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ASSOCIATION BETWEEN GENETIC VARIANTS IN ADHESION MOLECULES AND OUTCOMES AFTER HEMATOPOIETIC CELL TRANSPLANTS

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Abstract

Allogeneic hematopoietic cell transplant (HCT) is associated with a high morbidity and mortality. Adhesion molecules play an important role in endothelial activation and initiation of inflammatory response. We hypothesized that single nucleotide polymorphisms (SNPs) in the endothelial molecules may contribute to heterogeneity in HCT outcomes. We evaluated the association of 4 SNPs in *ICAM1* (rs5498), *PECAM1* (rs668 and rs1131012) and *SELL* (rs2229569) genes with acute and chronic graft versus host disease (GvHD) and those experiencing transplant related mortality (TRM) within one year among 425 allogeneic HCT recipient-donor pairs. Using a Fine and Gray proportional hazards model to evaluate the association between genetic variants and clinical outcomes, after adjustment for recipient age, race, diagnosis, disease status, gender mismatch, CMV serostatus, gender, donor type, conditioning regimen and year of transplant, only rs5498 in the *ICAM1* gene among both recipients and donors was associated with a decreased risk of TRM (p 0.02). None of the SNPs were associated with acute or chronic GvHD risk. These findings suggest that genetic variants in the vascular adhesion molecules may be used to identify patients at high risk for TRM.

Keywords

Adhesion molecules; hematopoietic cell transplantation; single nucleotide polymorphisms

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INTRODUCTION

Despite rapid advances in allogeneic hematopoietic cell transplant (HCT) techniques, the procedure continues to be associated with a high morbidity and mortality with transplant related mortality (TRM) ranging from 15% to as high as 50% (Bredeson, *et al.*, 2008, Canals, *et al.*, 2003, Schattenberg and Levenga, 2006). Despite extensive use of HLA matching, non-relapse TRM has remained relatively unchanged over the last decade and graft versus host disease (GvHD) continues to be a significant source of early TRM and morbidity and mortality associated with allogeneic HCT (Chien, *et al.*, 2009, Georges, *et al.*, 2002, Mielcarek, *et al.*, 2003, Mullighan and Bardy, 2007). The pathophysiology of acute GvHD involves priming the immune response by pre-transplant conditioning leading to tissue damage and induction of cytokine release, T cell activation and co-stimulation, alloreactive T cell expansion, differentiation and effector T cell trafficking to target tissues (Ferrara and Deeg, 1991). The pathophysiology of chronic GVHD is more complex. Four theories have been postulated based on experimental studies including thymic damage and defective negative selection of T cells generated from marrow progenitors after HCT, aberrant production of transforming growth factor- β , auto-antibody production, and deficiency of T-regulatory cells (Martin, 2008). The vascular endothelial cells come in intimate contact with lymphocytes during two timepoints; once during donor cell infusion and transmigration of lymphocytes through the endothelial monolayer and secondly, when the newly generated T-lymphocytes enter into the blood stream resulting in biphasic activation of endothelial cells during allogeneic HCT (Palomo, *et al.*, 2010). Experimental models suggest that vascular endothelial cell activation due to alloreactive T-lymphocytes may be important in the pathogenesis of acute and chronic GvHD (Biedermann, 2008). Several adhesion molecules such as platelet/endothelial cell adhesion molecule 1 (PECAM-1/CD31), intracellular adhesion molecule 1 (ICAM-1/CD54) and L-selectin (CD62L) are expressed on the surface of vascular endothelial cells and leukocytes and facilitate interactions between the two cell types (Carlos and Harlan, 1994). Several studies have shown increased serum concentrations of ICAM-1 and other adhesion molecules among patients who underwent allogeneic and autologous HCT (Cutler, *et al.*, 2010, Matsuda, *et al.*, 2001), though not all studies have been consistent (Carreras and Diaz-Ricart, 2011). The increased activation of the ICAM-1 in allogeneic HCT as compared to autologous HCT suggests that polymorphisms in the genes coding for these surface proteins may create alloantigens that may initiate or amplify the cascade leading to GvHD (Matsuda, *et al.*, 2001). Several coding single nucleotide polymorphisms (SNPs) with high allele frequency (> 10%) that result in an amino acid substitution that can influence protein structure and function have been identified in *ICAMI*, *PECAMI* and *SELL* genes. Several studies have evaluated the three common coding SNPs in *PECAMI*, with acute GvHD risk and overall survival and have found conflicting results (Mullighan and Bardy, 2007). Only one previous study on a Japanese population has evaluated the association between one coding SNP in *ICAMI* and *SELL* and acute GvHD and found no association between the coding SNPs in these genes and acute GvHD risk (Maruya, *et al.*, 1998). We hypothesized that an association between common coding SNPs of *PECAMI*, *ICAMI* and *SELL* genes with acute and chronic GvHD and TRM at one year might explain some variability in risks for these complications. We investigated these adhesion molecule SNPs in a cohort of recipients and donors who received an allogeneic HCT for hematologic malignancy at the University of Minnesota.

