Donor single nucleotide polymorphism in *ACAT1* affects the incidence of graft-versus-host disease after bone marrow transplantation

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Donor ACAT1 genotype and GVHD after BMT

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Abstract

Acyl-coenzyme A: cholesterol acyltransferase 1 (ACAT1) is an enzyme that converts cholesterol to cholesteryl esters. A recent in vivo study reported that inhibiting ACAT1 enzyme activity upregulates the membrane cholesterol levels of T cells, enhancing their cytotoxic function. In the present study, we investigated whether the presence of the *ACAT1* single nucleotide polymorphism rs11545566 in transplant donors affected the risk of graft-versus-host disease (GVHD) in 116 adult patients who underwent bone marrow transplantation from human leukocyte antigen-identical sibling donors, and who received GVHD prophylaxis with short-term methotrexate and cyclosporine. The frequencies of the AA, AG, and GG genotypes in the donors were 31%, 45%, and 24%, respectively. The cumulative incidences of grade II–IV acute GVHD on day 100 in patients whose donors had AA vs. non-AA genotypes were 6% and 18%, respectively, and those of extensive chronic GVHD at two years were 7% and 32%, respectively. Multivariate analyses demonstrated that donor rs11545566 non-AA genotypes showed a trend toward a higher incidence of grade II–IV acute GVHD (P = 0.079), and were significantly associated with a higher incidence of extensive chronic GVHD (P = 0.021). These results suggest that donor *ACAT1* rs11545566 genotype may be predictive of GVHD.

(199 words)

Keywords

Graft-versus-host disease; ACAT1; single nucleotide polymorphism; transplantation; donor

Introduction

Although allogeneic hematopoietic stem cell transplantation is a curative therapy for a variety of hematological disorders, its success is limited by the development of graft-versus-host disease (GVHD) [1,2]. Acute GVHD is mainly caused by alloreactive donor cytotoxic T cells [3,4]. Initially, the T-cell receptors (TCRs) of donor T cells recognize donor–recipient mismatched human leukocyte antigen (HLA) molecules or minor histocompatibility antigens, which are expressed on antigen-presenting cells [5]. This interaction induces phosphorylation of the cytoplasmic domains of CD3 subunits, and the subsequent activation of TCR signaling pathways results in T-cell activation, proliferation, and differentiation [6]. Finally, CD8- or CD4-positive cytotoxic T cells, as well as soluble inflammatory agents, cause destruction of target tissues [5]. Chronic GVHD is considered to have both alloimmune and autoimmune components. The polarization of CD4-positive T cells into T helper 2 cells is one of the crucial factors in the development of chronic GVHD [7]. The following processes are proposed to be involved in autoimmune attack: the failure of immune regulatory mechanisms due to fewer naïve regulatory T cells; B-cell dysregulation leading to the emergence of autoreactive B cells and the production of autoreactive antibodies; and the establishment of autoreactive T cells by exposure to autoantigens produced by the tissue damage that accompanies conditioning regimens or acute GVHD [5].

Acyl-coenzyme A: cholesterol acyltransferase 1 (ACAT1) is a key enzyme that

converts cholesterol to cholesteryl esters [8]. ACAT1 exists in various tissues, including hematopoietic cells [9]. In T cells, cholesterol is required for TCR clustering and the formation of the T-cell immunological synapse [10,11], and can regulate T-cell signaling and function [10-12]. A recent study demonstrated that inhibiting cholesterol esterification in T cells by the genetic ablation or pharmacological inhibition of ACAT1 led to potentiated effector function and enhanced proliferation of CD8-positive T cells [13]. In mice, ACAT1-deficient CD8-positive T cells were effective than wild-type CD8-positive T cells at controlling melanoma growth and metastasis [13]. The single nucleotide polymorphism (SNP) rs11545566 is located in the 5' untranslated region (−77G>A) of the *ACAT1* gene [14]. The expression level of *ACAT1* mRNA in white blood cells with the rs11545566 A allele is suggested to be higher than that in cells with the G allele (Genotype-Tissue Expression Portal, https://www.gtexportal.org/home/snp/rs11545566). Interestingly, the rs11545566 genotype has been shown to be associated with the plasma concentration of high-density lipoprotein cholesterol [14] and with the incidence of coronary artery disease [15], supporting the idea that rs11545566 affects ACAT1 enzyme activity.

We hypothesized that the genotypes of *ACAT1* rs11545566 in donors might impact the incidence of GVHD after allogeneic hematopoietic stem cell transplantation.

