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Impact of High-Frequency HLA Haplotypes on Clinical Cytomegalovirus Reactivation in Allogeneic Hematopoietic Stem Cell Transplantation

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A B S T R A C T

Some studies support the hypothesis that HLA genes and haplotypes evolved by natural selection through their protective abilities against specific infectious pathogens. However, very little is known regarding the impact of high-frequency HLA haplotypes on the risk of relevant infectious diseases among a given ethnic group. We evaluated the impact of high-frequency HLA haplotypes on cytomegalovirus (CMV) reactivation and infection in allogeneic hematopoietic stem cell transplantation (allo-HSCT) in a Japanese population as a model of infectious disease that has coexisted with humans. A total of 21,127 donor-patient pairs were analyzed. HLA-A-B-DRB1 haplotypes were estimated using the maximum probability algorithm. Seven haplotypes with >1% frequency were defined as high-frequency haplotypes (HfHPs). Homozygotes of HfHP and heterozygotes had significantly lower risk of CMV reactivation and infection (hazard ratio [HR] = 0.88, $P = .009$ and HR = 0.93, $P = .003$, respectively) than homozygotes of low-frequency HLA haplotypes (LfHPs). In subgroup analyses of a different donor source, these associations were statistically significant in unrelated donor transplants. Finally, CMV risk for homozygotes and heterozygotes of each HfHP was compared with that of homozygotes of LfHPs. The 2 most predominant HfHP groups (A*24:02-B*52:01-DRB1*15:02 group and A*24:02-B*07:02-DRB1*01:01 group) had a significantly lower risk of CMV reactivation and infection (HR = 0.86, $P < .001$ and HR = 0.91, $P = .033$, respectively). Our findings suggest that HfHPs may be protective against CMV reactivation and infection and that increased care regarding CMV reactivation and infection may be necessary for patients with LfHP after allo-HSCT.

INTRODUCTION

Human cytomegalovirus (CMV), officially known as human herpesvirus 5, is a member of the β herpesvirus subfamily of viruses that has coevolved with humans [1]. Primary infection, which usually occurs in early childhood without symptoms or

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with mild symptoms, is controlled by a healthy immune system. However, the infection is often not completely cleared, leading to latent infection that coexists with the host for life. Asymptomatic latent infection is established in hematopoietic progenitor cells and myeloid lineage cells [2-4]. CMV reactivation is associated with differentiation to a more mature myeloid cell type [5-7]. Although the immune system of healthy individuals will recognize and terminate reactivation, it is most common to observe viral complications after solid organ and allogeneic hematopoietic stem cell transplantation (allo-HSCT) [8,9]. CMV can cause severe diseases in immunocompromised individuals, including encephalitis, pneumonitis, hepatitis, uveitis, retinitis, and gastrointestinal disease [8-11]. CMV is the most common cause of congenital infection and a common cause of central nervous system sequelae in newborns, such as mental retardation, cerebral palsy, and seizures [12,13].

HLA is a key molecule in immune responses for recognizing and responding to foreign antigens, such as proteins related to infectious pathogens or tumor cells, particularly by activating T cells [14]. From an evolutionary point of view, high allelic diversity of individuals and populations at the HLA loci should be evolved and maintained to identify and process large numbers of foreign antigens [15]. Indeed, the 6p21.3 region of the short arm of human chromosome 6 is the most polymorphic region of the human genome. The level of polymorphism observed in some of the genes on this chromosome is very high, with over 4000, 5000, and 2000 variants reported in HLA-A, HLA-B, and HLA-DRB1, respectively, from around the world [16]. However, the distribution and frequency of these alleles markedly differ among populations and geographic regions. Some studies support the hypothesis that HLA genes and haplotypes evolved by natural selection from infectious pathogens [17-20]. However, the impact of high-frequency HLA haplotypes on infectious diseases has not been thoroughly explored.

The aim of this study was to evaluate the impact of high-frequency HLA haplotypes on CMV reactivation and infection in allo-HSCT in a Japanese population as a model of infectious disease that has coexisted with humans.

METHODS

Data Collection

We included patients who underwent their first allo-HSCT from related, unrelated, or umbilical cord blood donors between 1993 and 2014. We excluded patients who lacked data on survival status, CMV infection, and genotyping for HLA-A, HLA-B, and HLA-DRB1. Clinical data on HSCT recipients were collected by the Japan Society for Hematopoietic Cell Transplantation and the Japanese Data Center for Hematopoietic Cell Transplantation using the Transplant Registry Unified Management Program (TRUMP) [21-23]. The study was approved by the data management committee of TRUMP and by the Institutional Review Board of Hiroshima University.

HLA-A-B-DRB1 Haplotype Estimation

Histocompatibility data for genotyping for HLA-A, HLA-B, and HLA-DRB1 were obtained from the TRUMP database [21-23]. HLA-A-B-DRB1 haplotypes were estimated using the maximum probability algorithm. Eight possible haplotype combinations were determined from the results of HLA-A, HLA-B, and HLA-DRB1 genotyping in each patient. The probability of the 8 haplotype combinations was calculated using haplotype frequency data from a family study in a Japanese population [24]. The haplotype combination with the highest probability among the 8 combinations was used as the predicted haplotype of the patient [24].

Definition of High-Frequency Haplotype

HLA-A, HLA-B, and HLA-DRB1 haplotypes with a frequency of >1% in this cohort were defined as high-frequency haplotypes (HfHPs), and all other haplotypes were defined as low-frequency haplotypes (LfHPs).