MATERIAL AND METHODS

Patient Selection

All patients (pediatric and adult) who underwent an allogeneic HCT from an HLA-identical sibling donor, matched or mismatched URD or single umbilical cord blood (UCB) transplant

for treatment of hematological malignancies from January 1, 1998 to December 31, 2007 and their respective donors were eligible for study (n=587 donor-recipient pairs). After excluding patients for whom DNA samples were not available, 490 (83%) donor-recipient pairs were included in this study. This study was approved by the Institutional Review Board at the University of Minnesota and waiver of informed consent from individual patients was obtained for this study.

Clinical data and collection of DNA samples

All clinical data was obtained from the University of Minnesota Blood and Marrow Transplant Database which contains patient-approved prospective data on all patients and donors treated at our institution. Demographics and transplant characteristics, which included patient and donor age at transplant, patient and donor gender, type of donor (related, unrelated donor (URD) or umbilical cord blood (UCB)), graft source (bone marrow versus peripheral blood stem cell versus UCB), race, patient's underlying disease, disease status at transplant, HLA matching between donor and recipient, conditioning intensity (myeloablative or reduced intensity), conditioning regimen used for transplant, GvHD prophylaxis, cytomegalovirus (CMV) serological status of recipient and donor prior to transplant and survival at time of last follow up were collected. HLA- matching status for URD transplants was categorized as well matched, partially matched or mismatched based on classification proposed by Weisdorf et al (Weisdorf, *et al.*, 2008). HLA-matching status for cord blood transplants was based on antigen level HLA-A, B and allele level HLA-DRB1 typing. Transplant related mortality (TRM) was defined as mortality due to all causes other than disease relapse or progression. Causes of death were carefully evaluated and excluded one accidental death unrelated to HCT (n=1). Acute and chronic GvHD were defined using NIH consensus criteria (Filipovich, *et al.*, 2005, Przepiorka, *et al.*, 1995).

DNA samples from patients were obtained from peripheral blood samples collected prior to HCT. All patients with acute leukemias had no evidence of leukemic blasts in peripheral blood at the time of obtaining blood for DNA extraction. All donors donated a blood sample prior to stem cell mobilization or harvesting bone marrow. DNA was extracted from buffy coat using either the Puregene DNA extraction method (for samples collected prior to 2001) (Gentra Systems, Minneapolis, MN) or the Qiagen Mini Blood kit (all samples collected during or after 2001) (Qiagen Inc., San Jose, CA) as per manufacturer recommendations. All DNA samples were collected and stored at 4°C in the Molecular Diagnostics Laboratory at the University of Minnesota Medical Center, Fairview prior to use in the study. We genotyped 4 SNPs in adhesion molecules; K469E (rs5498) in the *ICAM1* gene, L98V (rs668) and R643G (rs1131012) in the *PECAM1* gene and P213S (rs2229569) in the *SELL* gene. All DNA samples were genotyped using the Sequenom iPLEX system at the Biomedical Genomics Center at the University of Minnesota. All primers and probes were designed using Sequenom primer design software. All SNPs had completion rates 95% and a concordance rate 95% (based on 61 pairs of duplicate samples).

STATISTICAL ANALYSIS

All statistical analyses were performed using SAS software version 9.1.3 (SAS Institute, Inc., Cary, NC) and R Statistical software version 2.4.1 (<http://cran.openourcesources.org/>) (R, 2008). We evaluated Hardy Weinberg proportions for all the 4 SNPs. Linkage disequilibrium (LD) between the 2 SNPs in the *PECAM1* gene was estimated using Haploview software (Barrett, *et al.*, 2005). Assuming an additive model for the SNPs (where each SNP was treated as a continuous variable with the homozygous major, heterozygote and homozygous minor genotypes indicating 0, 1 or 2 copies of the minor allele being present), the association between the individual SNPs and TRM at 1 year was evaluated using a proportional hazards model with disease relapse as the competing risk

