



# Donor *UNC-93 Homolog B1* genetic polymorphism predicts survival outcomes after unrelated bone marrow transplantation

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## Abstract

*UNC-93 homolog B1* (*UNC93B1*) is a key regulator of toll-like receptors (TLRs), pattern recognition receptors that sense invading pathogens and manage the innate immune response and deliver them from the endoplasmic reticulum to their respective endosomal signaling compartments. Several types of TLRs are known to contribute to the inflammatory process after allogeneic hematopoietic stem cell transplantation (SCT), so *UNC93B1* might play integral roles there. We investigated the influence of the *UNC93B1* single-nucleotide polymorphism (SNP) rs308328 (T>C) on transplant outcomes in a cohort of 237 patients undergoing unrelated HLA-matched bone marrow transplantation (BMT) for hematologic malignancies through the Japan Marrow Donor Program. The donor *UNC93B1* C/C genotype was associated with a better 3-year overall survival than the donor *UNC93B1* C/T or T/T genotype. An analysis of the *UNC93B1* rs308328 genotype may therefore be useful for selecting the donor, estimating the prognosis, and creating therapeutic strategies after allogeneic SCT.

## Introduction

Allogeneic hematopoietic stem cell transplantation (SCT) is expected to cure hematologic malignancies. However, life-threatening complications associated with allogeneic SCT,

such as severe infection, organ damage, and graft-versus-host disease (GVHD), remain obstacles to overcome [1]. Recently, increasing evidence has suggested that non-HLA genetic polymorphisms significantly influence outcomes after allogeneic SCT [2–13].

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Toll-like receptors (TLRs) are the most important family of receptors in the early to middle stages of infectious immunity, cooperatively recognizing patterns present in microorganisms and augmenting the synthesis of inflammatory mediators [14–17]. Previous studies [18–21] have suggested that the TLR -signaling pathway plays important roles in the anti-microbial immunity and GVHD after allogeneic SCT. TLR genes have several functional single-nucleotide polymorphisms (SNPs), which have been shown to be associated with the survival outcomes after allogeneic SCT in our recent reports [22, 23]. TLRs are present not only on the cell surface but also inside the cell, and such intracellular TLRs are translocated from the ER to endolysosomes, which are critically regulated by UNC-93 homolog B1 (UNC93B1), an ER protein with 12 membrane-spanning domains [24]. UNC93B1 is encoded by the *UNC93B1* gene on chromosome 11q13 and has one important SNP rs308328 (T>C) in an intronic region that is functional, and the major allele (T) has been reported to be associated with a lower UNC93B1 expression than T allele-negative individuals [25].

Given the above, we hypothesized that the SNP of *UNC93B1* rs308328 may be associated with clinical outcomes after allogeneic SCT through affecting the function of UNC93B1.

## Results

### Frequencies of *UNC93B1* genotypes

The rs308328 (T>C) polymorphism in the *UNC93B1* gene was genotyped in 237 patients with hematologic malignancies and their unrelated donors in this cohort (Table 1). The frequencies of C/C, C/T, and T/T in the rs308328 (T>C) polymorphism were 14%, 40%, and 46% in the patients and 14%, 41%, and 46% in the donors ( $P = 0.98$ ), respectively.

The prevalence of the *UNC93B1* rs308328 C/C genotype in the healthy Japanese population was 17% in according to a database of 1000 Genomes (<https://www.internationalgenome.org/home>).

The *UNC93B1* rs308328 genotype was also screened in 35 healthy Japanese volunteers in this study. The frequency of C/C in these volunteers was 11% (Table 1). The allele frequencies of this polymorphism in healthy controls, donors and patients did not differ to a statistically significant extent ( $P = 0.13$ ).