Endpoints

The primary endpoint of the study was clinical CMV reactivation, defined as the start of CMV preemptive therapy or occurrence of CMV infection [25]. Patients were monitored for pp65 antigenemia from the time of engraftment after allo-HSCT. Preemptive therapy was generally initiated when at least 2 CMV pp65 antigen-positive cells per 50,000 white blood cells were detected, as described previously in a study using the same registry data [26]. CMV infection was diagnosed according to published recommendations [25]. The secondary endpoint was overall survival (OS).

Statistical Analysis

The cumulative incidence of clinical CMV reactivation was assessed using a method described elsewhere to eliminate the effect of competing risks [27]. The competing event for clinical CMV reactivation was defined as death without clinical CMV reactivation. The probability of OS was estimated using the Kaplan-Meier method. Multivariable Cox regression analyses were conducted to evaluate the impact of HfHPs on clinical CMV reactivation and OS. Acute myelogenous leukemia and acute lymphoblastic leukemia in the first or second complete remission, chronic myelogenous leukemia in the first or second chronic phase or accelerated phase, myelodysplastic syndrome with refractory anemia or refractory anemia with ringed sideroblasts, adult T cell leukemia in complete remission, and malignant lymphoma in complete or partial remission were defined as standard-risk diseases. All other conditions were defined as high risk. Confounders considered were recipient age (0 to 15 years, 16 to 49 years, or ≥50 years), recipient sex, type of disease, disease status before transplantation (standard or high risk), type of graft-versus-host disease (GVHD) prophylaxis (cyclosporine based or tacrolimus based), type of conditioning regimen (myeloablative or reduced intensity), use of total body irradiation (yes or no), use of antithymocyte globulin (yes or no), CMV serostatus of recipients and donors (recipient-donor pairs), and the number of HLA mismatches at the HLA-A, HLA-B, and HLA-DRB1 loci (0 to 6). We adjusted for these confounders in all analyses in this study to ensure that the results were comparable. We also conducted analysis in which acute GVHD was evaluated as a time-varying covariate. Statistical significance was defined as $P < .05$. All analyses were performed using Stata version 14.2 (StataCorp, College Station, TX).

RESULTS

Patient Characteristics

A total of 21,127 patients whose haplotypes could be estimated were analyzed in this study. Table 1 shows the patients' transplant characteristics. For GVHD prophylaxis, tacrolimus-based regimens were used in 12,969 patients, in combination with methotrexate in 10,023 and with mycophenolate mofetil in 1253 patients; cyclosporine-based regimens were used in 7842 patients, in combination with methotrexate in 6948 and with mycophenolate mofetil in 352; and other regimens were used in 155 patients. The median follow-up period for survivors among the entire cohort was 3.8 years (range, 0.1 to 22.0 years).

Haplotype Distribution and Accuracy of HLA Estimation

Haplotype frequency in this cohort was comparable to that of a previous family study [24]. Table 2 lists the haplotypes with frequencies higher than 1% in this cohort. As expected from the previous report [8], there were 7 haplotypes with frequencies higher than 1% in this cohort. These 7 haplotypes were named HP 1 to HP 7 in descending order and defined as HfHPs. This method for HLA haplotype estimation is useful for populations with well-conserved dominant haplotypes, such as the Japanese population. Approximately two-fifths (41.3%) of Japanese have 1 of the 7 most common haplotypes (HPs 1 to 7) [24]. We confirmed the accuracy of our HLA estimation method using independent data obtained from 2118 healthy donors whose HLA haplotypes were identified in HLA family studies. The accuracy rate of this HLA haplotype estimation method was 99.4% for HPs 1 to 7.

Impact of High-Frequency HLA Haplotypes on Clinical CMV Reactivation

To evaluate the impact of HfHPs on clinical CMV reactivation, we first compared homozygotes of LfHPs (LL group) with heterozygotes of HfHPs and LfHPs (HL group) and homozygotes of HfHPs (HH group). The cumulative incidence of clinical CMV reactivation is shown in the curve in Figure 1A.