as described by Fine and Gray (Fine and Gray, 1999). Covariates considered included recipient age at transplant, race, diagnosis, disease status at transplant, gender mismatch, CMV serostatus of recipient and donor, recipient and donor sex, donor type, conditioning regimen (myeloablative or reduced intensity) and year of transplant. Since HLA matching was highly correlated with donor type, the final model was not adjusted for HLA matching. In addition to these covariates, we conducted additional analysis of TRM which was adjusted for acute GvHD as a time dependent covariate. SNPs that were significant in these analyses were evaluated for interactions between recipient and donor within the same SNP. For each of the 4 SNPs, we also considered if these SNPs could act as minor histocompatibility loci. Hence we evaluated whether non-identity (i.e.) genotype mismatch within each recipient-donor pair and non-compatibility (i.e.) recipient allele that could be recognized as foreign by the donor were associated with TRM using the models described above. Cumulative incidence estimates were calculated for TRM with disease relapse as the competing risk. We also evaluated the association between SNPs in adhesion molecules and acute and chronic GvHD where TRM was used as the competing risk. Cumulative incidence estimates for acute and chronic GvHD were calculated with TRM as the competing risk. A p value ≤ 0.05 was considered to be significant for this study.

RESULTS

The baseline characteristics of the study population are described in table 1. Briefly, a majority of the patients underwent myeloablative HCT from an HLA-identical sibling donor ($n=339$), 73% of the cohort were ≤ 21 years at the time of transplant and 86% of the cohort were Caucasian. Acute leukemia (acute myeloid leukemia and acute lymphoid leukemia) (51%) was the most common diagnosis. HLA matching was highly correlated with the donor source with all related sibling transplants (100%) and a majority of the unrelated donors (96%) undergoing 6/6 HLA matched transplants. Among 71 UCB recipients, 31 (44%) were mismatched at a single HLA locus (5/6), 32 (45%) were mismatched at 2 loci (4/6) and 8 (11%) received a 6/6 matched transplant. The cumulative incidence of TRM at 1 year was 24% (95% CI: 20%–28%), acute GvHD at 100 days was 41% (36%–46%) and chronic GvHD at 1 year was 29% (24%–33%). The linkage disequilibrium (r^2) between L98V (rs668) and R643G (rs1131012) in the *PECAM1* gene was 0.76 and 0.79 among recipients and donors respectively.

Association between SNPs in adhesion molecules and HCT outcomes

After adjustment for recipient age at transplant, race, diagnosis, disease status at transplant, gender mismatch, CMV serostatus of recipient and donor, recipient and donor sex, donor type, conditioning regimen (myeloablative or reduced intensity) and year of transplant, K469E (rs5498) in the *ICAM1* gene in both recipient and donors was associated with a decreased risk of TRM (Table 2a). None of the other SNPs tested were associated with TRM. There were no significant interactions between recipient and donor K469E ($p = 0.06$) in determining TRM risk. Non-identity and non-compatibility among recipients and donors for all four SNPs was not associated with TRM (Table 2b). After adjustment for acute GvHD as a time dependent covariate, rs5498 in recipients remained significantly associated with TRM (HR (95% CI): 0.68 (0.51 – 0.90); $p=0.01$) but was not significantly associated with TRM among donors (0.83 (0.61, 1.12) $p=0.23$). None of the SNPs tested in either donor or recipient were associated with acute GvHD (Tables 3a and 3b) or chronic GvHD (Tables 4a and 4b).

DISCUSSION

This is the first study to show an association between rs5498 in the *ICAM1* gene in either the recipient or donor and a decreased risk of TRM. This study did not find any association

between other known coding SNPs in adhesion molecules and risks of acute and chronic GvHD.

The rs5498 variant in *ICAM1* results in an amino acid change from Lysine (K) to glutamate (E) in codon 469 that can result in a change in function of the ICAM-1 protein. A recent study has shown that genetic polymorphisms in the *ICAM1* gene (including SNPs in strong linkage disequilibrium with rs5498) are associated with circulating levels of ICAM-1 (Bielinski, *et al.*, 2011). Activation of endothelial cells is essential in recruitment of leukocytes to the site of inflammation (Carlos and Harlan, 1994). Endothelial adhesion molecules such as ICAM-1 are expressed in a broad range of tissues including vascular endothelial cells and leukocytes and play a critical role in the initiation of the inflammatory process by recruiting neutrophils to the site of inflammation (Elangbam, *et al.*, 1997). Thus genetic variation in the *ICAM1* gene can modulate innate immune responses by influencing the amount of tissue damage induced by local inflammation in response to cellular interactions. Furthermore, rs5498 in recipients remained associated with TRM even after adjustment for risk of acute GvHD indicating that K469E may influence TRM through mechanisms independent of acute GvHD. This finding suggests that endothelial damage in the recipient that occurs as a part of the conditioning regimen may play an important role in determining ICAM-1 activation and subsequent TRM. Consistent with one previous report on rs5498 and acute GvHD in a Japanese population, the present study did not show any association between the rs5498 SNP and acute GvHD risk (Maruya, *et al.*, 1998). Since non-identity or non-compatibility of K469E was not associated with either acute or chronic GvHD and TRM, these findings suggest that the K469E has a functional effect on TRM and does not serve as a minor histocompatibility antigen.