Methods

Patients

A total of 116 adult Japanese patients and donors were consecutively enrolled in this study according to the following inclusion criteria: (1) the donor was an HLA-identical sibling, (2) the graft source was bone marrow, (3) short-term methotrexate and cyclosporine were given as GVHD prophylaxis, (4) donor genomic DNA samples were available, and (5) clinical data were available. HLA matching among donor-recipient pairs was confirmed in all patients either by a family study or genotyping. Informed consent was obtained from all patients. The study population included transplantation performed at the Nagoya University Hospital and the Japanese Red Cross Nagoya First Hospital between 1987 and 2015. The median follow-up for living patients was 5.8 years (range, 54 days to 30.7 years). The study was approved by the Ethics Committee of the Nagoya University School of Medicine and the Japanese Red Cross Nagoya First Hospital.

Definitions

Standard-risk diseases included acute myeloid leukemia in first complete remission, acute lymphoblastic leukemia without the Philadelphia chromosome in first complete remission, chronic myeloid leukemia in first chronic phase, myelodysplastic syndrome with an International Prognostic Scoring System score of 1.0 or lower, and non-malignant disease. All other malignant diseases were considered to be high-risk

diseases. Myeloablative conditioning or reduced-intensity conditioning was classified based on the report by the Center for International Blood and Marrow Transplant Research [16]. Acute GVHD was graded by established criteria [17]. Chronic GVHD was evaluated in patients who survived beyond day 100, and was classified based on traditional criteria [18]. Non-relapse mortality (NRM) was defined as mortality due to any cause other than relapse or disease progression.

Genotyping of *ACAT1* rs11545566

Genotyping of *ACAT1* rs11545566 was performed by polymerase chain reaction (PCR)-restriction fragment length polymorphism, using donor genomic DNA that was extracted from posttransplant patient peripheral blood mononuclear cells with the QIAamp DNA Blood Mini Kit (QIAGEN sciences, Germantown, MD, USA). The sense and antisense primers used for PCR were 5'-CTGGGGACCACCAATAGGAT-3' and 5'-GAGCACCACCGTTACCTGAG-3', respectively (Nihon Gene Research Laboratories, Sendai, Miyagi, Japan). Each PCR reaction mixture contained 0.2 μl of genomic DNA, 0.2 mmol/l of each of the four deoxyribonucleotides, 10 pmol of each *ACAT1* primer, 0.1 μl of TaKaRa Ex Taq polymerase (Takara Bio, Kusatsu, Shiga, Japan), and Ex Taq PCR Buffer in a volume of 20 μl. Amplification was performed with 30 denaturation cycles at 95°C for 10 sec, annealing at 60°C for 30 sec, and extension at 72°C for 20 sec. The 265-bp *ACAT1* fragment was digested for 1 h at 75°C

with 2.5 units of ApeKI (New England BioLabs, Ipswich, MA, USA) in a 20-μl reaction mixture, and the digested products were analyzed by electrophoresis on an agarose gel. The AA genotype of rs11545566 was identified by the presence of 165-, 50-, and 50-bp fragments, that seemed to be two bands at 165 and 50 bp. The GG genotype was identified by the presence of 117-, 50-, 50-, and 48-bp fragments, that seemed to be two bands at 117 and 48/50 bp. The AG genotype was identified by the presence of 165-, 117-, 50-, 50-, 50-, 50-, and 48-bp fragments, that seemed to be three bands at 165, 117, and 48/50 bp. All digested PCR products were classified in three genotypes by independent two researchers. All classified genotypes were identical between independent two researchers. Thirteen PCR products were picked at random and directly sequenced to check misclassification, and we confirmed that all products were accurately classified.

Quantitative reverse transcription PCR for *ACAT1*

Peripheral blood mononuclear cells were obtained from healthy volunteers with each *ACAT1* genotype. CD8-positive and CD4-positive cells were isolated from the peripheral blood mononuclear cells with Dynabeads (Thermo Fisher Scientific, Waltham, MA, USA). Total RNA was extracted from these cells using RNeasy Mini kit (QIAGEN sciences) and converted into cDNA using the Superscript III First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA, USA). Quantitative reverse

transcription PCR analysis of the expression of *ACAT1* was performed with 40 cycles of two-step PCR (95°C for 15 sec and 60°C for 60 sec) after initial denaturation (95°C for 10 min) using TaqMan Gene Expression Assays kit (Assay ID: Hs00162077_m1, Applied Biosystems, Foster City, CA, USA) and 7300 Real-time PCR System (Applied Biosystems). Expression level of *GAPDH* was measured as a reference level of target gene expression.