Table 1
Patient Characteristics

Characteristic	Total (N = 21,127)	Related (n = 4252)	Unrelated (n = 11,567)	Cord Blood (n = 5308)
Recipient age at transplant, yr				
Median (range)	44 (21-60)	44 (21-60)	43 (21-60)	46 (21-60)
CMV antibody (recipient and donor)				
Recipient negative/donor negative	1250 (6)	278 (7)	619 (5)	353 (7)
Recipient positive/donor negative	3819 (18)	409 (10)	1979 (17)	1431 (27)
Recipient negative/donor positive	1097 (5)	354 (8)	730 (6)	13 (0)
Recipient positive/donor positive	6528 (31)	2317 (54)	4115 (36)	96 (2)
Unknown	8433 (40)	894 (21)	4124 (36)	3415 (64)
No. of HLA mismatches				
0	10,120 (48)	2745 (65)	7222 (62)	153 (3)
1	4694 (22)	394 (9)	3751 (32)	549 (10)
2	3169 (15)	467 (11)	522 (5)	2180 (41)
3	2416 (11)	610 (14)	66 (1)	1740 (33)
4	605 (3)	23 (1)	4 (0)	578 (11)
5	111 (1)	7 (0)	2 (0)	102 (2)
6	12 (0)	6 (0)	0 (0)	6 (0)
Disease				
AML	8529 (40)	1777 (42)	4305 (37)	2447 (46)
ALL	3704 (18)	750 (18)	2074 (18)	880 (17)
ATL	942 (4)	196 (5)	510 (4)	236 (4)
CML	1423 (7)	116 (3)	1131 (10)	176 (3)
MDS	2069 (10)	353 (8)	1240 (11)	476 (9)
Other leukemia	196 (1)	35 (1)	100 (1)	61 (1)
MPD	315 (1)	66 (2)	188 (2)	61 (1)
NHL	2497 (12)	606 (14)	1194 (10)	697 (13)
Other lymphoma	284 (1)	64 (2)	152 (1)	68 (1)
MM/PCL	357 (2)	65 (2)	225 (2)	67 (1)
AA	586 (3)	153 (4)	359 (3)	74 (1)
Other disease	225 (1)	71 (2)	89 (1)	65 (1)
Disease risk at transplant				
Standard risk	9334 (44)	1705 (40)	5763 (50)	1866 (35)
High risk	10471 (50)	2222 (52)	5068 (44)	3181 (60)
N/A	1322 (6)	325 (8)	736 (6)	261 (5)
Stem cell source				
Bone marrow	12,832 (61)	1351 (32)	11,481 (99)	0 (0)
Peripheral blood	2987 (14)	2901 (68)	86 (1)	0 (0)
Cord blood	5308 (25)	0 (0)	0 (0)	5308 (100)
GVHD prophylaxis				
Cyclosporin based	7842 (37)	2567 (60)	3393 (29)	1882 (35)
Tacrolimus based	12,969 (61)	1585 (37)	8033 (69)	3351 (63)
Others/missing	316 (1)	100 (2)	141 (1)	75 (1)
Conditioning				
Reduced intensity	13,103 (62)	2298 (54)	8030 (69)	2775 (52)
Myeloablative	7501 (36)	1892 (44)	3094 (27)	2515 (47)
Missing	523 (2)	62 (1)	443 (4)	18 (0)
ATG				
ATG	1586 (8)	796 (19)	667 (6)	123 (2)
Non-ATG	19,460 (92)	3443 (81)	10,843 (94)	5174 (97)
Missing	81 (0)	13 (0)	57 (0)	11 (0)
Preconditioning				
TBI regimen	14,835 (70)	2403 (57)	8349 (72)	4083 (77)
Non-TBI regimen	5662 (27)	1837 (43)	2609 (23)	1216 (23)
Missing	630 (3)	12 (0)	609 (5)	9 (0)
Acute GVHD (grades II-IV)				
Yes	7875 (37)	1456 (34)	4710 (41)	1709 (32)
No	13,252 (63)	2796 (66)	6857 (59)	3599 (68)

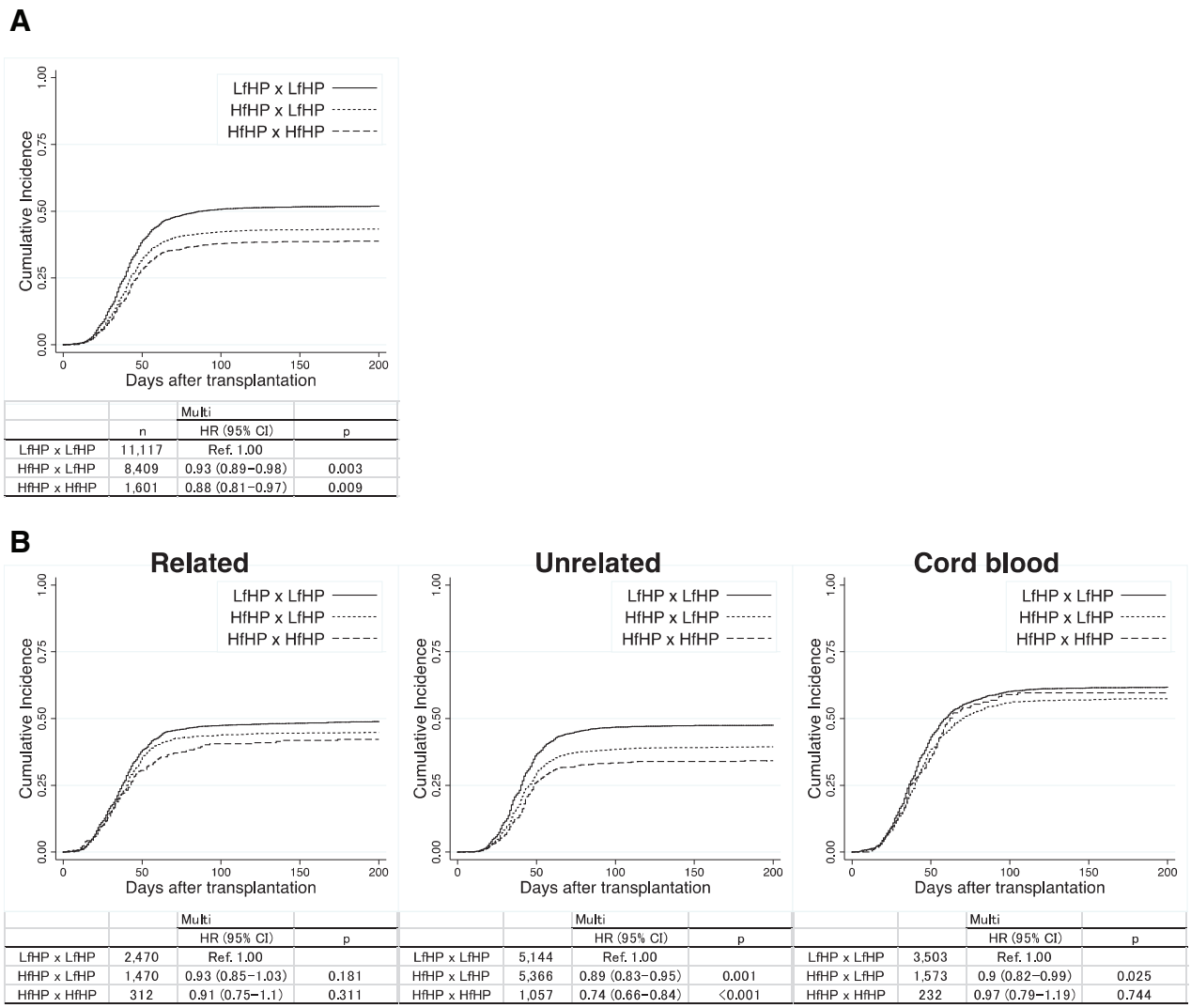
Values are presented as number (%) unless otherwise indicated.
AML indicates acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; ATL, adult T cell leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; MPD, myeloproliferative disorders; NHL, non-Hodgkin lymphoma; MM/PCL, multiple myeloma/plasma cell leukemia; AA, aplastic anemia; N/A, not applicable; ATG, antithymocyte globulin; TBI, total body irradiation.