### Transplant outcomes according to the *UNC93B1* rs308328 genotypes

Univariate analyses (Table 2) showed that the donor *UNC93B1* rs308328 C/C genotype was associated with a

**Table 1** Patient, donor, and healthy volunteers characteristics.

Variable	Value
Number of cases	237
Patient age, years, median (range)	48 (1–67)
Donor age, years, median (range)	34 (20–66)
Healthy volunteers age, years, median (range)	30 (22–54)
Year of HSCT, median (range)	2008 (2006–2009)
Patient <i>UNC93B1</i> genotype, n (%)	
C/C	32 (14)
C/T	95 (40)
T/T	110 (46)
Donor <i>UNC93B1</i> genotype, n (%)	
C/C	33 (14)
C/T	96 (41)
T/T	108 (46)
Healthy volunteers <i>UNC93B1</i> genotype, n (%)	
C/C	4 (17)
C/T	10 (43)
T/T	21 (44)
Patient sex, n (%)	
Male	137 (58)
Female	100 (42)
Donor sex, n (%)	
Male	157 (66)
Female	80 (34)
Patient/donor sex match, n (%)	
Sex-matched	131 (55)
Female/male	63 (27)
Male/female	43 (18)
Disease, n (%)	
AML	115 (49)
ALL	46 (19)
MDS	38 (16)
ML	24 (10)
CML	10 (4.2)
MPD	3 (1.3)
Multiple myeloma	1 (0.42)
Myeloid malignancies	166 (70)
Lymphoid malignancies	71 (30)
Disease stage, n (%)	
High risk	119 (50)
Standard risk	118 (50)
ABO matching, n (%)	
ABO-matched	141 (60)
Major mismatch	40 (17)
Minor mismatch	43 (18)
Bidirectional	13 (4.2)
Conditioning regimen, n (%)	
Myeloblastic	178 (75)

**Table 1** (continued)

Variable	Value
Reduced intensity	59 (25)
Pretransplantation CMV serostatus, <i>n</i> (%)	
CMV-positive recipient	181 (76)
Missing	16 (6.8)
PS, <i>n</i> (%)	
PS0–1	224 (95)
PS2–4	13 (5.5)
TNC, ×10 <sup>8</sup> /kg, median (range)	2.7 (0.54–6.3)

*HSCT* hematopoietic stem cell transplantation, *AML* acute myeloid leukemia, *ALL* acute lymphoblastic leukemia, *MDS* ML malignant lymphoma, *CML* chronic myeloid leukemia, *MPN* myeloproliferative neoplasm, *CMV* cytomegalovirus, *PS* performance status, *TNC* total number of nucleated cells harvested.

better 3-year overall survival (OS) than the donor *UNC93B1* rs308328 C/T or T/T genotype (77% vs. 58%; *P* = 0.04; Fig. 1A). Three years was set as the study time point according to the median follow-up period among the survivors (753 days; range, 6–1918 days). The donor *UNC93B1* rs308328 C/C genotype also exhibited a trend toward a lower 3-year transplant-related mortality (TRM; 13% vs. 27%, *P* = 0.06; Fig. 1C) than other genotypes but showed no marked reduction in the 3-year progression-free survival (PFS; 69% vs. 56%, *P* = 0.16; Fig. 1B) and 3-year relapse rate (18% vs. 18%, *P* = 0.72; Fig. 1D). The recipient *UNC93B1* rs308328 genotype did not significantly influence the transplant outcomes in this study (Table 2).

After adjusting for clinical factors in the multivariate model (Table 3), the donor *UNC93B1* rs308328 C/C genotype remained associated with a better 3-year OS than other genotypes (hazard ratio [HR], 0.37; 95% confidence interval [CI], 0.16–0.88; *P* = 0.03).

When the main causes of death were analyzed according to the *UNC93B1* rs308328 genotype, the donor *UNC93B1* rs308328 C/C genotype showed half the incidence of death attributed to infection compared with other genotypes (Fig. 2), although there were no significant differences.

