Predicting outcomes of HLA-identical allogeneic stem cell transplants from variable number of tandem repeat disparity between donors and recipients

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Background and Objectives. Detecting differences in the variable number of tandem repeats (VNTR) between a recipient and a donor has already been used to monitor the degree of chimerism after allogeneic stem cell transplantation (SCT). Alongside major histocompatibility complex disparity, the disparity of various polymorphous proteins encoded by several genes may play a critical role in the pathogenesis of graft-versus-host disease (GVHD) in allogenic SCT. However, the biological effect of VNTR disparity has scarcely been studied.

Design and Methods. Eighty-four patients receiving an SCT from an HLA-identical sibling (n=68) or an unrelated donor (n=16) were analyzed. The patients were transplanted because of acute myeloid leukemia (ns=48), acute lymphoblastic leukemia (n=8), chronic myeloid leukemia (n=15), non-Hodgkin's lymphoma (n=18) and myelodysplastic syndrome (n=3). Polymerase chain reaction analysis was performed to amplify three VNTR regions (D1S80, D1S111, and D17S5). These regions were classified as fully matched, partially matched, or mismatched between donors recipients.

Results. A strong correlation was observed between D1S80 matching status and transplant outcomes in terms of overall survival (p=0.0179) and non-relapse mortality (p=0.0305), but not for the D1S111 or D17S5 disparity. The fully matched D1S80 pairs showed a better overall survival (72% vs 38%) and lower non-relapse mortality (17% vs 50%) compared to the partially matched or mismatched pairs. In multivariate analyses, a fully matched D1S80 pair was found to be an independent favorable prognostic factor for overall survival (p=0.03) and non-relapse mortality (p=0.05). In addition, D1S80 disparity was significantly associated with the occurrence of gut chronic GVHD (p=0.05).

Interpretations and Conclusion. The present data suggest that disparities in D1S80 – located in chromosome 1 – are associated with an increased incidence of gut chronic GVHD and non-relapse mortality.

Key words: variable number of tandem repeats, disparity, allogeneic stem cell transplantation.

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The variable number of tandem repeats (VNTR) and short tandem repeats (STR) have been widely adopted to detect and monitor the degree of hematopoietic engraftment or chimerism with a high sensitivity and specificity after allogeneic stem cell transplantation (SCT).[1-5] However, when the VNTR between the donor and the recipient are completely identical, they cannot be used for monitoring chimerism. In the setting of HLA-identical SCT, alloreactive donor T-cells can recognize the disparate molecules of the recipient located at various polymorphic sites in the human genome, and thus play a major role in the pathogenesis of graft-versus-host disease (GVHD).[6,7] The minor histocompatibility antigen (mHA), one of the polymorphous proteins encoded by genes located throughout the human genome, has been identified as a major disparate molecule between the donor and the recipient.[6] Similarly, disparate VNTR or STR alleles between a donor and a recipient imply differences in the corresponding genes or chromosomes between the donor and the recipient. However, little is known about the biological role of the VNTR or STR disparity.

Recently, Alcoceba *et al.*[8] used microsatellite STR methods to reveal that disparities of *D13S317, D18S51*, and TPOX were associated with the severity of acute GVHD, while disparities of D16S539 were associated with chronic GVHD. Meanwhile, Stern *et al.*[9] reported that disparities of D13S317 were associated with increased incidence and severity of acute GVHD, suggesting the existence of an unknown mHA in chromosome 13. In this study we analyzed the effect of VNTR disparity at the D1S80, D1S111 and D17S5 loci between the donor and the recipient in terms of overall survival, non-relapse mortality, relapse and chronic GVHD after

allogeneic SCT from an HLA-identical sibling or unrelated donor.

Design and Methods

Patients' characteristics and transplantation procedures

A total of 84 patients who received an allogeneic SCT from an HLA-matched sibling (n=68) or unrelated donor (n=16) at Kyungpook National University Hospital between December 1996 and December 2004 were enrolled in the current study. The median age of the patients was 35.0 years (range 17~58 years), and the male to female ratio was 64:36 (n=54:30). The patients were transplanted for the following diseases: acute myeloid leukemia (AML, n=48, 56%), acute lymphoblastic leukemia (ALL, n=8, 10%), chronic myeloid leukemia (CML, n=15, 18%), non-Hodgkin's lymphoma (n=10, 12%), and high-risk myelodysplastic syndrome, including chronic myelomonocytic leukemia and refractory anemia with excess blasts (MDS, n=3, 4%). Forty-seven patients had a standard disease status (56%) and 37 had advanced disease (44%).

