HLA alleles are associated with Postherpetic Neuralgia but not with Herpes Zoster

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Objective: In some herpes zoster (HZ) patients, one symptom is problematic; postherpetic neuralgia (PHN), a persistence of pain such as spontaneous pain and stimulus-evoked pain, allodynia or hyperpathia, for more than 6 months after healing of the vesicular eruptions. In our previous study, we reported the association between HLA alleles, HLA-A*33 and B*44, and PHN patients. In this study, we aim to refine the association of these alleles with PHN or HZ using higher-resolution HLA typing technique with an increased sample size.

Methods: HLA allele frequencies were compared in 70 PHN patients, 80 HZ patients, 52 HZ(-) patients, and 140 Japanese controls using HLA typing kits of SSOP protocols.

Result and Conclusion: The allele frequencies of *HLA-A*3303*, *B*4403*, and *DRB1*1302* in PHN(+) patients were significantly higher than those in Japanese controls (P=0.00007, P=0.000002, and P=0.0003, respectively). The frequencies of above alleles in PHN(+) patients were also significantly higher than those in PHN(-) patients (P=0.03, P=0.006 and P=0.03, respectively). However, no association was found in comparison of HZ(+) patients and HZ(-). And the frequency of HLA-B*5101 in HZ(-) patients was significantly higher than those in HZ(+) and PHN(+) patients (P=0.003 and P=0.009, respectively) indicating that HLA-B*5101 functions as a protective allele to HZ.

Key words: HLA allele, Postherpetic Neuralgia, Herpes Zoster, Varicella-zoster Virus, Susceptible Locus

INTRODUCTION

Herpes zoster (HZ) is a common disease, which is generally limited to the dermatome innervated by a single sensory ganglion, and is accompanied with pain caused by reactivation of varicella-zoster virus (VZV). The main associated symptoms are severe neuralgia and eruption of belts of vesicles on one side of the body (1). VZV is a human specific alpha-herpes virus and causes a rash on the face and the body in childhood. Then the virus establishes a life-long latent infection in the sensory ganglia, after the primary infection. The reactivation of the virus causes HZ. The relationship between VZV and human immune responses has been studied (2). In herpes zoster patients, the major complication is the persistence of pain for more than 6 months after healing of the vesicular eruptions known as postherpetic neuralgia (PHN). PHN is more frequent in elder patients and subjects to the most severe HZ. Because of difficulties in controlling the pain of PHN and outcomes of this pain, the patients suffer from reduction of strength or quality of daily activity and occasionally cause depression (3).

Human histocompatibility leukocyte antigen (HLA) region is located on the human chromosome 6p21.3 regulating the immune response. The three genes, HLA-A, B and DRB1 are very close to each other within 3000 kb showing strong linkage disequilibrium (4). We have previously reported the positive associations of HLA alleles, *A*33*, *B*44*, *DRB1*1302* and the *HLA-A*33-B*44-DRB1*1302* haplotype with PHN (5). The results suggest the involvement of immune reaction to VZV as the HLA system is known to control various immune responses. The main risk factors for developing PHN are the decline of immune responses to VZV in HZ, and the severity of herpes zoster, suggesting that HLA may play a central role in the etiology of PHN (6). It is possible that the observed association with HLA may be caused by the association between HLA alleles and herpes zoster without subsequent PHN. Another possibility is that the HLA alleles are associated with the development of PHN rather than herpes zoster, either through defective immune response to VZV or excessive inflammatory response that eventually causes damage to the neurons. In order to distinguish these possibilities, the current study was designed to determine whether the patients with herpes zoster without subsequent PHN are associated with the candidates HLA alleles or not.

In this study, our objective is to assign HLA alleles associating with this disease using the 4 digits typing of HLA-A, B, and DRB1 of the HZ(+), HZ(-), PHN(+), PHN(-), and Japanese control samples.

PATIENTS AND METHODS

Population / sample recruitment

We recruited 150 HZ patients from the Department of Dermatology of Tokai University Hospital, Kanagawa, Japan. They were all tested as positive for herpes zoster. They had been followed–up for a

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minimum of 6 months to determine whether they developed PHN or not.

The data were analyzed either separately, or in combination: the overall 150 HZ(+) patients comprising 70 PHN(+) and 80 PHN(-) patient. 52 patients with an average age of 82 years old without herpes zoster were recruited as HZ negative controls (HZ(-)). 140 healthy Japanese volunteers with no past history of herpes zoster were additionally recruited as normal control subjects. This protocol was approved by the ethics committee of Tokai University and informed consent was obtained from all the patients and controls prior to the inclusion, and then peripheral blood samples were obtained.