## Discussion

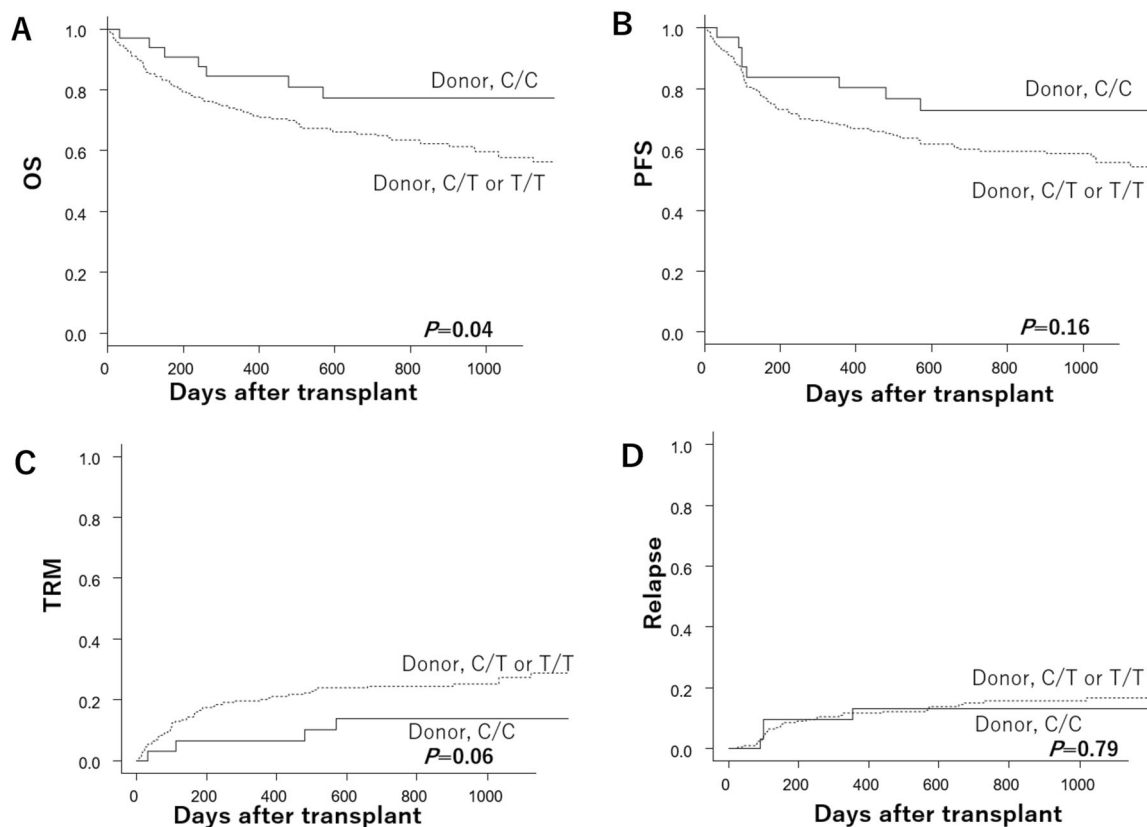
The present study showed that the donor *UNC93B1* rs308328 C/C genotype, which is presumed to have greater inducibility of *UNC93B1* than other genotypes [25], was associated with significantly better survival outcomes than other genotypes in patients with hematological malignancies receiving HLA fully matched unrelated bone marrow transplantation (BMT).

The mechanisms through which the donor *UNC93B1* rs308328 C/C genotype exerts its beneficial effects remain

**Table 2** Univariate analysis of the association between *UNC93B1* polymorphism and post HSCT outcomes.

Variable	3-Year OS, %	<i>P</i>	3-Year PFS, %	<i>P</i>	3-Year TRM, %	<i>P</i>	3-Year relapse, %	<i>P</i>	Grades II–IV acute GVHD, %	<i>P</i>	Grades III–IV acute GVHD, %	<i>P</i>	Chronic GVHD, %	<i>P</i>
Donor <i>UNC93B1</i> rs308328 genotype														
C/C (33)	77		69		13		18		30		5.2		23	
C/T (96)	48	0.08	50	0.51	34	0.11	16	1.00	38	1.00	1.4	0.78	25	0.86
T/T (108)	47	0.14	59	0.51	21	0.25	20	1.00	33	1.00	9.4	0.90	35	0.33
C/C (33)	77		69		13		18		30		5.2		23	
C/T or T/T (204)	58	0.04	54	0.16	27	0.06	18	0.79	35	0.61	11	0.32	30	0.36
Recipient <i>UNC93B1</i> rs308328 genotype														
C/C (32)	51		51		27		23		33		8.6		45	
C/T (95)	64	1.00	59	1.00	27	1.00	14	0.52	33	1.00	7.8	0.98	27	0.22
T/T (110)	60	1.00	60	1.00	24	1.00	16	0.81	37	1.00	1.4	0.98	26	0.22
C/C (32)	51		51		27		23		33		8.6		45	
C/T or T/T (205)	62	0.38	59	0.37	25	0.91	15	0.24	35	0.88	11	0.72	27	0.06

*HSCT* hematopoietic stem cell transplantation, *OS* overall survival, *PFS* progression-free survival, *TRM* transplant-related mortality, *GVHD* graft-versus-host disease.