Table 2
HLA-A-B-DRB1 Haplotype Frequency

Characteristic	A-B-DRB1 Haplotype	Frequency, %*	n†	Frequency, %†
HP 1	A*24:02-B*52:01-DRB1*15:02	8.44	4312	10.20
HP 2	A*24:02-B*07:02-DRB1*01:01	3.84	1963	4.66
HP 3	A*33:03-B*44:03-DRB1*13:02	4.45	1954	4.62
HP 4	A*24:02-B*54:01-DRB1*04:05	2.72	1245	2.95
HP 5	A*02:07-B*46:01-DRB1*08:03	1.81	875	2.07
HP 6	A*11:01-B*15:01-DRB1*04:06	1.37	588	1.39
HP 7	A*24:02-B*59:01-DRB1*04:05	1.07	498	1.18

* http://hla.or.jp/med/frequency_search/en/haplo/.
† This study.

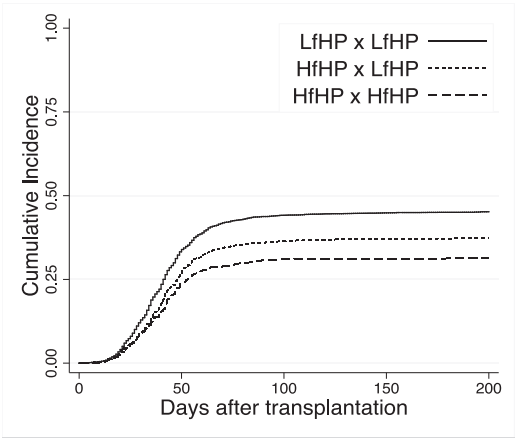
Multivariable analysis revealed that the HH group and HL group had a significantly lower risk of clinical CMV reactivation (hazard ratio [HR] = 0.88, $P = .009$ and HR = 0.93, $P = .003$, respectively) than the LL group. Although CMV reactivation is thought to be associated with the occurrence of acute GVHD, these same associations were observed in subgroup analysis in patients who did not have acute GVHD (Figure 2). Moreover, these associations were maintained after adjustment for the occurrence of acute GVHD as a time-varying covariate (HH group: HR = 0.91, $P = .009$; HL group: HR = 0.96, $P = .036$).



		Multi	
	n	HR (95% CI)	p
LfHP x LfHP	11,117	Ref. 1.00	
HfHP x LfHP	8,409	0.93 (0.89–0.98)	0.003
HfHP x HfHP	1,601	0.88 (0.81–0.97)	0.009

		Multi				Multi				Multi	
		HR (95% CI)	p			HR (95% CI)	p			HR (95% CI)	p
LfHP x LfHP	2,470	Ref. 1.00		LfHP x LfHP	5,144	Ref. 1.00		LfHP x LfHP	3,503	Ref. 1.00	
HfHP x LfHP	1,470	0.93 (0.85–1.03)	0.181	HfHP x LfHP	5,366	0.89 (0.83–0.95)	0.001	HfHP x LfHP	1,573	0.9 (0.82–0.99)	0.025
HfHP x HfHP	312	0.91 (0.75–1.1)	0.311	HfHP x HfHP	1,057	0.74 (0.66–0.84)	<0.001	HfHP x HfHP	232	0.97 (0.79–1.19)	0.744

Figure 1. Cumulative incidence and multivariable analysis of the impact of high-frequency HLA haplotypes on clinical CMV reactivation. (A) All subjects (n = 21,127). (B) Each donor source group (related transplant: n = 4252; unrelated transplant: n = 11,567; cord blood transplant: n = 5308). Multivariable analyses were adjusted for recipient age (0 to 15 years, 16 to 49 years, or ≥50 years), recipient sex, type of disease, disease status before transplantation (standard or high risk), type of GVHD prophylaxis (cyclosporine based or tacrolimus based), type of conditioning regimen (myeloablative or reduced intensity), use of total body irradiation (yes or no), use of antithymocyte globulin (yes or no), CMV serostatus of recipients and donors (recipient-donor pairs), and the number of HLA mismatches at the HLA-A, HLA-B, and HLA-DRB1 loci (0 to 6).



		Multi	
	n	HR (95% CI)	p
LfHP x LfHP	11,117	Ref. 1.00	
HfHP x LfHP	8,409	0.95 (0.89–1.01)	0.123
HfHP x HfHP	1,601	0.86 (0.76–0.98)	0.022

Figure 2. Cumulative incidence and multivariable analysis of the impact of high-frequency HLA haplotypes on clinical CMV reactivation in patients without acute GVHD. Subjects without acute GVHD (n = 13,252). Multivariable analyses were adjusted for recipient age (0 to 15 years, 16 to 49 years, or ≥50 years), recipient sex, type of disease, disease status before transplantation (standard or high risk), type of GVHD prophylaxis (cyclosporine based or tacrolimus based), type of conditioning regimen (myeloablative or reduced intensity), use of total body irradiation (yes or no), use of antithymocyte globulin (yes or no), CMV serostatus of recipients and donors (recipient-donor pairs), and the number of HLA mismatches at the HLA-A, HLA-B, and HLA-DRB1 loci (0 to 6).