PECAM-1 is a cell adhesion molecule that is expressed on surfaces of variety of hematopoietic cells (Newman, *et al.*, 1990, Novinska, *et al.*, 2006) and has a central role in the activation of lymphocytes following recognition of alloantigen and coordination of leukocyte adhesion and extravasation. This study found no association between individual SNPs or non-identity or non-compatibility of *PECAM1* SNPs and risk of acute or chronic GvHD and TRM. PECAM-1 has been extensively evaluated as a minor histocompatibility antigen due to its wide tissue distribution and structure of the extracellular domain that is important in determining histocompatibility. In addition, a few studies have evaluated the association between the individual coding SNPs in the *PECAM1* gene and acute GvHD. The first report by Behar et al showed that identical L98V genotypes between recipient and donors was associated with (previously reported as the L125V) and increased risk of acute GvHD (Behar, *et al.*, 1996) though subsequent studies showed that non-identity and non-compatibility were both not associated with acute GvHD (Nichols, *et al.*, 1996, Spellman, *et al.*, 2009). Subsequent studies reported both non-identity and non-compatibility of two additional polymorphisms, the R643G (previously reported as the R670G) polymorphism and the N563S polymorphisms to be associated with increased risk of acute GvHD (Balduini, *et al.*, 1999, Grumet, *et al.*, 2001, Maruya, *et al.*, 1998) though a larger multicenter study from the National Marrow Donor Program did not confirm this finding (Spellman, *et al.*, 2009). A study by Goodman et al also showed that the R643G polymorphism among donors was associated with increased risk of acute GvHD (Goodman, *et al.*, 2005) though this finding was not confirmed in a Tunisian population (Sellami, *et al.*, 2011). Since previous studies have reported complete linkage disequilibrium between the R643G and N563S polymorphisms (Grumet, *et al.*, 2001, Maruya, *et al.*, 1998) we genotyped only the R643G polymorphism in this study. Two studies have also shown no association between any of the *PECAM1* SNPs (L98V, R643G and N563S) and chronic GvHD risk (Sellami, *et al.*, 2011, Spellman, *et al.*, 2009).

L-selectin is a member of the selectin superfamily that is selectively expressed on all leukocytes (Carlos and Harlan, 1994). This study found no association between the P213S variant, non-identity or non-compatibility with risk of acute and chronic GvHD and TRM. Consistent with findings from this study, a previous study also found no association between the P213S variant in the *SELL* gene and acute GvHD in a Japanese population (Maruya, *et al.*, 1998).

Though SNPs in adhesion molecules may be associated with HCT outcomes among patients with specific HLA epitopes, in our modest sized cohort, we were unable to suitably address the association of these adhesion molecules with individual HLA types. This analysis suggests the need for further study into the biological significance of these SNPs and a need to examine somatic mutations within tumor cells that may also influence treatment outcomes following allogeneic HCT. Finally, TRM following allogeneic HCT includes several causes of death including GvHD, infection or organ toxicity from the conditioning regimen. We evaluated SNPs associated with TRM considering acute GvHD as a time dependent covariate to identify whether the SNPs were associated with TRM through GvHD dependent or GvHD independent pathways. However, our cohort size prevented subgroup analyses to evaluate the association of SNPs with individual causes of TRM.

This study found a significant association between genetic variation in *ICAM1* and risk of TRM after allogeneic HCT. Further studies are needed to comprehensively evaluate the role of common as well as other rare genetic variation in adhesion molecules in determining outcomes after allogeneic HCT.