**Fig. 1 Survival outcomes after transplantation according to the donor *UNC93B1* rs308328 genotype.** The Kaplan–Meier analysis of the overall survival rates (A), the progression-free survival rates (B), the transplant-related mortality rates (C), and relapse rates (D) after

transplantation according to the donor *UNC93B1* rs308328 genotype. The solid lines represent the donor C/C genotype, and the dashed lines represent the donor T/T or C/T genotype.

to be determined. One plausible explanation is that patients transplanted from donors with the *UNC93B1* C/C genotype may have been less susceptible to infection than those without the *UNC93B1* C/C genotype, considering that the incidence of death mainly attributed to infections was 3.0% vs. 8.3%, respectively (Fig. 2A). Evidence supporting this hypothesis may be seen in a previous report [26], in which two unrelated children possessing autosomal recessive deficiency of *UNC93B1* developed encephalitis due to herpes-simplex virus, wherein the antiviral cellular responses were impaired in the interferon- $\alpha$  (IFN- $\alpha$ ), IFN- $\beta$ , and IFN- $\lambda$  pathways. Experimental studies [26–32], have shown that a lack or suppression of *UNC93B1* inhibits the activation of TLR3, TLR7, and TLR9 to induce inflammatory mediators, leading to increased susceptibility to infections. In addition, mouse model studies using *UNC93B1* mutant mice, which lack the *UNC93B1* function as a result of H412R missense mutation, exhibited increased susceptibility to *Toxoplasma gondii* [27], *Trypanosoma cruzi* [28], *Leishmania major* [29], and cytomegalovirus (CMV) [31] infections in association with the reduced expression of inflammatory mediators, including IFN- $\gamma$  (*T. cruzi*, CMV, *L. major*), IFN- $\alpha$  (CMV) and interleukin

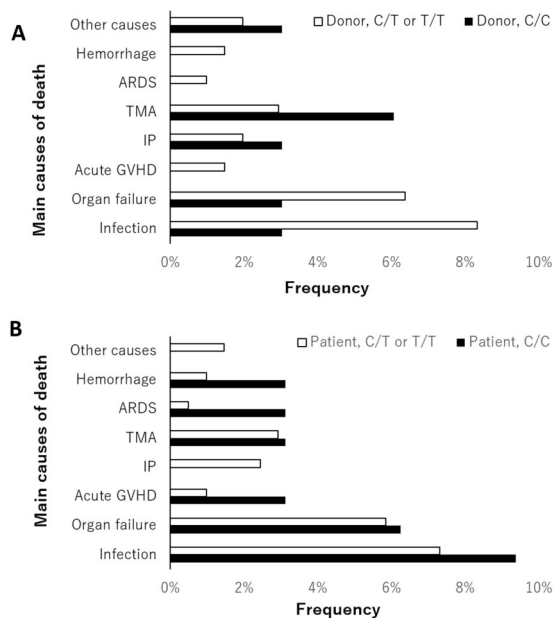
(IL)-12p40 (*T. gondii*, *T. cruzi*). In another experimental study [32], other *UNC93B1* mutant mice with a 54-amino acid deletion in exon 4, which are also deficient in functional *UNC93B1*, showed a decreased number of activated exudate macrophages and the decreased expression of CXC Chemokine Ligand (CXCL) 10, IFN- $\gamma$ , and type I IFN in the early phase of influenza A H1N1 infection. Given these previous findings, it is reasonable to assume from the present study that the donor *UNC93B1* C/C genotype, potentially having higher inducibility of *UNC93B1* than other genotypes, may enhance the functions of TLRs, thereby augmenting infectious immunity, which improved the survival outcomes after allogeneic SCT.