The transplant procedures were conducted as previously described.[10,11] Briefly, the conditioning regimens for the allogeneic recipients were busulfan/cytoxan (n=60) or fludarabine-based reduced intensity conditioning (n=24) The fludarabine-based regimens were as follows: (i) fludarabine, busulfan and cyclophosphamide (n=12); (ii) fludarabine, idarubicin and cytarabine (n=4); (iii) fludarabine and cyclophosphamide (n=2); (iiii) fludarabine and busulfan with antithymocyte globulin (n=6). Aphereses were performed on 61 patients (73%) from sibling donors, as previously described,[12,13] while 23 patients (27%) received a marrow harvest from sibling (n=7) or unrelated donors (n=16) as the stem cell source. Before the HLA-identical unrelated SCT, high-resolution DNA genotyping for HLA-A, B, C, and DR was performed (Biosewoom Inc. Seoul, Korea), and molecular matches confirmed for each HLA locus.

Prophylaxis against acute GVHD consisted of methotrexate and cyclosporine A (CSA; Cipol-N®, ChongKunDang, Seoul, Korea) in 77 patients, cyclosporine A alone in five patients, and FK506 plus short-term methotrexate in two patients. Prophylaxis against infections consisted of ciprofloxacin (250 mg *bid* p.o.)/metronidazole (500 mg *tid* p.o.)/fluconazole (100 mg *qd* p.o.), beginning with the initiation of conditioning, and acyclovir (600 mg *bid* p.o.) from day -1 until day +180. Co-trimoxazole was started after engraftment, and ursodeoxycholinic acid, used for prophylaxis against veno-occlusive disease was started at the same time as the conditioning regimen. Immunoglobulins (500 mg/kg) were infused intravenously every two weeks until day +100, then every month until 6 months. All patients received irradiated blood products that had been depleted of leukocytes using filters.

VNTR analyses for D1S80, D1S111, and D17S5 loci

Genomic DNA was extracted from peripheral blood samples from the recipient and donor before the SCT using a Wizard genomic DNA purification kit (Promega, Madison, WI, USA). All recipients and donors gave informed consent to all procedures. Polymerase chain reaction (PCR) analyses for three VNTR regions were then performed using primers that amplified a short fragment of DNA containing the polymorphic sites. The PCR primers for the D1S80 loci were 5'-GTC TTG TTG GAG ATG CAC GTG CCC CTT GC-3', and 5'-GAA ACT GGC CTC CAA ACA CTG CCC GCC G-3', while those for the D1S111 loci were 5'-TGT GAG TAG AGG AGA CCT CAC-3', and 5'-AAA GAC CAC AGA GTG AGG AGC-3', and those for the D17S5 loci were 5'-GGT CGA AGA GTG AAG TGC ACA G-3', and 5'-CAC AGT CTT TAT TCT TCA GCG-3'. The PCR were performed in a 20 μL reaction volume containing 17 μL of AccuPower PCR premix (Bioneer, Cheongwon, Korea), 1 μL of each primer, and 250 ng of genomic DNA. The PCR program for the D1S80 and D1S111 loci consisted of 30 cycles at 94°C for 30 sec, 65°C for 30 sec, and a final elongation step at 65°C for 20 min. The PCR program for the D17S5 loci consisted of 35 cycles at 94°C for 45 sec, 60°C for 30 sec, 72°C for 120 sec, and a final elongation step at 72°C for 2 min using a GeneAmp PCR 2400/9600 (Perkin Elmer, Roche Diagnostics Industry, Basel, Switzerland). The PCR products were separated on 2% agarose gel, then analyzed using the 1D-main software (Bioneer, Cheongwon, Korea). The current study was approved by the institutional research board of Kyungpook National University Hospital. VNTR data were available for 84 patients on the D1S80 loci, for 83 patients on the D1S111 loci, and for 78 patients on the D17S5 loci.

Definitions

The loci examined were classified as fully matched, partially matched, or mismatched. A fully matched pair was defined as completely identical bands between the donor and the recipient. A partially matched pair was defined as the co-existence of some identical bands and some different bands between the donor and the recipient, implying one identical allele and another non-identical allele. A mismatched pair was defined as completely different bands between the donor and the recipient. The day of the stem cell infusion was defined as day 0. Acute and chronic GVHD were diagnosed and graded using established criteria.[14,15] The date of onset of organ-specific acute or chronic GVHD was defined as the first day of the symptoms and/or signs of each organ-specific acute or chronic GVHD. Overall survival was defined as the time from transplantation until death from any cause. Non-relapse mortality was defined as a death unrelated to recurrence or disease progression. The cumulative incidence of relapse was defined as the time from transplantation until disease progression. High-risk diseases were defined as any disease that had relapsed after allogeneic or autologous SCT, acute leukemia in more than the first complete remission, Philadelphia positive acute lymphoblastic

leukemia, advanced phase chronic myelogenous leukemia and primary refractory or multiply relapsed malignancies.