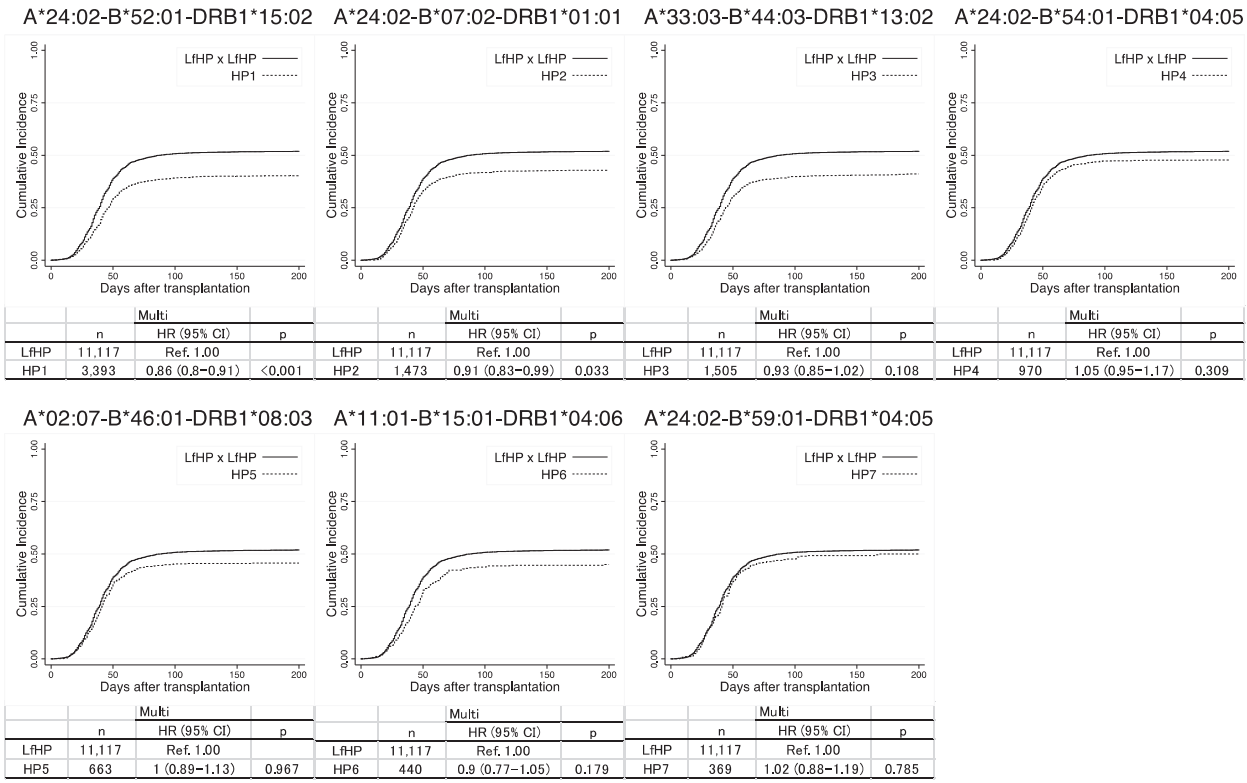
In subgroup analyses of different donor sources, these associations were statistically significant in unrelated donor transplants (HH group: HR = 0.74, $P < .001$; HL group: HR = 0.89, $P = .001$) and in cord blood transplants in the HL group (HR = 0.90, $P = .025$) (Figure 1B).

Impact of Individual High-Frequency HLA Haplotypes on Clinical CMV Reactivation

To evaluate the individual HfHPs (HPs 1 to 7), the CMV risk of homozygotes of each HfHP and heterozygotes of each HfHP and LfHP was compared with that of the LL group. For example, recipients who were homozygous for HP 1 or heterozygous for HP 1 and LfHP (HP 1 group) were compared with the LL group. The HP 1 (A*24:02-B*52:01-DRB1*15:02) group and HP 2 (A*24:02-B*07:02-DRB1*01:01) group had a significantly lower risk of clinical CMV reactivation than the LL group (HP 1 group: HR = 0.86, $P < .001$; HP 2 group: HR = 0.91, $P = .033$) (Figure 3). Results of the subgroup analyses for different donor sources are shown in Supplementary Table S1.

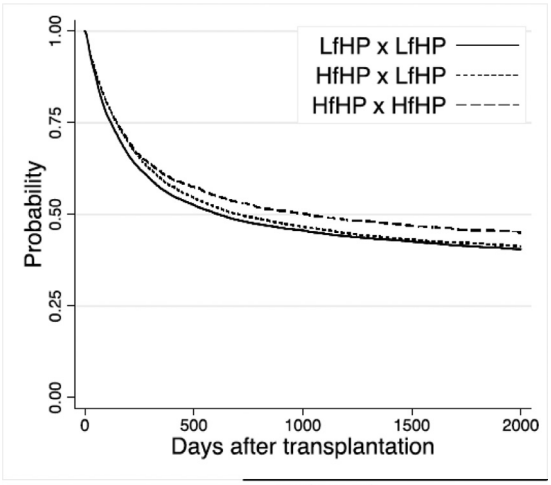
Overall Survival

The unadjusted 3-year OS rate was 44.8% (95% confidence interval [CI], 43.8% to 45.8%) in the LL group, 45.8% (95% CI, 44.7% to 47.0%) in the HL group, and 49.2% (95% CI, 46.7% to 51.8%) in the HH group (Figure 4). Although the unadjusted OS rate in the HH group was higher than that in the HL and LL groups, there was no significant difference between the HH and LL groups (HR of the HH group compared with the LL group for OS was 1.04, $P = .349$) in the multivariable analysis.