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REFERENCES

- Balduini CL, Noris P, Giorgiani G, Martinetti M, Klersy C, Spedini P, et al. Incompatibility for CD31 and human platelet antigens and acute graft-versus-host disease after bone marrow transplantation. *Br J Haematol.* 1999; 106:723–729. [PubMed: 10468865]
- Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics.* 2005; 21:263–265. [PubMed: 15297300]
- Behar E, Chao NJ, Hiraki DD, Krishnaswamy S, Brown BW, Zehnder JL, et al. Polymorphism of adhesion molecule CD31 and its role in acute graft-versus-host disease. *N Engl J Med.* 1996; 334:286–291. [PubMed: 8532023]
- Biedermann BC. Vascular endothelium and graft-versus-host disease. *Best Pract Res Clin Haematol.* 2008; 21:129–138. [PubMed: 18503981]
- Bielinski SJ, Reiner AP, Nickerson D, Carlson C, Bailey KR, Thyagarajan B, et al. Polymorphisms in the ICAM1 gene predict circulating soluble intercellular adhesion molecule-1(sICAM-1). *Atherosclerosis.* 2011; 216:390–394. [PubMed: 21392767]
- Bredeson CN, Zhang MJ, Agovi MA, Bacigalupo A, Bahlis NJ, Ballen K, et al. Outcomes following HSCT using fludarabine, busulfan, and thymoglobulin: a matched comparison to allogeneic transplants conditioned with busulfan and cyclophosphamide. *Biol Blood Marrow Transplant.* 2008; 14:993–1003. [PubMed: 18721762]
- Canals C, Martino R, Sureda A, Altes A, Briones J, Subira M, et al. Strategies to reduce transplant-related mortality after allogeneic stem cell transplantation in elderly patients: Comparison of reduced-intensity conditioning and unmanipulated peripheral blood stem cells vs a myeloablative regimen and CD34+ cell selection. *Exp Hematol.* 2003; 31:1039–1043. [PubMed: 14585367]

- Carlos TM, Harlan JM. Leukocyte-endothelial adhesion molecules. *Blood*. 1994; 84:2068–2101. [PubMed: 7522621]
- Carreras E, Diaz-Ricart M. The role of the endothelium in the short-term complications of hematopoietic SCT. *Bone Marrow Transplant*. 2011
- Chien JW, Zhao LP, Storer B, Martin PJ, Boeckh M, Warren EH, et al. Improving hematopoietic cell transplant outcomes in a new era of genomic research. *Biol Blood Marrow Transplant*. 2009; 15:42–45. [PubMed: 19147077]
- Cutler C, Kim HT, Ayanian S, Bradwin G, Revta C, Aldridge J, et al. Prediction of veno-occlusive disease using biomarkers of endothelial injury. *Biol Blood Marrow Transplant*. 2010; 16:1180–1185. [PubMed: 20184961]
- Elangbam CS, Qualls CW Jr, Dahlgren RR. Cell adhesion molecules--update. *Vet Pathol*. 1997; 34:61–73. [PubMed: 9150551]
- Ferrara JL, Deeg HJ. Graft-versus-host disease. *N Engl J Med*. 1991; 324:667–674. [PubMed: 1994250]
- Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant*. 2005; 11:945–956. [PubMed: 16338616]
- Fine J, Gray R. A proportional hazards model for the subdistribution of a competing risk. *Journal of The American Statistical Association*. 1999; 94:496–509.
- Georges GE, Maris M, Sandmaier BM, Malone DG, Feinstein L, Niederweiser D, et al. Related and unrelated nonmyeloablative hematopoietic stem cell transplantation for malignant diseases. *Int J Hematol*. 2002; 76(Suppl 1):184–189. [PubMed: 12430851]
- Goodman RS, Ewing J, Evans PC, Craig J, Poulton K, Dyer PA, et al. Donor CD31 genotype and its association with acute graft-versus-host disease in HLA identical sibling stem cell transplantation. *Bone Marrow Transplant*. 2005; 36:151–156. [PubMed: 15908974]
- Grumet FC, Hiraki DD, Brown BWM, Zehnder JL, Zacks ES, Draksharapu A, et al. CD31 mismatching affects marrow transplantation outcome. *Biol Blood Marrow Transplant*. 2001; 7:503–512. [PubMed: 11669217]
- Martin PJ. Biology of chronic graft-versus-host disease: implications for a future therapeutic approach. *Keio J Med*. 2008; 57:177–183. [PubMed: 19110529]
- Maruya E, Saji H, Seki S, Fujii Y, Kato K, Kai S, et al. Evidence that CD31, CD49b, and CD62L are immunodominant minor histocompatibility antigens in HLA identical sibling bone marrow transplants. *Blood*. 1998; 92:2169–2176. [PubMed: 9731077]
- Matsuda Y, Hara J, Osugi Y, Tokimasa S, Fujisaki H, Takai K, et al. Serum levels of soluble adhesion molecules in stem cell transplantation-related complications. *Bone Marrow Transplant*. 2001; 27:977–982. [PubMed: 11436109]
- Mielcarek M, Martin PJ, Leisenring W, Flowers ME, Maloney DG, Sandmaier BM, et al. Graft-versus-host disease after nonmyeloablative versus conventional hematopoietic stem cell transplantation. *Blood*. 2003; 102:756–762. [PubMed: 12663454]
- Mullighan CG, Bardy PG. New directions in the genomics of allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2007; 13:127–144. [PubMed: 17241919]
- Newman PJ, Berndt MC, Gorski J, White GC 2nd, Lyman S, Paddock C, et al. PECAM-1 (CD31) cloning and relation to adhesion molecules of the immunoglobulin gene superfamily. *Science*. 1990; 247:1219–1222. [PubMed: 1690453]
- Nichols WC, Antin JH, Lunetta KL, Terry VH, Hertel CE, Wheatley MA, et al. Polymorphism of adhesion molecule CD31 is not a significant risk factor for graft-versus-host disease. *Blood*. 1996; 88:4429–4434. [PubMed: 8977234]
- Novinska MS, Pietz BC, Ellis TM, Newman DK, Newman PJ. The alleles of PECAM-1. *Gene*. 2006; 376:95–101. [PubMed: 16581204]
- Palomo M, Diaz-Ricart M, Carbo C, Rovira M, Fernandez-Aviles F, Martine C, et al. Endothelial dysfunction after hematopoietic stem cell transplantation: role of the conditioning regimen and the type of transplantation. *Biol Blood Marrow Transplant*. 2010; 16:985–993. [PubMed: 20167280]