Statistical analysis

The results were analyzed according to information available as of February 2005. The univariate associations of the patients' characteristics, transplantation procedures, or transplant outcomes with the VNTR disparity were analyzed using Fisher's exact test or Mann-Whitney's U-test, as appropriate. The estimates of overall survival and the cumulative incidences of non-relapse mortality and relapse were calculated using the method of Kaplan and Meier. The differences in overall survival, non-relapse mortality and the probability of relapse were compared using a log-rank test according to each VNTR disparity for the D1S80, D1S111, and D17S5 loci. For the multivariate survival analyses, a Cox's proportional hazard model was adopted to define the prognostic factors for overall survival, non-relapse mortality, and the probability of relapse using a backwards conditional procedure until the p-value for the likelihood ratio test was >0.05. The following variables were included for the analyses: D1S80 disparity (fully matched pairs vs. partially matched or mismatched pairs), disease status (standard vs. advanced risk), transplanted dose of $CD34^+$ cells (6×10^6/Kg), donor type (sibling vs. unrelated donors), sex-mismatch (female-to-male versus others), and GVHD (acute GVHD grade 0~2 vs. 3 and 4 and development of chronic GVHD) for all patients. The hazard ratio (HR) and 95% confidence interval (CI) were also estimated. A cut off p-value of 0.05 was adopted for all the statistical analyses. The statistical data were generated using an SPSS software package (SPSS 11.5 Inc. Chicago, IL, USA).

Results

Overall transplant outcomes

With a median follow-up of 712 days (range, 83-2,229 days) for the surviving patients, the overall survival was calculated to be 48±6% with a non-relapse mortality of 40±6% and a cumulative incidence of relapse of 38±6% after 2 years. Out of the total 84 cases, 43 patients (51%) died of causes other than recurrence and disease progression (n=30, 36%) or of progressive disease (n=13, 15%).

When comparing the transplant outcomes according to the type of donor, no differences were observed in of overall survival (p=0.6942), non-relapse mortality (p=0.3223), or relapse (p=0.1759) between recipients of grafts from HLA-matched siblings or unrelated donors.

Frequency of VNTR disparity for D1S80, D1S111, and D17S5 loci

The VNTR disparity status between the donor and the recipient was as follows: for the D1S80 loci, there were 24 (29%) fully matched pairs, 27 (32%) partially matched pairs, and 33 (39%) mismatched pairs. For the D1S111 loci, there were 24 (29%) fully matched pairs, 38 (46%) partially matched pairs, and 21 (25%) mismatched pairs. For the D17S5 loci, there were 37 (47%) fully matched pairs, 17 (22%) partially matched pairs, and 24 (31%) mismatched pairs.

Table 1. Transplant outcomes (overall survival, non-relapse mortality, and relapse) according to VNTR disparities.

	Two-year OS (%)	*Two-year NRM (%)*	*Two-year Relapse (%)*
D1S80	0.0179	0.0305	0.2317
Fully matched (n=24, 29%)	72±10	17±9	25±10
Partially matched (n=27, 32%)	37±11	52±12	41±11
Mismatched (n=33, 39%)	38±9	50±10	43±11
D1S111	0.6327	0.6334	0.8000
Fully matched (n=24, 29%)	50±11	35±11	36±11
Partially matched (n=38, 46%)	50±8	40±9	31±8
Mismatched (n=21, 25%)	45±12	46±13	57±16
D17S5	0.8470	0.9814	0.9953
Fully matched (n=37, 47%)	48±9	43±9	37±9
Partially matched (n=17, 22%)	52±12	33±12	40±14
Mismatched (n=24, 31%)	36±12	50±14	39±13

**OS: overall survival; NRM: non-relapse mortality.*

The transplant outcomes according to each VNTR disparity for the D1S80, D1S111, and D17S5 loci are summarized in Table 1. Briefly, the difference was significant in favor of the fully matched D1S80 pairs compared to the partially matched or mismatched pairs with respect to overall survival (p=0.0179; Figure 1A) and non-relapse mortality (p=0.0305), but not for relapse. No difference in transplant outcomes was found according to VNTR disparity for the D1S111 or D17S5 loci (Figures 1B and C).

VNTR disparity at D1S80 loci and transplant outcomes

The patients' characteristics and brief descriptions of the transplantation procedures according to the VNTR disparity at the D1S80 loci are summarized in Table 2, Although no differences in the patients' characteristics or transplant procedures were noted among the fully matched, partially matched, and mismatched pairs, overall survival and the cumulative incidence of myeloid engraftment were significantly better in the fully matched pairs. No differences were noted in acute GVHD, cytomegalovirus reactivation, opportunistic infection, or relapse, (Table 3).