		Multi				Multi				Multi				Multi		
	n	HR (95% CI)	p		n	HR (95% CI)	p		n	HR (95% CI)	p		n	HR (95% CI)	p
LfHP	11,117	Ref. 1.00		LfHP	11,117	Ref. 1.00		LfHP	11,117	Ref. 1.00		LfHP	11,117	Ref. 1.00	
HP1	3,393	0.86 (0.8–0.91)	<0.001	HP2	1,473	0.91 (0.83–0.99)	0.033	HP3	1,505	0.93 (0.85–1.02)	0.108	HP4	970	1.05 (0.95–1.17)	0.309
LfHP	11,117	Ref. 1.00		LfHP	11,117	Ref. 1.00		LfHP	11,117	Ref. 1.00					
HP5	663	1 (0.89–1.13)	0.967	HP6	440	0.9 (0.77–1.05)	0.179	HP7	369	1.02 (0.88–1.19)	0.785				

Figure 3. Cumulative incidence and multivariable analysis of the impact of individual high-frequency HLA haplotypes on clinical CMV reactivation. All subjects (n = 21,127). The results for each high-frequency haplotype (HP 1 to HP 7) group are shown. Multivariable analyses were adjusted for recipient age (0 to 15 years, 16 to 49 years, or ≥50 years), recipient sex, type of disease, disease status before transplantation (standard or high risk), type of GVHD prophylaxis (cyclosporine based or tacrolimus based), type of conditioning regimen (myeloablative or reduced intensity), use of total body irradiation (yes or no), use of antithymocyte globulin (yes or no), CMV serostatus of recipients and donors (recipient-donor pairs), and the number of HLA mismatches at the HLA-A, HLA-B, and HLA-DRB1 loci (0 to 6).



	n	HR (95% CI)	p
LfHP x LfHP	11,117	Ref. 1.00	
HfHP x LfHP	8,409	1.08 (1.03–1.12)	<0.001
HfHP x HfHP	1,601	1.04 (0.96–1.12)	0.349

Figure 4. Overall survival. All subjects (n = 21,127). Kaplan-Meier curves estimating the unadjusted probability of overall survival in all subjects are shown along with the results of the multivariable analysis for overall survival in all subjects. Multivariable analyses were adjusted for recipient age (0 to 15 years, 16 to 49 years, or ≥50 years), recipient sex, type of disease, disease status before transplantation (standard or high risk), type of GVHD prophylaxis (cyclosporine based or tacrolimus based), type of conditioning regimen (myeloablative or reduced intensity), use of total body irradiation (yes or no), use of antithymocyte globulin (yes or no), CMV serostatus of recipients and donors (recipient-donor pairs), and number of HLA mismatches at the HLA-A, HLA-B, and HLA-DRB1 loci (0 to 6).

In contrast, the HL group had significantly higher risk than the LL group (HR = 1.08, $P < .001$) (Figure 4).

DISCUSSION

Allo-HSCT is an established curative therapy for hematologic malignancies and other hematologic disorders. Although accumulated evidence from extensive recent research indicates a role for HLA allele matching and haplotype matching between recipients and donors in transplant outcomes [28-31], the association between a particular HLA allele or HLA haplotype and the occurrence of infection has been scarcely explored. Du et al. [32] demonstrated using multivariable analysis that HLA-DRB1*09, grade II to IV acute GVHD, and CMV-seronegative donors were associated with an increased incidence of CMV infection and disease after allo-HSCT, although their sample size was relatively small. We could not replicate this association. One possible explanation for this is that the distribution of HLA haplotypes containing HLA-DRB1*09 is different between Japanese and Chinese populations [33]. In this study, we focused on the frequency of HLA haplotype in a Japanese population and analyzed the association between the frequency and risk of CMV clinical reactivation using a large cohort of allo-HSCT. We demonstrated that homozygotes of HfHP and heterozygotes of HfHP and LfHP had a statistically significantly lower risk of clinical CMV reactivation than homozygotes of LfHP after adjustment for potential confounders, including acute GVHD and CMV seropositivity. Therefore, increased care regarding CMV reactivation may be necessary for patients with LfHP after allo-HSCT.