Statistical analysis

The chi-squared test was used to compare categorical variables. The probabilities of acute and chronic GVHD stratified by rs11545566 genotype were estimated on the basis of cumulative incidence curves [19]. The Fine and Gray proportional hazards model was used for multivariate analyses of acute GVHD, chronic GVHD, relapse, and NRM [20]. The Cox proportional hazards model was used for multivariate analyses of overall survival (OS) [21]. The following variables were evaluated: patient age (≥ 35 vs. <35); donor-recipient sex combination (female to male vs. others); disease (high-risk disease vs. standard-risk disease); patient cytomegalovirus serostatus (positive vs. negative); conditioning regimen (myeloablative conditioning vs. reduced-intensity conditioning); and rs11545566 genotype (non-AA genotypes vs. AA genotype). The history of grade II-IV acute GVHD was included as a variable in the chronic GVHD analysis. All variables at $P < 0.1$ on univariate analysis were entered into the multivariate stepwise

analyses. All tests were two-sided. *P* values < 0.05 were considered significant, and those between 0.05 and 0.1 were considered as suggestive of a trend. The data were analyzed using STATA (StataCorp. 2012; Stata Statistical Software: Release 12.1. Stata Corporation, College Station, TX, USA) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [22], the latter of which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria).

Results

Comparison of the expression level of *ACAT1* mRNA in T cells among rs11545566 genotypes

We initially investigated the expression level of *ACAT1* mRNA in CD8-positive and CD4-positive T cells of each rs11545566 genotype. In CD8-positive T cells, the relative expression level of *ACAT1* mRNA varied among rs11545566 genotypes (*P* = 0.098; Figure 1a, left). The relative expression level in CD8-positive cells of the non-AA genotypes was significantly lower than that of the AA genotype (*P* = 0.027; Figure 1a, right). In CD4-positive T cells, the relative expression level of *ACAT1* mRNA significantly varied among rs11545566 genotypes (*P* = 0.025; Figure 1b, left). The relative expression level in CD4-positive cells of the non-AA genotypes was lower than that of the AA genotype, but it was not significant (*P* = 0.098; Figure 1b, right).

Frequencies of *ACAT1* rs11545566 genotypes

The frequencies of the A and G alleles in the donors were 52% (n = 124) and 48% (n = 108), respectively. The frequencies of the AA, AG, and GG genotypes in the donors were 31% (n = 36), 45% (n = 52), and 24% (n = 28), respectively. As shown in Table 1, there were no significant differences in patient characteristics when categorized by the three donor rs11545566 genotypes. These data are

Acute GVHD

Of the 116 evaluable patients, 17 developed grade II-IV acute GVHD. The cumulative incidences of grade II-IV acute GVHD on day 100 in patients who had received bone marrow transplantation (BMT) from donors with genotypes AA (n = 36), AG (n = 52), and GG (n = 28) were 6% (1% to 17%), 17% (10% to 31%), and 18% (6% to 34%), respectively (P = 0.169, Figure 2a). The incidences of grade II-IV acute GVHD in patients transplanted from donors with AG genotype and GG genotype were not significantly different (P = 0.912). The cumulative incidences of grade II-IV acute GVHD on day 100 in patients transplanted from donors with AA (n = 36) and non-AA (AG or GG) (n = 80) genotypes were 6% (1% to 17%) and 18% (11% to 28%), respectively (P = 0.06, Figure 2b).

In a univariate analysis, there was a significant association between a higher incidence of grade II-IV acute GVHD and higher patient age (P = 0.011), and there was

a trend toward a higher incidence of grade II-IV acute GVHD in patients transplanted from donors with a non-AA genotype (P = 0.082). In a multivariate analysis, only higher patient age was significantly associated with a higher incidence of grade II-IV acute GVHD (P = 0.011). There was a trend toward a higher incidence of grade II-IV acute GVHD in patients transplanted from donors with a non-AA genotype (P = 0.079) (Table 2).