As shown in Table 1 and Figure 2A, non-relapse mortality was significantly lower among the fully matched pairs (17±9%) than among the partially matched (52±12%) or mismatched pairs (50±10%; p=0.0305) at the D1S80 loci, although the incidence of relapse was not different among the three groups (p=0.2317). In general, GVHD and opportunistic infections are considered the two main causes of non-relapse mortality after allogeneic SCT; VNTR disparity at the D1S80 loci did not seem to influence the incidence of opportunistic infections or the incidence or severity of acute/chronic GVHD (Table 3). Interestingly, however, when including organ-specific incidences of GVHD in the analyses,

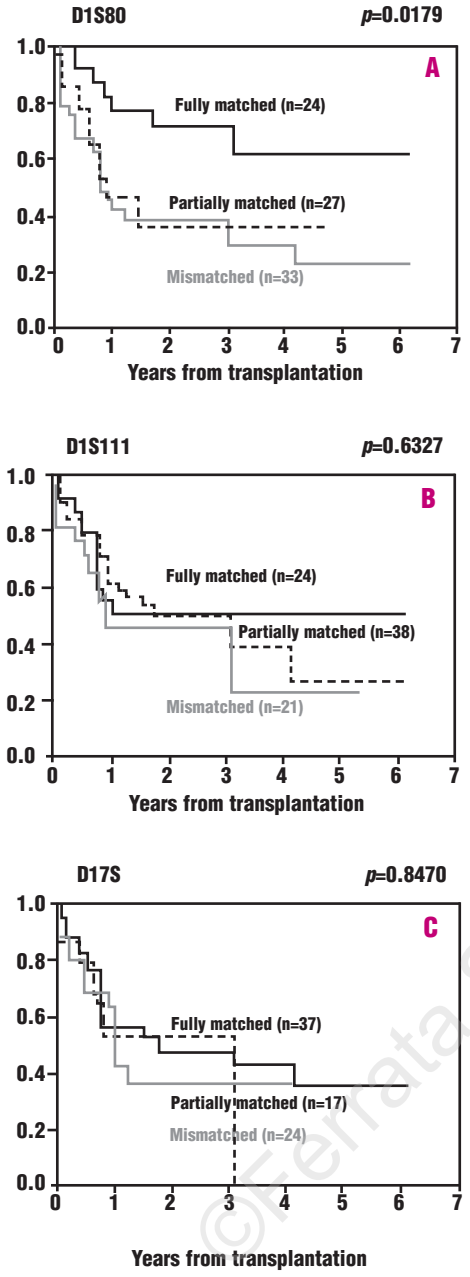

Figure 1. Overall survival according to VNTR disparity (A, D1S80; B D1S111; C, D17S5)

the occurrence of chronic GVHDof the gut significantly differed depending on the VNTR disparity at the D1S80 loci. No patients with fully matched D1S80 pairs developed chronic GVHD of the gut, while 22% and 16% of the patients with partially matched and mismatched pairs, respectively, developed this organ-specific GVHD (p=0.05; Table 3 and Figure 2B). When comparing the patients with fully matched D1S80 pairs to those with D1S80 disparities, the difference was even more significant, as shown in Figure 2C (0% in fully matched pairs vs. 32% in pairs with some type of disparity p=0.0144).

When analyzing the causes of death according to the

Table 2. Patients' characteristics and transplantation procedures according to VNTR disparity for D1S80 loci.

Disparity of D1S80	*Fully matched pairs (n=24, 29%)*	*Partially matched pairs (n=27; 32%)*	*Mismatched pairs (n=33, 39%)*	*p value*
Sex (F/M, %)	12/12 (50:50)	8/19 (30:70)	10/23 (30:70)	0.22
Female to male/ Others	2/10 (8/92)	8/19 (30/70)	6/27 (18/82)	0.21
Age (median, range)	37.0 (17~54)	32 (17~49)	33 (18~58)	0.66
Diagnosis (%)				
AML/ALL	14/2 (58/8)	16/1 (59/4)	18/5 (55/15)	0.40
CML/MDS	3/3 (13/13)	6/0 (22/0)	6/0 (18/0)	
NHL	2 (8)	4 (15)	4 (12)	
Advanced disease	9 (38)	13 (48)	15 (46)	0.73
Conditioning (%)				
BuCy	16 (67)	21 (78)	23 (70)	0.65
Fludarabine-based RIST	8 (33)	6 (22)	10 (30)	
GVHD prophylaxis				
CSA/MTX	21 (88)	26 (96)	30 (91)	0.38
CSA alone	2 (8)	0 (0)	3 (9)	
FK506/sMTX	1 (4)	1 (4)	0 (0)	
Source of stem cells				
Sibling/PBSC	19 (79)	20 (74)	22 (67)	0.64
Sibling/BM	2 (9)	3 (11)	2 (6)	
Unrelated/BM	3 (12)	4 (15)	9 (27)	
Infused cell dose (mean±S.E.)				
MNC ($\times10^8$/Kg)	7.72±1.06	7.07±0.84	5.69±0.82	0.25
$CD34^+$ cells ($\times10^6$/Kg)	7.81±0.98	6.26±0.90	6.66±1.01	0.28
$CD3^+$ cells ($\times10^8$/Kg)	2.21±0.36	2.41±0.39	1.87±0.26	0.69