The mechanisms underlying our finding that homozygotes of HfHP and heterozygotes of HfHP and LfHP had a statistically significantly lower risk of clinical CMV reactivation than homozygotes of LfHP have not been investigated. One possible mechanism is that the frequency of CMV-protective haplotypes was elevated through natural selection for thousands of years in the Japanese people and their ancestors. HLA is the human version of the major histocompatibility complex. HLA genes are the most variable genes in the human genome and are generally inherited as a haplotype. It is widely accepted that HLA genes and their haplotypes have been under balancing selection and that the mechanism is related to protectivity against infectious disease [34]. This is also true for major histocompatibility complex genes in other vertebrates [34]. In a large case-control study in Gambia, Hill et al. [17,18] reported that HLA-Bw53 and DRB1*13:02-DQB1*05:01 haplotypes are protective against malaria infection. These HLA and haplotypes are common in West African populations but rare in other racial groups, suggesting that malaria infection affected the development of HLA genes and haplotype distribution in West Africa. HLA-Bw53 is also prevalent among tribes in Maharashtra, where malaria infection is common, but is less prevalent in the general population in Maharahtra [35]. Other HLA alleles, such as HLA-B*1513 [19], HLA-B35 [36], and HLA-A*02:11 [37], have been linked to protection against malaria infection in population-based studies where malaria infection is endemic. Recent genome-wide association studies using high-throughput genotyping technology have improved the efficiency of detecting loci that are associated with the risk for developing various diseases, including infectious diseases such as human immunodeficiency virus 1, hepatitis B and C, dengue, tuberculosis, and leprosy [38]. The many protective and susceptible loci detected in these studies were in genes or regions associated with human immunity, including HLA. These studies have implicated loci linked to innate and acquired immunity in human host defense against infectious disease and confirmed an important role for HLA in the susceptibility to a number of infectious diseases. These protective and susceptible alleles may have a selective effect on selection pressure produced, for example, by intermittent epidemics [39]. Although most HLA associations with infectious disease presumably show small effects, they are important from an evolutionary point of view [39]. Although CMV infection is not a life-threatening infection in healthy adults, congenital CMV infection is the most common intrauterine infection during pregnancy and causes severe sequelae such as neurologic disorders [12,13]. CMV infection also causes life-threatening events in immunodeficient individuals [8,10,11,40,41]. These findings suggest that alleles linked to protection from CMV infection may have a weak selective advantage that has shaped the distribution of HLA genes and haplotypes. Another possible mechanism is that high-frequency haplotypes have immunologic advantages, such as superior antigen presenting ability, which would suggest that HfHPs are less susceptible to not only CMV infection but also potentially many other infections. To prove this hypothesis, similar analysis is needed for other infections. In this study, there were insufficient data in the Japanese registry to perform analyses on other infections. Moreover, HLA or other HfHP genes or a summation of their effects may explain our findings.

This study has several limitations. The Japan Society for Hematopoietic Cell Transplantation has published guidelines for the diagnosis and management of CMV in transplant patients. Although surveillance and treatment of CMV at each transplant center were conducted according to these guidelines, as described in the Methods section, compliance to the guidelines was not confirmed for each center. Therefore, the results of this study should be interpreted with caution. Second, associations between a low risk of clinical CMV reactivation and

high-frequency HLA haplotypes were maintained after adjustment for acute GVHD as a time-varying covariate. The associations were also observed in subgroup analysis in patients who did not have acute GVHD (Figure 2). However, because GVHD is expected to lie somewhere along the causal path leading to clinical CMV reactivation, we were unable to conduct analysis that completely excluded the effects of GVHD. Third, because this was a hypothesis-testing study, we used all data to test the hypothesis that high-frequency HLA haplotypes are associated with a low risk of CMV clinical reactivation. HPs 1 to 7 are also high-frequency haplotypes in the Korean population [42]; however, the associations observed in this study have yet to be evaluated in the Korean population. These should be examined in a future study. Meanwhile, HPs 1 to 7 are not frequent in other populations.

In conclusion, our findings suggest that HfHPs may be protective against CMV reactivation and infection and that increased care regarding CMV reactivation and infection may be necessary for patients with LfHP after allo-HSCT. Validation in independent cohorts is necessary to strengthen the generalizability of the results. Further immunologic experimental studies are needed to elucidate the biologic mechanisms of these findings.