Chronic GVHD

Of the 110 evaluable patients who survived for at least 100 days after transplantation, 43 developed extensive chronic GVHD. The cumulative incidences of extensive chronic GVHD at 2 years after transplantation in patients who had received BMT from donors with genotypes AA (n = 36), AG (n = 49), and GG (n = 25) were 7% (1% to 19%), 35% (21% to 50%), and 27% (9% to 48%), respectively (P = 0.022, Figure 3a). The incidences of extensive chronic GVHD in patients transplanted from donors with AG genotype and GG genotype were not significantly different (P = 0.495). The cumulative incidences of extensive chronic GVHD at 2 years after transplantation in patients transplanted from donors with AA (n = 36) and non-AA (n = 74) genotypes were 7% (1% to 19%) and 32% (21% to 45%), respectively (P = 0.008, Figure 3b).

In a univariate analysis, there was a significant association between a higher incidence of extensive chronic GVHD and higher patient age (P = 0.026), high-risk

disease (P = 0.017), history of grade II-IV acute GVHD (P = 0.010), and transplants from donors with a non-AA genotype (P = 0.023). A trend toward a higher incidence of extensive chronic GVHD was observed in male patients with female donors (P = 0.091). In a multivariate analysis, high-risk disease (P = 0.014), and non-AA donor rs11545566 genotype (P = 0.021) were significantly associated with a higher incidence of extensive chronic GVHD (Table 2).

Other outcomes

In 100 evaluable patients with malignant disease, the relapse rate at 5 years after transplantation was 27% (18% to 37%). The relapse rates at 5 years after transplantation in patients transplanted from donors with AA (n = 32) and non-AA (n = 68) genotypes were 34% (16% to 52%) and 24% (14% to 36%), respectively. Multivariate analysis demonstrated that high-risk disease (P = 0.014) was the only factor significantly associated with higher relapse rate (Table 2). The donor *ACAT1* rs11545566 genotype did not affect the relapse rate.

In 100 evaluable patients with malignant disease, NRM at 5 years after transplantation was 24% (15% to 34%). The NRM at 5 years after transplantation in patients transplanted from donors with AA (n = 32) and non-AA (n = 68) genotypes were 24% (9% to 42%) and 24% (14% to 36%), respectively. Multivariate analysis demonstrated that higher patient age (P = 0.023) was the only factor significantly

associated with higher NRM (Table 2). The donor *ACAT1* rs11545566 genotype did not affect NRM.

In 116 evaluable patients, OS at 5 year after transplantation was 63% (95% CI, 53% to 71%). The OS at 5 year after transplantation in patients transplanted from donors with AA (n = 36) and non-AA (n = 80) genotypes were 67% (46% to 77%) and 62% (51% to 72%), respectively. Multivariate analysis demonstrated that higher patient age ($P = 0.015$), high-risk disease ($P = 0.004$), and history of grade II-IV acute GVHD ($P = 0.038$) were significantly associated with lower OS (Table 2). The donor *ACAT1* rs11545566 genotype did not affect OS.

Discussion

In the present study, donor *ACAT1* SNP rs11545566 non-AA genotypes showed a trend toward association with a higher incidence of grade II-IV acute GVHD, and were significantly associated with a higher incidence of extensive chronic GVHD after BMT from HLA-matched siblings.

Previous studies have demonstrated that membrane lipids can regulate T cell signaling and function [10,11,23]. Cholesterol, a key component of membrane lipids, has been shown to be required for TCR clustering and for the formation of the T cell immunological synapse [10,11]. A recent study showed that these processes were enhanced after the membrane cholesterol level of CD8-positive T cells was increased by

inhibiting the enzyme activity of ACAT1, thus enhancing the production of cytokines and cytolytic granules and the killing and proliferation of CD8-positive T cells [13]. The putative promoter region of *ACAT1* has been identified in the first 648 bp flanking exon 1 of the gene and just upstream of rs11545566 [12]. Although this putative promoter region contains neither a typical TATA box nor a typical CCAAT box, its promoter activity was confirmed by luciferase assay. It is not clear whether the SNP rs11545566 plays a direct role in controlling the transcription of *ACAT1*. However, the associations of the rs11545566 genotype with plasma concentrations of high-density lipoprotein cholesterol [14] and with the incidence of coronary artery disease [15] suggest that this genotype may affect the degree of cellular ACAT1 activity. Thus, our finding that non-AA rs11545566 donor genotypes were associated with the development of GVHD might reflect a high responsiveness of donor cytotoxic T cells to alloantigens as a consequence of elevated membrane cholesterol levels due to the low ACAT1 enzyme activity of non-AA genotypes. Future in vitro and in vivo studies are required to clarify the impact of T cell cholesterol metabolism on the pathogenesis of GVHD.