AML: acute myeloid leukemia; ALL: acute lymphoblastic leukemia; CML: chronic myeloid leukemia; MDS: myelodysplastic syndrome; NHL: non-Hodgkin's lymphoma; BuCy: busulfan/cyclophosphamide; RIST: reduced intensity conditioning stem cell transplantation; CSA: cyclosporine A; MTX: methotrexate; PBSC: peripheral blood stem cells; BM: bone marrow; MNC: mononuclear cells.

three different levels of VNTR disparity at the D1S80 loci (Table 3), only four patients (17%) with fully matched D1S80 pairs died of causes other than recurrence or disease progression while 26 patients (43%) with partially matched or mismatched pairs died of non-relapse, non-progression causes (p=0.02). In addition, non-relapse mortality from GVHD and/or opportunistic infections occurred in 17% of the fully matched D1S80 pairs, but in 38% of the partially matched or mismatched D1S80 pairs (p=0.05). No difference in mortality from disease progression was noted between the patients with fully matched D1S80 pairs (12%) and the patients with disparate D1S80 pairs (17%; p=0.93).

Multivariate survival analysis

In a multivariate survival analysis using a Cox's proportional hazard model, a fully matched D1S80 pair was found to have a favorable prognostic impact on overall survival and non-relapse mortality, but not on relapse (Table 4). Patients with disparate D1S80 pairs had a lower overall survival than did those with fully matched pairs (p=0.03, HR 2.217 [1.128~4.826]). Disparate D1S80 pairs were also found to be associated with a higher non-relapse mortality (p=0.05, HR 2.551 [1.000~7.021]) and severe grade 3 or 4 acute GVHD (p=0.004, HR 3.802 [1.538~9.346]. Therefore,

Table 3. Transplantation outcomes according to VNTR disparity at the D1S80 loci.

Disparity of D1S80	*Fully matched pairs (n=24, 29%)*	*Partially matched pairs (n=27; 32%)*	*Mismatched pairs (n=33, 39%)*	*p value*
Follow-up duration (days)	347 (15~2181)	232 (17~1290)	479 (15~2181)	
Engraftment				
Myeloid	13.0 (10~24)	13.5 (10~25)	16 (10~30)	0.01
Megakaryocyte	14 (9~161)	14 (10~56)	17 (0~42)	0.26
Acute GVHD (n=81)	(24)	(26)	(31)	
Overall	21 (88)	23 (89)	22 (71)	0.19
≥grade 2	17(71)	20 (77)	20 (65)	0.59
≥grade 3	4 (17)	7 (27)	8 (26)	0.64
Skin,≥ stage 2	12 (50)	14 (54)	15 (48)	0.92
Liver,≥ stage 1	4 (17)	9 (31)	13 (42)	0.14
Gut,≥ stage 1	14 (58)	16 (62)	17 (55)	0.88
Chronic GVHD (n=71)	(23)	(23)	(25)	
Limited + extensive	16 (70)	19 (83)	20 (80)	0.60
Extensive	9 (39)	11 (48)	11 (44)	0.87
Skin involvement	11 (48)	13 (57)	11 (44)	0.68
Hepatic involvement	11 (48)	12 (52)	14 (56)	0.85
Gut involvement	0 (0)	5 (22)	4 (16)	0.05
Infectious events				
CMV reactivation	15 (63)	15 (56)	17 (52)	0.71
Infectious events	11 (46)	10 (37)	18 (55)	0.40
Bacterial infections	6 (25)	7 (26)	10 (30)	0.91
Viral infections	6 (25)	3 (11)	10 (30)	0.21
Fungal infections	3 (13)	3 (11)	4 (12)	1.00
Survival				
Relapse	8 (33)	10 (26)	6 (29)	0.84
Deaths	7 (29)	15 (56)	21 (64)	0.03
Causes of death				
Non-relapse mortalities	4 (17)	11 (41)	15 (45)	0.063
GVHD and/or infection	4 (17)	10 (37)	13 (39)	0.154
Others	0 (0)	1 (4)1	2 (6)2	0.78
Progression	3 (12)	4 (15)	6 (18)	0.93