- 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; 15:825–828. [PubMed: 7581076]
- R, D., Core, Team. Foundation for Statistical Computing. Vienna, Austria: 2008. R: A Language and Environment for Statistical Computing.
- Schattenberg AV, Levenga TH. Differences between the different conditioning regimens for allogeneic stem cell transplantation. *Curr Opin Oncol.* 2006; 18:667–670. [PubMed: 16988592]
- Sellami MH, Torjemane L, Ladeb S, Kaabi H, Ben Ahmed A, Cherif G, et al. Investigation of the effect of donor platelet endothelial cell adhesion molecule 1 polymorphism on the graft-vs.-host disease occurrence in Tunisian recipients of hematopoietic stem cells. *Clin Biochem.* 2011; 44:699–703. [PubMed: 21349261]
- Spellman S, Warden MB, Haagenson M, Pietz BC, Goulmy E, Warren EH, et al. Effects of mismatching for minor histocompatibility antigens on clinical outcomes in HLA-matched, unrelated hematopoietic stem cell transplants. *Biol Blood Marrow Transplant.* 2009; 15:856–863. [PubMed: 19539218]
- Weisdorf D, Spellman S, Haagenson M, Horowitz M, Lee S, Anasetti C, et al. Classification of HLA-matching for retrospective analysis of unrelated donor transplantation: revised definitions to predict survival. *Biol Blood Marrow Transplant.* 2008; 14:748–758. [PubMed: 18541193]

Table 1

Demographic and transplant characteristics of patients who underwent allogeneic hematopoietic stem cell transplant at the University of Minnesota: 1998–2007

Variable	N (%)
N	425 (100)
Recipient Age at Transplant (Years)	
<20	116 (27.3)
20 – 40	99 (23.3)
>=40	210 (49.4)
Median age (range), years	39.9 (0.7 – 69.8)
Donor Age at Transplant (Years)	
<20	95 (24.2)
20–40	113 (28.8)
>=40	185 (47.1)
Not known	32
Median age (range), years	39.1 (0.3 – 75.3)
Race	
African American	12 (2.9)
Asian	12 (2.9)
Caucasian	363 (86.4)
Hispanic	13 (3.1)
Native American	5 (1.2)
Mixed	5 (1.2)
Missing	15 (2.6)
Diagnosis	
Acute lymphoid leukemia	87 (20.5)
Acute myeloid leukemia	129 (30.4)
Chronic myeloid leukemia	65 (15.3)
Lymphoma	74 (17.4)
Myelodysplastic syndrome	37 (8.7)
Others	33 (7.8)
Disease Status at Transplant	
Complete response	254 (60.1)
Partial response	23 (5.4)
Relapse / primary induction failure	109 (25.8)
Myelodysplastic syndrome	37 (8.8)
Missing	2
CMV Serological status	
Recipient positive / donor positive	91 (21.4)
Recipient positive / donor negative	132 (31.1)