In addition to T cells, B cells also play a role in the development of chronic GVHD [24]. The proliferation of pro-B cells was significantly enhanced in *Acat1* knockout mice in comparison with age-matched wild-type mice, and the number of pre-B cells in bone marrow was significantly higher in the *Acat1* knockout mice [25]. These data

suggest that cholesterol homeostasis is involved in the increase of B cell progenitors. However, less attention has been directed to the role of cholesterol homeostasis in B-cell activation. It remains to be determined whether the association between *ACAT1* SNP rs11545566 and the incidence of chronic GVHD is explained by the dysregulation of T cells, B cells, or both.

This study cohort consisted of a remarkably homogeneous population of adult Japanese patients who had received bone marrow from HLA-identical sibling donors with GVHD prophylaxis consisting of short-term methotrexate and cyclosporine. This homogeneity made it possible to perform analyses without the need to consider HLA incompatibility, stem cell source differences, or transplant procedure biases. However, the small study cohort may have resulted in false positives [26]. Thus, it is important to perform additional studies with larger numbers of patients with their donor's DNA. A candidate gene study, as described in this study, is one of attractive approaches to find non-HLA gene polymorphisms associated with GVHD [27]. The major advantage of a candidate gene study approach is its cost effectiveness and its focus on a particular gene polymorphism with known functional consequences. On the other hand, Martin et al. raised an alarm as follows: careful consideration must be given to the number of SNPs and genetic models that can be tested while accounting appropriately for multiple comparisons, the minimum minor allele frequency, the anticipated effect size, the available numbers of patients and donors, and the need to replicate any new discoveries

in an independent cohort [26]. Another limitation of this study is that it used the traditional classification criteria for the diagnosis of chronic GVHD, rather than the latest National Institute of Health criteria [28]. This was due to inclusion of patients who underwent transplantation before the National Institute of Health criteria came into widespread use in Japan. Nonetheless, the frequencies of the rs11545566 A and G alleles in donors were approximately equal (A allele, 52%; G allele, 48%), which is consistent with a previous report (A allele, 50%; non-AA genotypes, 50%) [14]. This suggests that donor rs11545566 may impact transplant outcomes in many patients. However, because several donor non-HLA gene polymorphisms besides rs11545566 are associated with the incidence of GVHD [27,29-31], prioritization of their clinical importance will be required.

In conclusion, the present study suggests that donor *ACAT1* SNP rs11545566 non-AA genotypes may be associated with an increased incidence of GVHD in HLA-matched sibling BMT with short-term methotrexate and cyclosporine. Because HLA-matched sibling BMT is decreasing in recent years (www.jdchct.or.jp), the study results may be applicable to the small patient population. However, our findings may provide patients an opportunity to receive optimal strategies in terms of the selection of the GVHD prophylaxis and/or the tapering schedule of immunosuppressant drugs according to the *ACAT1* genotypes. A validation study is needed to confirm the impact of *ACAT1* polymorphisms on transplant outcomes. Additional knowledge about the

ACAT1 genotype may provide valuable information regarding donor choice and GVHD prophylaxis.

(3169 words)

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Conflicts of interest

H. Kiyoi received research funding from FUJIFILM Co., Kyowa Hakko Kirin Co., Bristol-Myers Squibb, Ltd., Otsuka Pharmaceutical Co., Ltd., Perseus Proteomics Inc., and Daiichi Sankyo Co., Ltd., donations from Zenyaku Kogyo Co., Ltd., Nippon Shinyaku Co., Ltd., Eisai Co., Ltd., Chugai Pharmaceutical Co., Ltd., Astellas Pharma Inc., Kyowa Hakko Kirin Co., Takeda Pharmaceutical Co., Ltd., and Sumitomo Dainippon Pharma Co., Ltd., and honoraria from Bristol-Myers Squibb, Ltd., Pfizer

Japan Inc., and Astellas Pharma Inc. These companies were not directly involved in any part of this study. The remaining authors have no financial conflicts of interest with regard to this study.

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Figure legends

Figure 1

Comparison of the expression level of *ACAT1* mRNA in T cells among rs11545566 genotypes. The normalized expression level of *ACAT1* of the AA genotype was used as a reference. The relative expression levels of *ACAT1* in **(a)** CD8-positive T cells and **(b)** CD4-positive T cells were compared among three rs11545566 genotypes (AA, n = 3; AG, n = 4; GG, n = 3) using one-way ANOVA (left) and between the AA (n = 3) and non-AA (n = 7) genotypes using a t-test (right). Data of two independent experiments (mean ± S.E.M.) each performed in triplicate are shown.