There is a contrasting report that the *UNC93B1* mutation with a 54-amino acid deletion in exon 4 was associated with the increased cardiac expression of IFN- $\beta$  and markers of tissue injury and fibrosis early after coxsackievirus strain B serotype 3 (CVB3), which is a picornavirus that induces myocarditis [33]. However, one or more alternative pathways may mediate the cardiac IFN- $\beta$  upregulation in the case of CSV3. For example, melanoma differentiation-associated gene 5 (MDA5) detects the double-stranded RNA replicative form in picornavirus-infected cells and

**Table 3** Multivariate analysis of the association between *UNC93B1* polymorphism and post HSCT outcomes.

Variable	OS			PFS			TRM			Relapse		
	Adjusted HR	95% CI	P	Adjusted HR	95% CI	P	Adjusted HR	95% CI	P	Adjusted HR	95% CI	P
Donor <i>UNC93B1</i> rs308328 genotype, C/C vs. C/T or T/T	0.37	0.16–0.88	0	0.50	0.94–4.20	0.07	0.43	0.77–6.80	0.1	0.77	0.45–3.70	0.64
Recipient <i>UNC93B1</i> rs308328 genotype, C/C vs. C/T or T/T	1.50	0.80–2.80	0.2	1.67	0.35–1.10	0.1	1.00	0.37–2.70	1.00	2.00	0.21–1.30	0.2
ABO major mismatch	0.91	0.50–1.60	0.8	1.03	0.55–1.70	0.9	1.35	0.32–1.70	0.49	0.67	0.65–3.40	0.3
ABO minor mismatch	0.81	0.42–1.60	0.5	1.15	0.47–1.60	0.7	1.28	0.32–1.90	0.6	0.83	0.47–3.10	0.70
ABO bidirectional	0.70	0.25–2.00	0.5	1.32	0.27–2.10	0.60	1.52	0.14–3.10	0.60	0.71	0.31–6.00	0.7
Recipient age	1.00	1.00–1.00	<0.01	1.00	1.00–1.00	<0.01	1.00	1.00–1.10	0	1.00	0.98–1.00	0.9
Donor age	1.00	0.96–1.00	0.9	1.00	0.97–1.00	0.80	1.00	0.96–1.00	1	1.00	0.96–1.00	1
TNC	0.94	0.73–1.20	0.7	1.12	0.70–1.10	0.39	1.22	0.58–1.20	0.3	0.83	0.86–1.60	0.33
Pretransplantation CMV serostatus												
CMV-positive recipient	1.00	0.55–2.00	0.9	0.91	0.57–1.90	0.9	1.16	0.39–1.90	0.7	0.71	0.53–3.90	0.47
Missing	1.80	0.63–5.40	0.3	0.59	0.63–4.70	0.3	0.50	0.58–7.00	0.3	0.91	0.15–8.10	0.92
Disease stage standard risk/high risk	2.70	1.60–4.80	<0.01	0.34	1.70–5.00	<0.01	0.32	1.60–6.30	<0.01	0.67	0.56–3.90	0.43
Myeloid malignancies	1.40	0.79–2.50	0.3	0.67	0.88–2.60	0.1	0.67	0.70–3.30	0.28	0.91	0.40–2.80	0.90
Conditioning regimen, MAC vs. RIC	0.86	0.49–1.50	0.60	1.05	0.56–1.60	0.9	1.14	0.45–1.70	0.71	1.05	0.38–2.40	0.91
PS2–4	2.40	0.98–5.70	0.1	0.45	0.95–5.30	0.1	0.63	0.44–5.30	0.47	0.59	0.37–7.70	0.5
Year of HSCT	1.30	0.69–2.30	0.5	0.67	0.85–2.60	0.2	0.83	0.60–2.40	0.60	0.71	0.58–3.60	0.4
Recipient/donor sex match												
Female/male	0.79	0.46–1.40	0.4	1.14	0.53–1.50	0.6	1.09	0.48–1.80	0.80	1.11	0.37–2.20	0.8
Male/female	1.50	0.84–2.70	0.2	0.63	0.93–2.80	0.1	0.83	0.59–2.70	0.6	0.50	0.83–5.00	0.1

*HSCT* hematopoietic stem cell transplantation, *HR* hazard ratio, *CI* confidence interval, *OS* overall survival, *PFS* progression-free survival, *TRM* transplant-related mortality, *TNC* total number of nucleated cells harvested, *CMV* cytomegalovirus, *MAC* myeloablative conditioning, *RIC* reduced-intensity conditioning, *PS* performance status.