HLA classes I and II allele genotyping

Genomic DNAs of all individuals were purified from their peripheral blood using an extraction kit (QIAamp Blood Kit, QIAGEN, Hilden, Germany). Detection of alleles at HLA-A, -B, and -DRB1, were performed by combiniation of polymerase chain reaction (PCR) and sequence-specific oligonucleotide probes (SSOP) protocols with the Luminex 100xMap flow cytometry dual-laser system to quantitate fluorescent-labeled oligonucleotides attached to color-coded microbeads kits (LABType SSO A, B, DRB1 Locus Typing Test, ONE LAMBDA, INC., Hannover, Germany).

Statistical analyses

The association of HLA alleles of Japanese patients with PHN was assessed by the Chi-square contingency table analysis with Yates' correction, as by the standard P value (*P*) test. Odds Ratio (O.R.) was calculated from the cross product ratio of the entries in the Chi-square 2×2 table. The corrected *P* value (*Pc*) was also obtained by multiplying the P value (P) by the number of measured alleles in each locus tested. $P<0.05$ was accepted as statistically significant (7).

RESULTS AND DISCUSSION

In genotyping of HLA-A, -B, -DRB1 loci, the frequency of each HLA locus was determined in 150 HZ(+) patients comprising 70 PHN(+) patients and 80 PHN(-) patients, 52 HZ(-) patients, and the 140 Japanese controls. In this study, a total of 10 alleles of HLA-A locus, 31 alleles of HLA-B locus, and 26 alleles of HLA-DRB1 locus were detected and genotyped (Table 1). The allele frequencies of HLA-A, B, and DRB1 alleles among the all subjects are shown in Table 1. In the Table 2, the data shows the significance of the comparison between the different samples ($P<0.05$).

The allele frequencies of *HLA-A*3303*, *B*4403* and *DRB1*1302* in PHN(+) patients were significantly higher than those in Japanese controls (P=0.00007 (Pc=0.0007, OR=4.27) for *HLA-A*3303*, P=0.000002 (Pc=0.00005, OR=6.14) for *HLA-B*4403*, P=0.0003 (Pc=0.007, OR=4.05) for *HLA-DRB1*1302*, respectively).

The frequencies of above alleles in PHN(+) patients were also significantly higher than those in PHN(-) patients but significances were disappeared after multiple test correction (P=0.03 (Pc=Not Significant, OR=2.44), P=0.006 (Pc=N.S., OR=3.14), P=0.03 (Pc=N.S., OR=2.47), respectively). However, the frequencies of those alleles in HZ(+) patients have no statistical differences comparing with those of HZ(-) patients.

Our previous report indicated the positive correlation between PHN and *HLA-A33*, *B44*, and *DRB1*1302* (4). However, the number of the PHN patients subjected in the previous study was not substantial (n=32) enough to confirm the association evidence. To strengthen the statistical power, we recruited 70 PHN patients and assigned new groups (HZ(+), HZ(-)) in the current study. Furthermore, we added the HZ(+) and the HZ(-) samples and also used 4 digit HLA-A, B, and DRB1 genotyping to emphasize the correlation of the PHN with the *HLA-A33*, *B44*, *DRB1*1302*. These results indicated that the positive correlations with the disease were only observed for *HLA-A*3303*, *B*4403*, *DRB1*1302* alleles. Embedded in a haplotype, these alleles are typical of the Japanese population (haplotype frequency around 5%, data not shown) (8). However, this haplotype had a higher frequency in PHN(+) group (21.7%) (Data not shown). On the other hand, the case with the PHN(-) vs. HZ(-) and PHN(-) vs. Japanese control group, the 3 alleles did not show a positive correlation. As a result, the alleles were not susceptible to HZ, but susceptible to PHN basically confirming the previously reports with high statistical power (4, 9, 10).

For the case of *HLA-B*5101* allele, there area correlations in comparison of PHN(+) vs. HZ(-), and HZ(+) vs. HZ(-) (P=0.009 (Pc=N.S., OR=0.31), P=0.003 (Pc=N.S., OR=0.33), respectively) suggesting that this allele is the protective allele of HZ, because this allele frequency was founded to be higher in the HZ(-) group.