Figure 2

Impact of donor *ACAT1* rs11545566 genotype on the incidence of acute GVHD. **(a)** Cumulative incidences of grade II-IV acute GVHD in patients transplanted from donors with *ACAT1* rs11545566 AA (solid line, n = 36), AG (dashed line, n = 52), and GG (dotted line, n = 28) genotypes are shown. **(b)** Cumulative incidences of grade II-IV acute GVHD in patients transplanted from donors with *ACAT1* rs11545566 AA (solid line, n = 36) and non-AA (dashed line, n = 80) genotypes are shown.

Figure 3

Impact of donor *ACAT1* rs11545566 genotype on the incidence of chronic GVHD. **(a)**

Cumulative incidences of extensive chronic GVHD in patients transplanted from donors with *ACAT1* rs11545566 AA (solid line, n = 36), AG (dashed line, n = 49), and GG (dotted line, n = 25) genotypes are shown. **(b)** Cumulative incidences of extensive chronic GVHD transplanted from donors with *ACAT1* rs11545566 AA (solid line, n = 36) and non-AA (dashed line, n = 74) genotypes are shown.

Table 1 Patient characteristics categorized by ACAT1 rs11545566 genotype of the donors

	Donor genotype			P-value
	AA	AG	GG	
Number of patients	36	52	28	
Median age (range), y	35 (16 − 58)	36 (15 − 51)	35 (16 − 56)	
Age at transplantation, n (%)				
15 − 20 y	5 (14)	8 (15)	4 (14)	
21 − 30 y	5 (14)	11 (21)	7 (25)	
31 − 40 y	12 (33)	16 (31)	8 (29)	0.83
41 − 50 y	9 (25)	15 (29)	6 (21)	
51 − 58 y	5 (14)	2 (4)	3 (11)	
Sex, n (%)				
Male	23 (64)	32 (62)	12 (43)	0.18
Female	13 (36)	20 (38)	16 (57)	
Sex mismatch between patient and donor, n (%)				
Male patient from female donor	12 (33)	17 (33)	5 (18)	0.31
Other combinations	24 (67)	35 (67)	23 (82)	
Diagnosis, n (%)				
Acute myeloid leukemia	12 (33)	16 (31)	7 (25)	
Acute lymphoblastic leukemia	5 (14)	8 (15)	6 (21)	0.65
Chronic myeloid luekemia	9 (25)	18 (34)	7 (25)	
Myelodysplastic syndrome	6 (17)	2 (4)	2 (7)	
Other malignancies[#1]	0 (0)	1 (2)	1 (4)	
Non-malignancies[#2]	4 (11)	7 (14)	5 (18)	
Disease risk, n (%)				
Standard-risk	28 (78)	41 (79)	20 (71)	0.74
High-risk	8 (22)	11 (21)	8 (29)	
Cytomegalovirus serostatus, n (%)				
Positive	26 (72)	45 (87)	22 (79)	0.31
Negative	6 (17)	2 (4)	4 (14)	
Unknown	4 (11)	5 (10)	2 (7)	
Conditioning regimen, n (%)				
MAC	35 (97)	50 (96)	25 (89)	0.31
RIC	1 (3)	2 (4)	3 (11)	

#1 Other malignancies include multiple myeloma and mantle cell lymphoma.

#2 Non-malignancies include aplastic anemia, pure red cell aplasia, and paroxysmal nocturnal hemoglobinuria.

Table 2 Multivariate analysis of risk factors for transplant outcome

Outcome and significant factor		Hazard ratio (95% confidence interval)	P-value
Grade II-IV acute GVHD			
Age	<35 y	1	
	≥35 y	7.14 (1.58 − 32.2)	0.011
Donor rs11545566 genotype	AA	1	
	Non-AA	3.70 (0.88 − 15.6)	0.079
Extensive chronic GVHD			
Disease risk	Standard-risk	1	
	High-risk	3.12 (1.29 − 7.74)	0.014
Donor rs11545566 genotype	AA	1	
	Non-AA	5.62 (1.29 − 24.5)	0.021
Relapse rate			
Disease risk	Standard-risk	1	
	High-risk	2.67 (1.22 − 5.85)	0.014
NRM			
Age	<35 y	1	
	≥35 y	3.45 (1.18 − 10.0)	0.023
OS rate			
Age	<35 y	1	
	≥35 y	2.25 (1.17 − 4.33)	0.015
Disease risk	Standard-risk	1	
	High-risk	2.51 (1.33 − 4.19)	0.004
Acute GVHD	0 or I	1	
	II − IV	2.08 (1.03 − 4.19)	0.038