GVHD, graft-versus-host disease. [1].Others denote veno-occlusive disease (n=1). [2]Others denote veno-occlusive disease (n=1) and hemorrhagic uremic syndrome/thrombotic thrombocytopenic purpura (n=1). [3]p=0.02 when analyzed between fully matched D1S80 pairs versus disparate D1S80 pairs, including partially matched and mismatched pairs, based on χ^2 test. [4]p=0.05 when analyzed between fully matched D1S80 pairs versus disparate D1S80 pairs, including partially matched and mismatched pairs, based on χ^2 test.

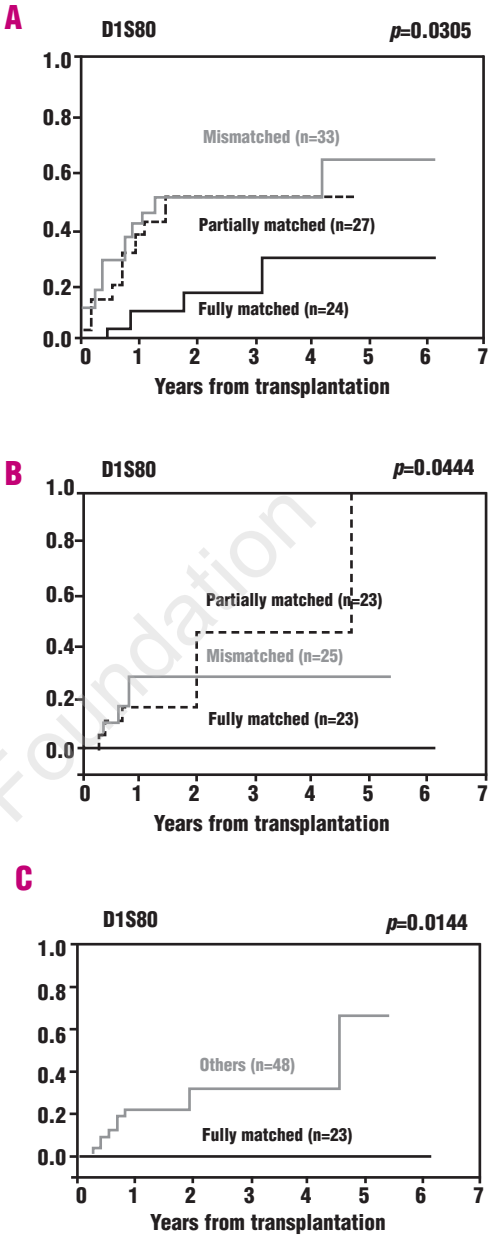

Figure 2. Cumulative incidence of non-relapse mortality (A) and occurrence of chronic GVHD of the gut (B,C) according to D1S80 disparity.

although VNTR disparity at the D1S80 loci did not affect the incidence of relapse, it did affect the disease risk (p=0.01, HR 3.155 [1.319~7.576]).

Discussion

This study examined the issue of VNTR disparity, especially at the D1S80 loci, and its clinical impact on transplant outcomes after HLA-identical SCT. VNTR disparity at the D1S80 loci was found to be significantly associated with overall survival, non-relapse mortality and the occurrence of chronic GVHD of the gut, thus influencing non-relapse mortality mainly through GVHD- or opportunistic infection-related deaths.

In the case of HLA-identical allogeneic SCT, the HLA antigens are genotypically identical between the donor and the recipient, yet GVHD occurs frequently and can be unpredictably fatal. This implies that T-cell recognition can occur beyond a mismatch in HLA molecules. In addition to major histocompatibility complex-disparity, the disparity of various polymorphous proteins encoded by genes located throughout the human genome may also play a critical role in the pathogenesis of GVHD.[7] Alloreactivity, the key mechanism in the pathogenesis of GVHD, is known to occur through the T-cell receptor recognition by donor T cells of different host-derived peptides that are bound to the HLA molecules on host antigen-presenting cells.[7] Similarly, disparate VNTR or STR alleles between the donor and the recipient imply disparities in their corresponding genes or chromosomes in the donor and recipient genome. However, the biological effect of VNTR disparity in a transplant setting has hardly been studied even though

Table 4. Multivariate survival analysis of prognostic factors for overall survival and cumulative incidence of non-relapse mortality or relapse in all patients.