Moreover, the correlations were shown for the *HLA-A*02* allele for PHN(+) vs. Japanese control, HZ(+) vs. Japanese control, and PHN(+) vs. PHN(-) suggesting that this allele could be the protective allele of PHN (P=0.0001, Pc=0.001 and OR=0.26, P=0.003, Pc=0.03 and OR=0.48, P=0.009, Pc=N.S. and OR=0.36, respectively).

It is reported that the increased risk of HZ and PHN is accompanied with the declination of immune response over the aging (6). Also, it is reported that the increased risk of PHN is associated with the severity of the peripheral nerves damage (11). Further analyses need to be performed to demonstrate whether the HLA genes themselves are the causality or other genes in linkage disequilibrium with HLA alleles are responsible (12).

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Table 1 Allele frequencies of HLA-A, B, DRB1 in all samples.

HLA Locus	PHN+		PHN-		HZ+		HZ-		control	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
HLA-A	2n=124		2n=150		2n=300		2n=104		2n=280	
*0101	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.9)	1	(0.4)
*02	12	(9.7)	30	(20.0)	42	(14.0)	14	(13.5)	67	(23.9)
*1101	9	(7.3)	10	(6.7)	19	(6.3)	3	(2.9)	26	(9.3)
*24	38	(30.6)	56	(37.3)	94	(31.3)	42	(40.4)	86	(30.7)
*2601	6	(4.8)	12	(8.0)	18	(6.0)	8	(7.7)	20	(7.1)
*2602	3	(2.4)	1	(0.7)	4	(1.3)	0	(0.0)	4	(1.4)
*2603	4	(3.2)	3	(2.0)	7	(2.3)	3	(2.9)	7	(2.5)
*3001	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.4)
*3101	17	(13.7)	14	(9.3)	31	(10.3)	11	(10.6)	28	(10.0)
*3303	21	(16.9)	13	(8.7)	34	(11.3)	8	(7.7)	15	(5.4)
HLA-B	2n=128		2n=140		2n=268		2n=104		2n=280	
*0702	7	(5.5)	9	(6.4)	16	(6.0)	3	(2.9)	15	(5.4)
*1301	2	(1.6)	0	(0.0)	2	(0.7)	2	(1.9)	2	(0.7)
*1302	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.4)
*1501	13	(10.2)	9	(6.4)	22	(8.2)	8	(7.7)	19	(6.8)
*1507	1	(0.8)	0	(0.0)	1	(0.4)	0	(0.0)	0	(0.0)
*1511	0	(0.0)	1	(0.7)	1	(0.4)	2	(1.9)	3	(1.1)
*1518	3	(2.3)	2	(1.4)	5	(1.9)	2	(1.9)	8	(2.9)
*2704	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.4)
*2705	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	2	(0.7)
*3501	6	(4.7)	10	(7.1)	16	(6.0)	5	(4.8)	21	(7.5)
*3701	0	(0.0)	1	(0.7)	1	(0.4)	1	(1.0)	1	(0.4)
*3901	3	(2.3)	7	(5.0)	10	(3.7)	0	(0.0)	6	(2.1)
*3902	0	(0.0)	1	(0.7)	1	(0.4)	0	(0.0)	0	(0.0)
*3923	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.4)
*4001	6	(4.7)	5	(3.6)	11	(4.1)	1	(1.0)	18	(6.4)
*4002	7	(5.5)	18	(12.3)	25	(9.3)	5	(4.8)	25	(8.9)
*4003	1	(0.8)	0	(0.0)	1	(0.4)	0	(0.0)	1	(0.4)
*4006	3	(2.3)	6	(4.3)	9	(3.4)	2	(1.9)	8	(2.9)
*4402	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
*4403	22	(17.2)	10	(7.1)	32	(23.9)	6	(5.8)	11	(3.9)
*4601	5	(3.9)	10	(7.1)	15	(5.6)	9	(8.7)	15	(5.4)