Figure 1

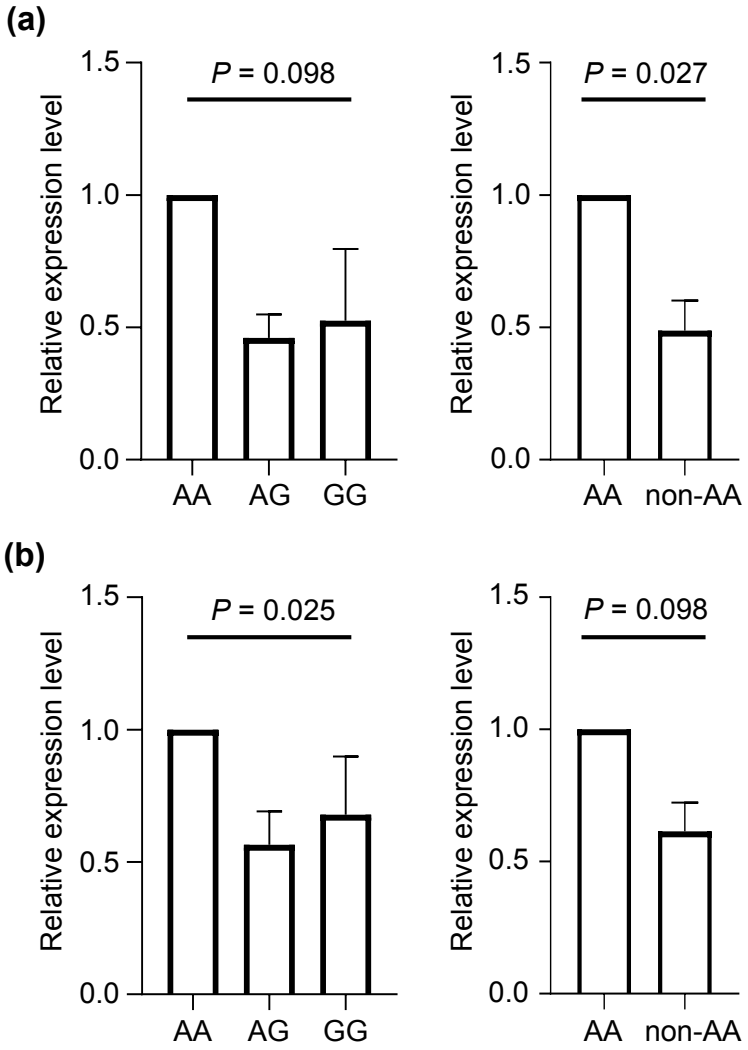

(a)
Relative expression level
1.5
1.0
0.5
0.0
P = 0.098
AA
AG
GG
P = 0.027
non-AA
(b)
P = 0.025
P = 0.098