Variable	N (%)
Recipient negative / donor positive	61 (14.4)
Recipient negative / donor negative	140 (32.9)
Missing	1
Conditioning	
Myeloablative	339 (79.8)
Reduced Intensity	86 (20.2)
Donor Type	
Sibling	277 (65.2)
Unrelated donor	77 (18.1)
Cord blood	71 (16.7)
HLA matching	
HLA matched transplants (6/6)	359 (84.5)
Partially matched HLA transplants (5/6)	32 (7.5)
Mismatched HLA transplants (4/6)	34 (8.0)
Stem Cell Source	
Marrow	149 (35.0)
PBSC	202 (47.5)
Cord blood	74 (17.4)
GvHD Prophylaxis	
CSA/MMF	109 (25.7)
CSA/MTX	227 (53.5)
T-cell depletion	42 (9.9)
Other	46 (10.9)
Missing	1
Acute Graft versus host disease	
No aGvHD	212 (50.1)
Grade I	41 (9.7)
Grade II	109 (25.8)
Grade III/IV	61 (14.4)
Missing	2
Chronic graft versus host disease	136 (32.0)
Follow up in years (Survivors), median (range)	3.1 (0.0 – 8.6)

Abbreviations CMV: cytomegalovirus; PBSC: peripheral blood stem cell; CSA: cyclosporine; MMF: mycophenolate mofetil; MTX: methotrexate

Table 2

a: Association between individual SNPs in adhesion molecules and one year transplant related mortality after adjustment for clinical covariates				
SNPs	Gene	Alleles	Minor allele Frequency	Hazard Ratio (95% CI); p-value (n=425 donor-recipient pairs)
Recipient SNPs				
rs5498	<i>ICAM1</i>	A/G	0.42	0.67 (0.50, 0.89); p = 0.01
rs668	<i>PECAM1</i>	C/G	0.45	1.16 (0.88, 1.53); p = 0.29
rs1131012	<i>PECAM1</i>	G/A	0.50	1.21 (0.91, 1.60); p = 0.18
rs2229569	<i>SELL</i>	C/T	0.14	0.73 (0.45, 1.19); p = 0.21
Donor SNPs				
rs5498	<i>ICAM1</i>	A/G	0.43	0.73 (0.55, 0.95); p = 0.02
rs668	<i>PECAM1</i>	C/G	0.46	0.89 (0.67, 1.18); p = 0.42
rs1131012	<i>PECAM1</i>	G/A	0.49	0.98 (0.74, 1.29); p = 0.86
rs2229569	<i>SELL</i>	C/T	0.14	0.81 (0.52, 1.27); p = 0.36

b: Association between non-identity and non-compatibility in adhesion molecules and one year transplant related mortality after adjustment for clinical covariates			
SNPs	Gene	Frequency	Hazard Ratio (95% CI); p-value (n=425 donor-recipient pairs)
Non-identity **			
rs5498	<i>ICAM1</i>	0.48	1.04 (0.70, 1.55); p = 0.86
rs668	<i>PECAM1</i>	0.50	1.05 (0.71, 1.56); p = 0.80
rs1131012	<i>PECAM1</i>	0.67	1.04 (0.68, 1.60); p = 0.85
rs2229569	<i>SELL</i>	0.33	0.71 (0.45, 1.12); p = 0.14
Non-compatibility ***			
rs5498	<i>ICAM1</i>	0.12	0.94 (0.54, 1.64); p = 0.84
rs668	<i>PECAM1</i>	0.15	1.18 (0.69, 2.01); p = 0.55
rs1131012	<i>PECAM1</i>	0.30	0.88 (0.57, 1.35); p = 0.55
rs2229569	<i>SELL</i>	0.02	Not-estimable

* This model was adjusted for recipient age at transplant, race, diagnosis, disease status at transplant, gender mismatch, CMV serostatus of recipient and donor, recipient and donor sex, donor type, conditioning regimen (myeloablative or reduced intensity) and year of transplant.

**

Non-identity = mismatch in genotypes between recipient donor pairs.

Non-compatibility = Recipient genotypes that could be recognized as foreign by the donor.

Table 3

a: Association between individual SNPs in adhesion molecules and grade II–IV acute graft versus host disease (aGvHD) after adjustment for clinical covariates				
SNPs	Gene	Alleles	Minor allele Frequency	Hazard Ratio (95% CI); p-value (n=425 donor-recipient pairs)
Recipient SNPs				
rs5498	<i>ICAM1</i>	A/G	0.42	0.95 (0.76, 1.18); p = 0.63
rs668	<i>PECAM1</i>	C/G	0.45	0.92 (0.75, 1.12); p = 0.40
rs1131012	<i>PECAM1</i>	G/A	0.50	0.86 (0.71, 1.06); p = 0.16
rs2229569	<i>SELL</i>	C/T	0.14	0.85 (0.61, 1.19); p = 0.35
Donor SNPs				
rs5498	<i>ICAM1</i>	A/G	0.43	1.10 (0.88, 1.38); p = 0.41
rs668	<i>PECAM1</i>	C/G	0.46	1.00 (0.80, 1.24); p = 0.99
rs1131012	<i>PECAM1</i>	G/A	0.49	1.06 (0.85, 1.33); p = 0.61
rs2229569	<i>SELL</i>	C/T	0.14	0.74 (0.53, 1.03); p = 0.08