**Fig. 2** The main causes of death were analyzed according to the *UNC93B1* rs308328 genotype. ARDS acute respiratory distress syndrome, TMA thrombotic microangiopathy, IP interstitial pneumonia, GVHD graft-versus-host disease.

induces the production of inflammatory cytokines, including IFN- $\beta$  [30]. Since different immune system cascades are activated depending on the type of pathogen, another cohort study to validate the current findings is desired in order to confirm the impact of *UNC93B1* on allogeneic SCT, considering the type of pathogen, as this is beyond the scope of the present study.

Some reports have suggested that *TLR4* polymorphisms may have an impact directly on the infection and graft rejection after transplantation [34–38]. We showed that the donor *TLR4* +3725G/G genotype, which induces a low expression of TLR4, was significantly associated with a lower incidence of fatal infections than the donor G/C and C/C genotypes in another cohort of 367 patients who underwent unrelated HLA-matched BMT for hematologic malignancies through the Japan Marrow Donor Program (JMDP) [22]. The *UNC93B1* gene and the *TLR4* gene are not predicted to be in linkage disequilibrium because the *UNC93B1* gene is located on chromosome 11q13, while the *TLR4* gene is located on chromosome 9q32–q33. Furthermore, the *UNC93B1* gene does not affect the function of TLR directly. Therefore, it is reasonable to consider that the donor *UNC93B1* rs308328 C/C genotype, which putatively has higher inducibility of *UNC93B1* than others, improves the outcomes of transplantation without the effects of *TLR4* SNPs.

Several limitations associated with the present study warrant mention. First, detailed information on the infections, including the types, severity, treatments, and therapeutic

appropriateness, was not obtained in the current study. Second, the functional roles of the SNP of *UNC93B1* rs308328 in BMT remain purely speculative due to the lack of data using blood samples from BMT recipients and their donors. Further studies including more samples from patients, donors, and healthy individuals need to be conducted.

Another limitation is that our results for the *UNC93B1* gene may be a coincidence in the analysis of polymorphisms in many genes associated with the *UNC93B1* gene, especially other *TLR*-related genes, although the fact that *UNC93B1* is an important protein for infectious immunity that integrates the functions of TLRs and that the *UNC93B1* gene plays a functional role suggests that the *UNC93B1* gene polymorphism is probably responsible for the observed phenotype. Therefore, further studies using a separate cohort should be warranted to clarify whether polymorphisms in *TLR*-related genes are involved in the outcomes of BMT in an integrated manner.

In conclusion, the findings of the present study suggested that the donor *UNC93B1* rs308328 C/C genotype predicted better survival outcomes after SCT than other genotypes. Therefore, the donor *UNC93B1* rs308328 C/C genotype in donors may be a valuable tool for selecting donors and evaluating pretransplantation risks that, combined with other currently known risk factors, can form the basis for carrying out suitable tailoring of transplantation strategies. Considering the plausible functional roles of these polymorphisms, they may be candidates for future prophylactic and therapeutic strategies for complications after allogeneic SCT and may lead to the development of molecular targeted therapy [39]. Further studies are warranted to ascertain whether or not the findings of this study can be extended to other stem cell sources or to HLA-mismatched transplantation and to validate the present findings in other ethnic groups.

## Materials and methods

### Patients

A total of 237 patients and their unrelated donors were included in the study. The patients underwent HLA-matched BMT for hematologic malignancies through the JMDP with T cell-replete marrow from HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQB1, and HLA-DPB1 allele-matched donors between May 2006 and April 2009. No patient had a history of any previous transplantation. The final clinical data analyses of these patients were completed by September 27, 2011.