Figure 2

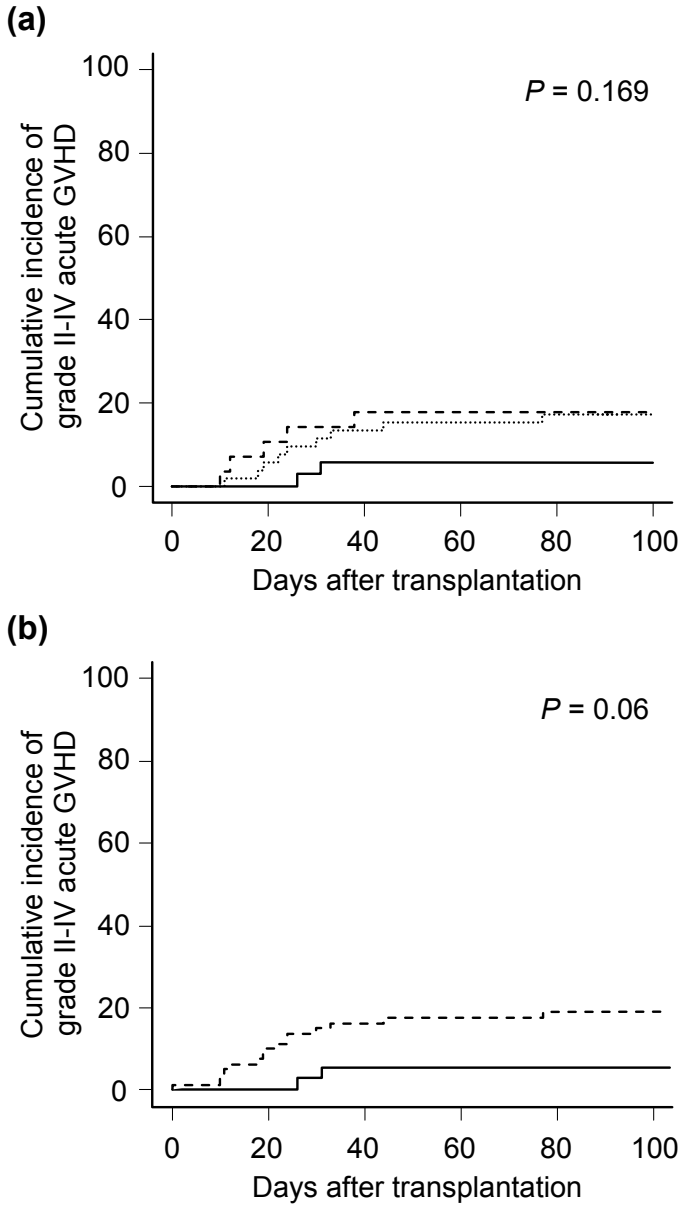
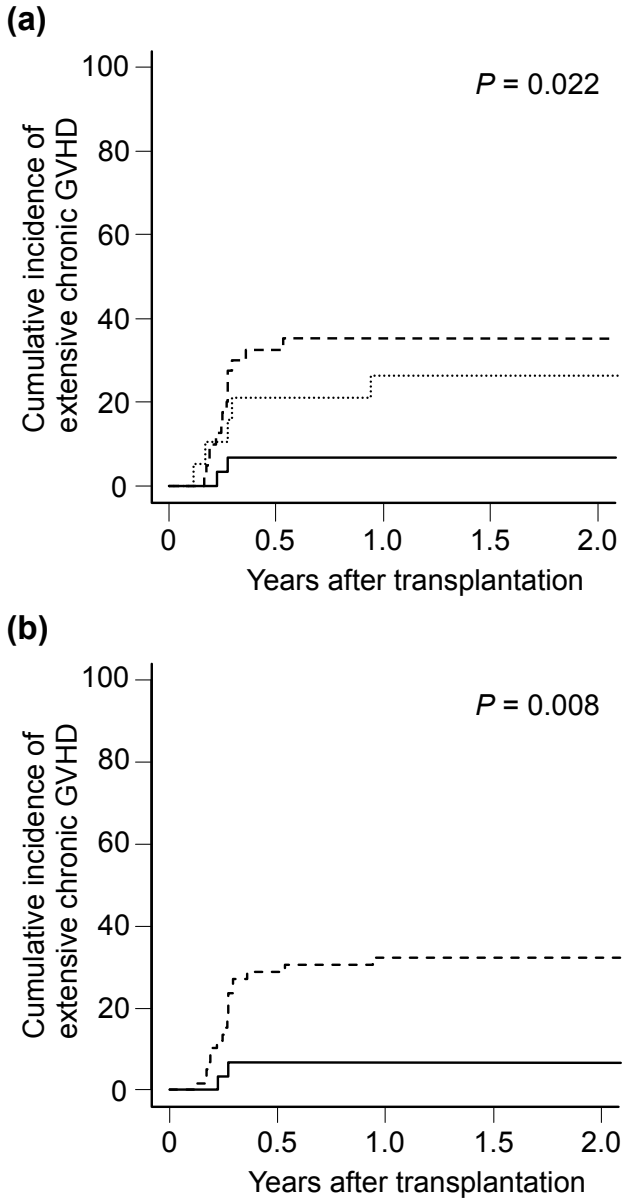

(a)
100
80
60
40
20
0
Cumulative incidence of grade II-IV acute GVHD
P = 0.169
0
20
40
60
80
100
Days after transplantation
(b)
100
80
60
40
20
0
Cumulative incidence of grade II-IV acute GVHD
P = 0.06
0
20
40
60
80
100
Days after transplantation

Figure 3


(a)
100
80
60
40
20
0
Cumulative incidence of extensive chronic GVHD
P = 0.022
0
0.5
1.0
1.5
2.0
Years after transplantation
(b)
100
80
60
40
20
0
Cumulative incidence of extensive chronic GVHD
P = 0.008
0
0.5
1.0
1.5
2.0
Years after transplantation