b: Association between non-identity and non-compatibility in adhesion molecules and grade II–IV acute graft versus host disease (aGvHD) after adjustment for clinical covariates			
SNPs	Gene	Frequency	Hazard Ratio (95% CI); p-value (n=425 donor-recipient pairs)
Non-identity**			
rs5498	<i>ICAM1</i>	0.48	1.27 (0.92, 1.76); p = 0.15
rs668	<i>PECAM1</i>	0.50	1.07 (0.77, 1.48); p = 0.70
rs1131012	<i>PECAM1</i>	0.67	0.96 (0.69, 1.34); p = 0.82
rs2229569	<i>SELL</i>	0.33	1.29 (0.94, 1.77); p = 0.12
Non-compatibility**			
rs5498	<i>ICAM1</i>	0.12	1.18 (0.77, 1.81); p = 0.45
rs668	<i>PECAM1</i>	0.15	0.93 (0.59, 1.47); p = 0.76
rs1131012	<i>PECAM1</i>	0.30	0.87 (0.62, 1.22); p = 0.42
rs2229569	<i>SELL</i>	0.02	0.89 (0.23, 3.39); p = 0.86

* This model was adjusted for recipient age at transplant, race, diagnosis, disease status at transplant, gender mismatch, CMV serostatus of recipient and donor, recipient and donor sex, donor type, conditioning regimen (myeloablative or reduced intensity) and year of transplant.

** Non-identity = mismatch in genotypes between recipient donor pairs.

Non-compatibility = Recipient genotypes that could be recognized as foreign by the donor.

Table 4

a: Association between individual SNPs in adhesion molecules and chronic graft versus host disease (cGvHD) after adjustment for clinical covariates				
SNPs	Gene	Alleles	Minor allele Frequency	Hazard Ratio (95% CI); p-value (n=425 donor-recipient pairs)
Recipient SNPs				
rs5498	<i>ICAM1</i>	A/G	0.42	0.99 (0.77, 1.28); p=0.95
rs668	<i>PECAM1</i>	C/G	0.45	0.79 (0.61, 1.02); p=0.07
rs1131012	<i>PECAM1</i>	G/A	0.50	0.89 (0.71, 1.11); p=0.31
rs2229569	<i>SELL</i>	C/T	0.14	0.73 (0.48, 1.10); p=0.13
Donor SNPs				
rs5498	<i>ICAM1</i>	A/G	0.43	0.95 (0.73, 1.23); p=0.69
rs668	<i>PECAM1</i>	C/G	0.46	0.85 (0.65, 1.11); p=0.24
rs1131012	<i>PECAM1</i>	G/A	0.49	1.11 (0.85, 1.46); p=0.44
rs2229569	<i>SELL</i>	C/T	0.14	1.01 (0.67, 1.50); p=0.97

b: Association between non-identity and non-compatibility in adhesion molecules and chronic graft versus host disease (cGvHD) after adjustment for clinical covariates			
SNPs	Gene	Frequency	Hazard Ratio (95% CI); p-value (n=425 donor-recipient pairs)
Non-identity **			
rs5498	<i>ICAM1</i>	0.48	0.80 (0.55, 1.15); p=0.23
rs668	<i>PECAM1</i>	0.50	0.75 (0.50, 1.11); p=0.15
rs1131012	<i>PECAM1</i>	0.67	1.02 (0.70, 1.48); p=0.91
rs2229569	<i>SELL</i>	0.33	0.91 (0.61, 1.36); p=0.65
Non-compatibility **			
rs5498	<i>ICAM1</i>	0.12	0.78 (0.43, 1.43); p=0.43
rs668	<i>PECAM1</i>	0.15	1.19 (0.81, 1.73); p=0.38
rs1131012	<i>PECAM1</i>	0.30	1.18 (0.67, 2.09); p=0.57
rs2229569	<i>SELL</i>	0.02	NA

* This model was adjusted for recipient age at transplant, race, diagnosis, disease status at transplant, gender mismatch, CMV serostatus of recipient and donor, recipient and donor sex, donor type, conditioning regimen (myeloablative or reduced intensity) and year of transplant.

** Non-identity = mismatch in genotypes between recipient donor pairs.

Non-compatibility = Recipient genotypes that could be recognized as foreign by the donor.