The diagnosed diseases were acute myeloid leukemia (AML) ( $n = 115$ ; 49%), acute lymphoblastic leukemia (ALL) ( $n = 46$ ; 19%), myelodysplastic syndrome (MDS)

( $n = 38$ ; 16%), malignant lymphoma (ML) ( $n = 24$ ; 10%), chronic myeloid leukemia (CML) ( $n = 10$ ; 4%), myeloproliferative neoplasm (MPN) ( $n = 3$ ; 1%), and multiple myeloma (MM) ( $n = 1$ ; 0.4%) (Table 1). Relapse or secondary cases were defined as high-risk diseases. Myeloid malignancies included AML, MDS, CML, and MPD. Lymphoid malignancies included ALL, ML, and MM.

The conditioning regimen depends on the type of underlying disease and the patient's condition. The combination of cyclophosphamide (CY) and total body irradiation (TBI) was mainly classified as myeloablative conditioning (MAC), whereas the combination of fludarabine and melphalan was mainly classified as reduced-intensity conditioning (RIC) [40]. Cyclosporin- or tacrolimus-based therapy was selected as GVHD prophylaxis [41, 42]. Patients who were used anti-T cell therapy, such as anti-thymocyte globulin or ex vivo T cell depletion were excluded from this study.

All patients and donors provided their informed consent at the time of transplantation to participate in molecular studies of this nature according to the Declaration of Helsinki. This project was approved by the Institutional Review Board of Aichi Medical University School of Medicine and the JMDP.

### UNC93B1 genotyping

Real-time polymerase chain reaction (PCR) genotyping for *UNC93B1* was done with the TaqMan-Allelic discrimination method in a Step One Plus Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) as described previously [7], and the results were analyzed using the Allelic Discrimination software program (Applied Biosystems). We purchased the specific probe designed for SNP rs308328 (T>C) (product No. C\_2623961\_1) and TaqMan genotyping master mix from Applied Biosystems.

### Data management and statistical analyses

The JMDP collected the data using a standardized report form. Follow-up reports were submitted at 100 days, 1 year, and annually after transplantation. Pre-transplant cytomegalovirus (CMV) serostatus was routinely measured only in patients but not in their donors. Neutrophil engraftment was confirmed by an absolute neutrophil count of more than  $0.5 \times 10^9/L$  for at least three consecutive days. Acute GVHD (aGVHD) was diagnosed and graded in accordance with the established criteria [43]. Chronic GVHD (cGVHD) was classified on the basis of the Seattle criteria [44].

We calculated the OS from the date of transplantation to death, regardless of cause, and defined disease relapse as the number of days from transplantation to disease relapse or progression. The TRM was defined as death due to any cause without relapse or disease progression, including

infections, toxicities, and other non-relapse- or disease progression-related causes of death. The PFS was defined as the survival without disease relapse or progression. Any patients who were alive at the last follow-up date were censored. Data concerning the clinical and microbiologic features of infections, postmortem changes, prophylaxis of infections, and therapy of GVHD given on an institutional basis were not available for this study.

The EZR software package [45] was used in all statistical analyses. The probabilities of the OS and PFS were calculated with the Kaplan–Meier method, and comparisons between groups were made with the log-rank test. The occurrence of TRM, disease relapse, aGVHD, and cGVHD was compared using the Gray test [46] and analyzed using a cumulative incidence analysis [47], considering relapse, death without disease relapse, death without aGVHD, death without cGVHD, and death without engraftment as respective competing risks. A multivariate Cox model was created for the OS, TRM, relapse, grades II–IV aGVHD, grades III–IV aGVHD, and cGVHD, using stepwise selection at a significance level of 5% to evaluate the hazard ratio (HR) associated with the *UNC93B1* rs308328 genotype. The recipient age at the time of BMT, sex, pretransplantation CMV serostatus, performance status, disease characteristics (i.e., disease type, disease lineage, and disease risk at transplantation), donor characteristics (i.e., age, sex, sex compatibility, and ABO compatibility), transplant characteristics (i.e., MAC or RIC and total nucleated cell count harvested per recipient weight), and year of transplantation were included as variables. The median was used as the cut-off point for continuous variables.

The chi-square test and Mann–Whitney test were used to compare the results of the two groups. The Hardy–Weinberg equilibrium for the *UNC93B1* rs308328 gene polymorphism was tested using the Haploview program. For both the univariate and multivariate analyses  $P < 0.05$  was considered to indicate statistical significance.

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### Compliance with ethical standards

**Conflict of interest** The authors declare no competing interests.

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