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SUPPLEMENTARY MATERIALS

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REFERENCES

1. Sun JC, Lanier LL. The natural selection of herpesviruses and virus-specific NK cell receptors. *Viruses*. 2009;1:362.
2. Mendelson M, Monard S, Sissons P, Sinclair J. Detection of endogenous human cytomegalovirus in CD34+ bone marrow progenitors. *J Gen Virol*. 1996;77(pt 12):3099–3102.
3. Sindre H, Tjoonnfjord GE, Rollag H, et al. Human cytomegalovirus suppression of and latency in early hematopoietic progenitor cells. *Blood*. 1996;88:4526–4533.
4. Kondo K, Xu J, Mocarski ES. Human cytomegalovirus latent gene expression in granulocyte-macrophage progenitors in culture and in seropositive individuals. *Proc Natl Acad Sci USA*. 1996;93:11137–11142.
5. Taylor-Wiedeman J, Sissons P, Sinclair J. Induction of endogenous human cytomegalovirus gene expression after differentiation of monocytes from healthy carriers. *J Virol*. 1994;68:1597–1604.
6. Minton EJ, Tysoe C, Sinclair JH, Sissons JG. Human cytomegalovirus infection of the monocyte/macrophage lineage in bone marrow. *J Virol*. 1994;68:4017–4021.
7. Hahn G, Jores R, Mocarski ES. Cytomegalovirus remains latent in a common precursor of dendritic and myeloid cells. *Proc Natl Acad Sci USA*. 1998;95:3937–3942.
8. Cook M, Briggs D, Craddock C, et al. Donor KIR genotype has a major influence on the rate of cytomegalovirus reactivation following T-cell replete stem cell transplantation. *Blood*. 2006;107:1230–1232.
9. Legendre C, Pascual M. Improving outcomes for solid-organ transplant recipients at risk from cytomegalovirus infection: late-onset disease and indirect consequences. *Clin Infect Dis*. 2008;46:732–740.
10. Emery VC. Investigation of CMV disease in immunocompromised patients. *J Clin Pathol*. 2001;54:84–88.
11. Soderberg-Naucler C. Treatment of cytomegalovirus infections beyond acute disease to improve human health. *Expert Rev Anti Infect Ther*. 2014;12:211–222.
12. Stagno S, Whitley RJ. Herpesvirus infections of pregnancy. Part II: Herpes simplex virus and varicella-zoster virus infections. *N Engl J Med*. 1985;313:1327–1330.
13. Fowler KB, Stagno S, Pass RF, Britt WJ, Boll TJ, Alford CA. The outcome of congenital cytomegalovirus infection in relation to maternal antibody status. *N Engl J Med*. 1992;326:663–667.
14. Bjorkman PJ, Parham P. Structure, function, and diversity of class I major histocompatibility complex molecules. *Annu Rev Biochem*. 1990;59:253–288.
15. Little AM, Parham P. Polymorphism and evolution of HLA class I and II genes and molecules. *Rev Immunogenet*. 1999;1:105–123.
16. Robinson J, Halliwell JA, Hayhurst JD, Flicek P, Parham P, Marsh SG. The IPD and IMGT/HLA database: allele variant databases. *Nucleic Acids Res*. 2015;43:D423–D431.
17. Hill AV, Allsopp CE, Kwiatkowski D, et al. Common west African HLA antigens are associated with protection from severe malaria. *Nature*. 1991;352:595–600.
18. Hill AV, Elvin J, Willis AC, et al. Molecular analysis of the association of HLA-B53 and resistance to severe malaria. *Nature*. 1992;360:434–439.
19. Hirayama K, Zaidi AS, Lokman Hakim S, et al. Molecular analysis of HLA-B in the Malaysian aborigines. *Tissue Antigens*. 1996;48:692–697.
20. Matzaraki V, Kumar V, Wijmenga C, Zhernakova A. The MHC locus and genetic susceptibility to autoimmune and infectious diseases. *Genome Biol*. 2017;18:76.
21. Atsuta Y. Introduction of Transplant Registry Unified Management Program 2 (TRUMP2): scripts for TRUMP data analyses, part I (variables other than HLA-related data). *Int J Hematol*. 2016;103:3–10.
22. Kuwatsuka Y. Quality control and assurance in hematopoietic stem cell transplantation data registries in Japan and other countries. *Int J Hematol*. 2016;103:20–24.
23. Atsuta Y, Suzuki R, Yoshimi A, et al. Unification of hematopoietic stem cell transplantation registries in Japan and establishment of the TRUMP System. *Int J Hematol*. 2007;86:269–274.
24. Ikeda N, Kojima H, Nishikawa M, et al. Determination of HLA-A, -C, -B, -DRB1 allele and haplotype frequency in Japanese population based on family study. *Tissue Antigens*. 2015;85:252–259.
25. Ljungman P, Boeckh M, Hirsch HH, et al. Definitions of cytomegalovirus infection and disease in transplant patients for use in clinical trials. *Clin Infect Dis*. 2017;64:87–91.
26. Takenaka K, Nishida T, Asano-Mori Y, et al. Cytomegalovirus reactivation after allogeneic hematopoietic stem cell transplantation is associated with a reduced risk of relapse in patients with acute myeloid leukemia who survived to day 100 after transplantation: the Japan Society for Hematopoietic Cell Transplantation Transplantation-related Complication Working Group. *Biol Blood Marrow Transplant*. 2015;21:2008–2016.
27. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med*. 1999;18:695–706.
28. Petersdorf EW, Malkki M, Gooley TA, Martin PJ, Guo Z. MHC haplotype matching for unrelated hematopoietic cell transplantation. *PLoS Med*. 2007;4:e8.
29. Petersdorf EW. Role of major histocompatibility complex variation in graft-versus-host disease after hematopoietic cell transplantation. *F1000Res*. 2017;6:617.
30. Morishima Y, Kashiwase K, Matsuo K, et al. Biological significance of HLA locus matching in unrelated donor bone marrow transplantation. *Blood*. 2015;125:1189–1197.
31. Morishima S, Ogawa S, Matsubara A, et al. Impact of highly conserved HLA haplotype on acute graft-versus-host disease. *Blood*. 2010;115:4664–4670.
32. Du J, Liu J, Gu J, Zhu P. HLA-DRB1*09 is associated with increased incidence of cytomegalovirus infection and disease after allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2007;13:1417–1421.
33. Li XF, Zhang X, Chen Y, Zhang KL, Liu XJ, Li JP. An analysis of HLA-A, -B, and -DRB1 allele and haplotype frequencies of 21,918 residents living in Liaoning, China. *PLoS One*. 2014;9:e93082.
34. Garrigan D, Hedrick PW. Perspective: detecting adaptive molecular polymorphism: lessons from the MHC. *Evolution*. 2003;57:1707–1722.

35. Ghosh K. Evolution and selection of human leukocyte antigen alleles by *Plasmodium falciparum* infection. *Hum Immunol*. 2008;69:856–860.
36. Contu L, Carcassi C, Orru S, et al. HLA-B35 frequency variations correlate with malaria infection in Sardinia. *Tissue Antigens*. 1998;52:452–461.
37. Mehra NK, Jaini R, Rajalingam R, Balamurugan A, Kaur G. Molecular diversity of HLA-A*02 in Asian Indians: predominance of A*0211. *Tissue Antigens*. 2001;57:502–507.
38. Chapman SJ, Hill AV. Human genetic susceptibility to infectious disease. *Nat Rev Genet*. 2012;13:175–188.
39. Hill AV. Immunogenetics: defence by diversity. *Nature*. 1999;398:668–669.
40. Ljungman P, de la Camara R, Cordonnier C, et al. Management of CMV, HHV-6, HHV-7 and Kaposi-sarcoma herpesvirus (HHV-8) infections in patients with hematological malignancies and after SCT. *Bone Marrow Transplant*. 2008;42:227–240.
41. Clark DA, Emery VC, Griffiths PD. Cytomegalovirus, human herpesvirus-6, and human herpesvirus-7 in hematological patients. *Semin Hematol*. 2003;40:154–162.
42. Park H, Lee YJ, Song EY, Park MH. HLA-A, HLA-B and HLA-DRB1 allele and haplotype frequencies of 10 918 Koreans from bone marrow donor registry in Korea. *Int J Immunogenet*. 2016;43:287–296.