	Risk factor	2 Yr rate (%)	HR [95% CI]	p value
Overall Survival				
D1S80 disparity	Fully matched	72±10	1.0	0.03
	Others	38±7	2.217 [1.128~4.826]	
Non-Relapse Mortality				
D1S80 disparity	Fully matched	17±9	1.0	0.05
	Others	50±8	2.551 [1.000~7.021]	
Acute GVHD ≥ grade 3	Grades 0~2	40±7	1.0	0.004
	Grades 3,4	61±13	3.802 [1.538~9.346]	
Relapse				
Disease risk	Standard risk	28±8	1.0	0.01
	Advanced risk	57±10	3.155 [1.319~7.576]	

**The analysis included D1S80 disparity (fully matched pairs vs. partially matched or mismatched pairs), disease status (standard vs. advanced risk), transplanted dose of $CD34^+$ cells (6×10^6/Kg), donor type (sibling vs. unrelated donors), and GVHD (acute GVHD grades 0 to 2 vs. 3 and 4 and development of chronic GVHD).*

the detection of VNTR has been used to monitor the degree of chimerism after allogeneic SCT. Huge numbers of single nucleotide polymorphisms (SNP) exist throughout the human genome. As a result, each SNP can result in the alteration of the biological function of the corresponding proteins via single amino acid substitutions, and many other changes may influence the immunogenic properties of the peptides that can be recognized by T cells in the context of HLA molecules.[6] In a recent study by Alcoceba *et al.*[8], microsatellite STR methods using PowerPlex16® (Promega, Madison, WI, USA) at 16 STR loci revealed that disparities of D13S317, D18S51, and TPOX were associated with the severity of acute GVHD , while disparities of D16S539 were associated with chronic GVHD. Although the number of loci discrepancies was not related to any clinical parameter, the overall survival was strikingly associated with the number of disparities at the 16 STR. Similarly, Stern *et al.,*[9] reported that disparate D13S317 pairs at 10 STR loci were associated with an increased incidence and severity of acute GVHD.

In the current study, a VNTR method was used to target novel loci, such as D1S80, D1S111, and D17S5, which are different from those used in the PowerPlex 16® system. Disparate alleles at the D1S80 loci were found to affect the transplant outcomes, especially the non-relapse mortality. A possible explanation of how VNTR disparity affects transplantation outcome might be alloreactivity, which is the combined effect of the recognition by donor T-cells of several different kinds of non-self host peptides. Generally, mHA are accepted as the accumulated phenotypic expression of various genetic variations that are predominantly SNP.[16] Thus, even though the present study did not focus on a specific SNP in a specific gene, disparate alleles based on the VNTR or STR seemed to reflect the genetic signatures of each individual's SNP. In the current study, the fully matched D1S80 pairs showed the lowest incidence of non-relapse mortality and chronic GVHD of the gut. Conversely, patients with disparate D1S80 pairs showed an increased incidence of chronic GVHD of the gut and mortality from GVHD and/or opportunistic infections, resulting in a lower survival. This implies that chronic GVHD of the gut is an important cause of non-relapse mortality after SCT and that D1S80 disparity may have an influence on this.

In a previous study, it was suggested that the skin, liver, gut, and lungs were more susceptible to alloreactive T-cell responses leading to GVHD, and that different factors may play a role in this tissue specificity of GVHD,[6] which could explain the higher incidence of chronic GVHD of the gut in the disparate D1S80 pairs. One possible explanation is the expression of gut-specific homing molecules in the donor T cells.[6] It has already been found that some T-cell subsets expressing cutaneous lymphocyte-associated antigens can result in specific homing of T cells to the skin.[17] Another possibility is selective gene expression in specific tissues, such as the gut or skin, which can lead to tissue-specific mHA even though these mHA have not yet been discovered.[6,7,16] The D1S80 loci may be associated with gut-specific antigen expression, thereby recruiting alloreactive donor T cells into the gut. It seems from the present data that disparities at D1S80 – located in chromosome 1 – are associated with an increased incidence of chronic GVHD of the gut and non-relapse mortality, suggesting the existence of unknown genes of mHA targeting the gut or cytokine/cytokine receptor in chromosome 1. In conclusion, the present findings suggest an association between D1S80 disparity and the outcomes after HLA-identical allogeneic SCT, including chronic GVHD oh the gut and non-relapse mortality. Thus, further study is strongly warranted to reach a final conclusion on the association of VNTR disparity, especially at the D1S80 loci, with transplant outcomes in patients under-going HLA-identical allogeneic SCT. The present data also suggest the existence of unknown genes for mHA targeting the gut or cytokine/cytokine receptor in chromosome 1.