*4801	2	(1.6)	5	(3.6)	7	(2.6)	2	(1.9)	7	(2.5)
*5101	9	(7.0)	11	(7.9)	20	(14.9)	18	(17.3)	24	(8.6)
*5201	12	(9.4)	18	(12.3)	30	(11.2)	11	(10.6)	34	(12.1)
*5401	10	(7.8)	9	(6.4)	19	(7.1)	8	(7.7)	24	(8.6)
*5502	2	(1.6)	1	(0.7)	3	(1.1)	5	(4.8)	9	(3.2)
*5601	0	(0.0)	2	(1.4)	2	(0.7)	0	(0.0)	2	(0.7)
*5603	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.4)
*5801	1	(0.8)	2	(1.4)	3	(1.1)	0	(0.0)	1	(0.4)
*5901	2	(1.6)	0	(0.0)	2	(0.7)	1	(1.0)	7	(2.5)
*6701	3	(2.3)	0	(0.0)	3	(1.1)	0	(0.0)	3	(1.1)
HLA-DRB1	2n=138		2n=150		2n=288		2n=104		2n=280	
*0101	9	(6.5)	7	(4.7)	16	(5.6)	2	(1.9)	18	(6.4)
*0301	0	(0.0)	1	(0.7)	1	(0.3)	0	(0.0)	0	(0.0)
*0401	0	(0.0)	0	(0.0)	0	(0.0)	4	(3.8)	4	(1.4)
*0403	4	(2.9)	6	(4.0)	10	(3.5)	5	(4.8)	9	(3.2)
*0405	14	(10.1)	13	(8.7)	27	(9.4)	7	(6.7)	39	(13.9)
*0406	5	(3.6)	4	(2.7)	9	(3.1)	6	(5.8)	6	(2.1)
*0407	1	(0.7)	0	(0.0)	1	(0.3)	0	(0.0)	3	(1.1)
*0408	0	(0.0)	1	(0.7)	1	(0.3)	0	(0.0)	2	(0.7)
*0410	2	(1.4)	3	(2.0)	5	(1.7)	0	(0.0)	2	(0.7)
*0802	6	(4.3)	5	(3.3)	11	(3.8)	1	(1.0)	11	(3.9)
*0803	9	(6.5)	18	(12.0)	27	(9.4)	6	(5.8)	20	(7.1)
*0901	14	(10.1)	30	(20.0)	44	(15.3)	18	(17.3)	51	(18.2)
*1001	0	(0.0)	1	(0.7)	1	(0.3)	0	(0.0)	1	(0.4)
*1101	5	(3.6)	2	(1.3)	7	(2.4)	3	(2.9)	4	(1.4)
*1201	6	(4.3)	1	(0.7)	7	(2.4)	4	(3.8)	6	(2.1)
*1202	3	(2.2)	1	(0.7)	4	(1.4)	2	(1.9)	4	(1.4)
*1302	19	(13.8)	10	(6.7)	29	(10.1)	6	(5.8)	12	(4.3)
*1401	2	(1.4)	2	(1.3)	4	(1.4)	6	(5.8)	10	(3.6)
*1402	1	(0.7)	1	(0.7)	2	(0.7)	0	(0.0)	0	(0.0)
*1403	2	(1.4)	2	(1.3)	4	(1.4)	1	(1.0)	7	(2.5)
*1405	2	(1.4)	0	(0.0)	2	(0.7)	1	(1.0)	7	(2.5)
*1406	2	(1.4)	1	(0.7)	3	(1.0)	0	(0.0)	2	(0.7)
*1501	5	(3.6)	13	(8.7)	18	(6.3)	10	(9.6)	17	(6.1)
*1502	8	(5.8)	16	(10.7)	24	(8.3)	14	(13.5)	28	(10.0)
*1601	2	(1.4)	0	(0.0)	2	(0.7)	0	(0.0)	0	(0.0)
*1602	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	3	(1.1)

Table 2 The significance of the comparison between the different samples.

	PHN+ vs. control			HZ+ vs. control			PHN+ vs. PHN-			PHN+ vs. HZ-			HZ+ vs. HZ-		
	P	Pc	O.R	P	Pc	O.R	P	Pc	O.R	P	Pc	O.R	P	Pc	O.R
HLA-A															
*02	0.0001	0.001	0.26	0.003	0.03	0.48	0.009	N.S.	0.36	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
*24	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	0.02	N.S.	0.39	N.S.	N.S.	N.S.
*3303	0.00007	0.0007	4.27	0.002	0.02	2.75	0.03	N.S.	2.44	0.02	N.S.	2.82	N.S.	N.S.	N.S.
HLA-B															
*4403	0.000002	0.00005	6.14	0.0003	0.008	3.68	0.006	N.S.	3.14	0.005	N.S.	4.02	N.S.	N.S.	N.S.
*5101	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	0.009	N.S.	0.31	0.003	N.S.	0.33
HLA-DRB1															
*1302	0.0003	0.007	4.05	0.006	N.S.	2.69	0.03	N.S.	2.47	0.03	N.S.	0.69	N.S.	N.S.	N.S.

NS, not significant; OR, odds ratio

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