1.4-1.7)	
DRB1*04	18 (10.1%)	8(8%)	0.8(0.3-1.8)	
DRB1*07	5 (2.8%)	8(8%)	3.0(1.0-9.5)	
DRB1*08	19 (10.7%)	3(3%)	0.3(0.1-0.9)	0.29
DRB1*09	44 (24.7%)	26(26%)	1.1(0.6-1.9)	
DRB1*10	4 (2.2%)		1.6(1.4-1.7)	
DRB1*11	14 (7.9%)	12(12%)	1.6(0.7-3.6)	
DRB1*12	15(8.4%)	14(14%)	1.8(0.8-3.8)	
DRB1*13	11(6.2%)	4(4%)	0.6(0.2-2.0)	
DRB1*14	15(8.4%)	12(12%)	1.5(0.7-3.3)	
DRB1*15	24 (13.5%)	12(12%)	0.9(0.4-1.8)	
DRB1*16	3 (1.7%)		1.6(1.4-1.7)	
DRB1*17	1(0.6%)	1(1%)	1.8(0.1-28.9)	

Table 3. Significant Risks of Cervical Cancer Associated with Class I and II Multilocus Genotypes

Loci	Alleles	Control group (n=178),n(%)	High risk group (n=100),n(%)	P	OR(95%CI)	$P_{correct}$
A-B-DRB1	A*11-B*46-DRB1*14	0(0%)	3(3.0%)	0.046	2.8(2.4-3.3)	1.00
	A*02-B*46DRB1*-09	12(6.7%)	0(0%)	0.005	0.6(0.6-0.7)	0.81
	A*24-B*15-DRB1*09	0(0%)	4(4.0%)	0.016	2.9(2.4-3.4)	1.00
	A*26-B*40-DRB1*09	0(0%)	4(4.0%)	0.016	2.9(2.4-3.4)	1.00
A-B	A*02-B*46	18(10.1%)	3(3.0)	0.034	0.3(0.1-1.0)	1.00

All ORs in this table are significant; *P < 0.05, Holm's test; result is significant after adjusting for multiple comparisons

HLA-DRB1*11 were 8.7% and 6%, respectively (Liu etal.,2005).

In this study we investigated the following HLA allele types: 10 HLA-A, and 21 HLA-B (Table 1), and 13 HLA-DRB1 (Table 2). Of the above HLA polymorphisms, we found that HLA-B*07 was a high risk allele type (OR = 8.7, 95% CI = 1.8-41.1). Its allele frequency was 9.0% in the high risk group, but only 1.1% in the control group (Table 2).

We also analyzed the risks of different combinations of allele types (Table 3). The three HLA –A, -B, and -DRB1 allele types created 134 different combinations, or haplotypes. The two HLA –A, and -B allele types gave rise to 61 haplotypes, while HLA –B, and -DRB1 gave rise to 83 haplotypes. None was present with a high frequency in the cervical cancer group after using the Holm procedure.

Discussion

In this investigation, we selected Wufeng County, Hubei Province, a special area with a high incidence of cervical cancer, as our study location since it is a rich source of women with a history of familial cervical cancer resulting from genetic causes. By comparing familial cervical cancer HLA genotypes with healthy controls, we tried to identify high risk HLA alleles. Our results indicated that HLA-B*07 is an allele associated with a very high risk of cervical cancer (OR, 95% CI: 8.7, 1.8-41.1).

In this study, women with the HLA-B*07 allele all had serum type B7. This surprising finding is in line with the high frequency of the B7 serum type in the original report on familial cervical cancer from 30 years ago, where two of the three sisters with cervical cancer had serum type B7(Bruinse etal., 1981). Our results thus verify the hypothesis in that report that genetic factors are related to the origin of familial cervical cancer. The B7 serum type can thus be used as a high risk factor for screening for cervical cancer.

HLA-B*07 has been reported as a cervical cancer risk factor in other studies. Hildesheim (Hildesheim etal.,1998) found that HLA-B*07 was a risk factor associated with cervical displasia (CIN) in women in the Portland area of the US. In the UK, Duggan-Keen (Duggan-Keen etal., 1996)found that cervical cancer patients with HLA-B*07 had a worse prognosis than patients in the control group. Moreover, they found that 31.8% of patients with HLA-B*07 (7 of 22) had a HPV16 variant. Ellis etal. (1995)found that a haplotype involving HLA-B*07 (HLA-B*0702-Cw*0702-DQB1*0301) increased the risk of cervical squamous cancer (OR,2.8; 95%CI,1.7–4.5). Wang et al.(2001) also found that co-occurrence of HLA-B*07 and HLA-DQB1 increased the risk of cervical cancer.

The mechanism by which serum type B7 or B*07 increases the risk of cervical cancer, has been explored from different angles. One study (Sidney etal., 1996) has indicated that the HPV16 variant changes the B7 epitope which in turn causes a decrease in CTL antigen recognition, inducing immune escape of abnormal cells. Bhattacharya et al. (2007) found that HLA-B*07 increased the infection rate of HPV16/18 (its OR was 4.73 in the control group, and 6.14 in the cervical cancer group). This means that people with HLA-B*07 have a weak ability to clear HPV and thus have a persistent infection of high risk HPV. The rate of co-occurrence of HLA-B*07 with the well known P53Pro73Pro risk factor in the Bhattacharya et al. study was also markedly increased (7.0% vs 1.3%), and the incidence of cervical cancer was also greater when both of these risk factors was present. However, in the absence of HLA-B*07, the occurrence of P53Pro72Pro in the cervical cancer group was not significantly different from the control group. This suggests that there is a synergetic effect between HLA-B*07 and P53Pro72Pro in the genesis of cervical cancer.

While the B7 serotype and B*07 allele have been reported as risk factors for cervical cancer, we are the first to suggest that the B*07 allele may be an important biomarker for familial cervical cancer. In future studies, we will focus on HLA-B*07 subtypes in order to ascertain which subtype is the most reliable molecular biomarker.

In our previous work, we found that allele B*6701 only occurred in women with cervical cancer (6%, Pcorrect = 0.003, OR = 4.8, 95% CI = 4.0-5.7). By combining the occurrence of B*6701 with other genetic factors and other environmental factors, we constructed a cervical cancer risk evaluation model which gave prediction rates with an accuracy as high as 80% .

HLA-B*6701 and HLA- B*07 share a common epitope (LIVMAFWY), the second residue of which is proline and the C terminal residue of which is a hydrophobic aliphatic or aromatic amino acid. This means these epitopes respond in a similar way to HPV antigen presentation. That is, they both induce HPV immune tolerance probably due to decreased HPV antigen presentation. The relationship between epitopes and HPV presentation is an issue worthy of further study.

We found that HLA-B*07 is a special allele. Its risk for cervical cancer was extremely high as a single allele and its OR was even higher than the OR multiple other high risk alleles co-presented. HLA-B*07 combined with environmental factors such as the HPV high risk type is probably one of the main reasons why Wufeng county has

a high incidence of cervical cancer and familial cervical cancer. This conclusion needs further verification in this area using a larger sample population.

The many risk alleles and haplotypes present in the local population explains why Wufeng county is a high incidence region for cervical cancer. HLA-B*07 may form the basis for development of useful genetic biomarkers for screening cervical cancer.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (No.30872935; 81072123) All authors declare that there is no conflict of interest in this study.

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