D-H K and N-Y L contributed equally to the work and assume primary responsibility for it. D-H K was responsible for the design of the study, supervision of data collection, data analysis, and writing the manuscript. N-YL was responsible for the supervision of laboratory investigations, data interpretation, data analysis, and critical revision of the manuscript. H-D J and D-H K were involved in the laboratory investigations, interpretation of the data and critical revision of the manuscript.

S-K S, J-H B, J-G K were involved, to varying degrees, in the interpretation of data and critical revision of the manuscript. J-S S and K-BL were involved in critical revision of the manuscript. I-H S was involved in the design of study, statistical analysis of the data and critical revision of the manuscript.

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References

1. Martinelli G, Trabetti E, Zaccaria A, Farabegoli P, Buzzi M, Testoni N, et al. In vitro amplification of hypervariable DNA regions for the evaluation of chimerism after allogeneic BMT. Bone Marrow Transplant 1993;12:115-20.
2. van Leeuwen JE, van Tol MJ, Joosten AM, Schellekens PT, van den Bergh RL, Waaijer JL, et al. Relationship between patterns of engraftment in peripheral blood and immune reconstitution after allogeneic bone marrow transplantation for (severe) combined immunodeficiency. Blood 1994;84:3936-47.
3. Sreenan JJ, Pettay JD, Tbakhi A, Totos G, Sandhaus LM, Miller ML, et al. The use of amplified variable number of tandem repeats (VNTR) in the detection of chimerism following bone marrow transplantation. A comparison with restriction fragment length polymorphism (RFLP) by Southern blotting. Am J Clin Pathol 1997;107:292-8.
4. Rothberg PG, Gamis AS and Baker D. Use of DNA polymorphisms to monitor engraftment after allogeneic bone marrow transplantation. Clin Lab Med 1997; 17:109-18.
5. Thiede C. Diagnostic chimerism analysis after allogeneic stem cell transplantation: new methods and markers. Am J Pharmacogenomics 2004;4:177-87.
6. Falkenburg JH, van de Corput L, Marijt EW, Willemze R. Minor histocompatibility antigens in human stem cell transplantation. Exp Hematol 2003; 31:743-51.
7. Chao NJ. Minors come of age: minor histocompatibility antigens and graft-versus-host disease. Biol Blood Marrow Transplant 2004;10:215-23.
8. Alcoceba M, Diez-Campelo M, Martin-Jimenez P, Sarasquete ME, Armellini A, Blazquez L, et al. Allogeneic transplantation with identical MHC: clinico-prognostic value of discrepancies of microsatellite DNA regions between recipient and donor. Blood 2004;104:912a[abstract].
9. Stern M, Meyer-Monard S, Bucher C, Buser A, Heim D, Rovo A, et al. Prognostic value of discrepancies in micro-satellite DNA regions between donor and recipient in allogeneic stem cell transplantation. Bone Marrow Transplant 2005;35:S44.
10. Sohn SK, Kim DH, Kim JG, Lee NY, Suh JS, Lee KS, et al. Transplantation outcome in allogeneic PBSCT patients according to a new chronic GVHD grading system, including extensive skin involvement, thrombocytopenia, and progressive-type onset. Bone Marrow Transplant 2004;34:63-8.
11. Kim DH, Kim JG, Sohn SK, Sung WJ, Suh JS, Lee KS, et al. Clinical impact of early absolute lymphocyte count after allogeneic stem cell transplantation. Br J Haematol 2004;125:217-24.
12. Sohn SK, Kim JG, Chae YS, Kim DH, Lee NY, Suh JS, et al. Large-volume leukapheresis using femoral venous access for harvesting peripheral blood stem cells with the Fenwal CS 3000 Plus from normal healthy donors: predictors of $CD34^+$ cell yield and collection efficiency. J Clin Apheresis 2003; 18:10-5.
13. Sohn SK, Kim JG, Sung WJ, Kim DH, Suh JS, Lee KS, et al. Harvesting peripheral blood stem cells from healthy donors on 4^{th} day of cytokine mobilization. J Clin Apheresis 2003;18:186-9.
14. Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant 1995;15:825-8.
15. Shulman HM, Sullivan KM, Weiden PL, McDonald GB, Striker GE, Sale GE, et al. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. Am J Med 1980;69:204-17.
16. Roopenian D, Choi EY, Brown A. The immunogenomics of minor histocompatibility antigens. Immunol Rev 2002;190:86-94.
17. Fuhlbrigge RC, Kieffer JD, Armerding D and Kupper TS. Cutaneous lymphocyte antigen is a specialized form of PSGL-1 expressed on skin-homing T cells. Nature 1997; 389:978-81.