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Impact of Donor and Recipient Sex and Parity on Outcomes of HLA-Identical Sibling Allogeneic Hematopoietic Stem Cell Transplantation

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ABSTRACT

Allogeneic hematopoietic stem cell transplantation (SCT) may cure patients with hematologic malignancies, but it carries significant risks. Careful donor selection is an important component of the clinical transplantation decision-making process and includes evaluation of HLA typing and other criteria, the most controversial of which is parity. We examined the effect of donor sex and parity on outcomes of HLA-identical sibling SCT. Because the effect of recipient sex/parity has never been explicitly evaluated, we also analyzed the effect of recipient sex/parity on outcomes of transplantation. We found that (1) parous female donors result in an increased risk of chronic graft-versus-host disease (GVHD) in all recipients, (2) the magnitude of this increased risk is similar in male and female recipients, and (3) nulliparous female donors increase the risk of chronic GVHD in male recipients to a degree comparable to that from parous donors. A decrease in the risk of relapse was not observed, and there was no effect on overall survival, acute GVHD, or transplant-related mortality. Recipient parity had no independent effect on any endpoint. Until the effects of pregnancy on the maternal immune system are better understood, it is appropriate whenever possible to avoid parous female donors and to choose male donors for male recipients in HLA-identical related donor SCT.

KEY WORDS

Allogeneic stem cell transplantation • Graft-versus-host disease • Pregnancy

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (SCT) is a curative therapy for patients with hematologic malignancies but results in significant morbidity and mortality. Donor selection is an important way that the risks may be decreased and is therefore a key component of the clinical practice of transplantation. In general, HLA-identical siblings are the preferred donors, but some patients have more than one HLA-matched sibling. Thus, it is important to understand the contribution of donor factors other than HLA matching to outcomes after SCT. Criteria proved or hypothesized to affect outcomes after SCT include age, cytomegalovirus (CMV) serostatus, ABO compatibility, and sex and parity. Of these, sex/parity is the most controversial, and it is not clear which of these factors should outweigh the others. Some investigators have found an increased risk of acute or

Table 1. *Summary of Previous Studies of Sex and/or Parity in Allogeneic SCT*

Study	Year	n	Sex/Parity Combinations Considered in Study	GVHD Endpoint	Results†
Gale et al [1]	1987	2036	Sex mismatching Alloimmunized* female donor → male vs female recipients	Acute	Increased risk of aGVHD in female → male transplants vs all other combinations Increased risk of aGVHD in alloimmunized female → male vs female recipients Increased risk of aGVHD in non-alloimmunized female → male vs female recipients No increased risk of aGVHD in alloimmunized vs non-alloimmunized female → female recipients
Flowers et al [2]	1990	136	Donor sex/parity → any recipient Parous female donor → male vs female recipients	Acute	Increased risk of aGVHD in recipients of parous vs nulliparous female donor grafts No increased risk of aGVHD in recipients of parous female vs male donor grafts No increased risk of aGVHD in recipients of parous female donor → male vs female recipients
Atkinson et al [3]	1986	2534	Sex mismatching Alloimmunized donor	Chronic	Increased risk of cGVHD in alloimmunized female donors → male vs female recipients
Weisdorf et al [4]	1991	469	Sex mismatching Alloimmunized donor	Acute	Increased risk of aGVHD in all combinations other than female donor/female recipient Increased risk of aGVHD in alloimmunized vs non-alloimmunized donor into all recipients
Nash et al [5]	1992	446	Sex mismatching Donor parity/recipient sex	Acute	Increased risk of aGVHD in female recipients of male grafts and in male recipients of parous female grafts
Carlens et al [6]	1998	451	Female donor → male recipient vs all others Alloimmunized female donor vs all other combinations	Chronic	Increased risk of cGVHD in alloimmunized vs non-alloimmunized donor into all recipients
Remberger et al [7]	2002	679	Alloimmunized female donor → male recipient vs all other combinations	Chronic	Increased risk of cGVHD in male recipients of alloimmunized female donor transplants
Przepiorka et al [8]	1999	160	Donor sex Female donor → male recipient vs all other combinations Alloimmunized donor Alloimmunized donor → male recipient vs all other combinationss	Acute	No effect of donor sex or parity on aGVHD
Bross et al [9]	1984	136	Sex mismatching	Acute	Sex mismatching (either direction) increases risk of aGVHD
Randolph et al [10]	2004	3238	Sex mismatching	Acute and chronic	Increased risk of aGVHD and cGVHD in male recipients of female grafts

**Alloimmunized* refers to previous pregnancy or transfusion.
†aGVHD indicates acute graft-versus-host disease; cGVHD, chronic GVHD.

chronic graft-versus-host disease (GVHD) associated with donor parity [1-7], although it is uncertain whether this risk applies just to male recipients or to all patients. Conversely, in other studies, parity was not a risk factor for GVHD [8]. Further, some studies have focused on sex mismatching only, without incorporating parity [9,10]. These results, summarized in Table 1, are generally from single centers, contain small

numbers of patients, or were reported before the era of combination GVHD prophylaxis with a calcineurin inhibitor plus methotrexate. Thus, it is difficult to synthesize the results into a cohesive model of the effect of donor parity on outcomes of related-donor SCT that is applicable to current practice. In a National Marrow Donor Program analysis of unrelated donor data, parity was identified as an independent risk factor for chronic GVHD [11].

It is hypothesized that pregnancy-induced alloimmunization is the mechanism underlying the increased risk of GVHD imparted by parous donors. It is clear that there is maternal exposure to fetal antigens: fetal cells or DNA have been detected in the maternal circulation in 50% to 100% of women during pregnancy [12-15] and are identified as soon as 5 weeks' gestation [16]. Further, there are ample data to demonstrate that alloimmunization occurs, as evidenced by clinical phenomena such as rhesus isoimmunization and neonatal alloimmune thrombocytopenia, and by the postpregnancy development of antibodies and/or cytotoxic T-cells that recognize epitopes of the rhesus D protein [17], platelet antigens [18], HLA [19-21], and autosomal [22] and Y-chromosome–encoded (H-Y) [22-26] minor histocompatibility antigens.

The role of previous pregnancies in recipients of allogeneic SCT has never been evaluated, although this also has the potential to influence outcomes and may interact with donor parity. Residual recipient immunity may remain despite myeloablative conditioning therapy [27], and this persistent immunity is the presumed cause of graft rejection [28,29].

This study investigated the effect of donor and recipient sex/parity on outcomes of HLA-identical sibling allogeneic SCT in a large cohort of patients. This is also the first study to evaluate the effect of recipient sex/parity on outcomes of SCT.

METHODS

Patients

The study population consisted of adults (age ≥18 years) who were reported to the Center for International Blood and Marrow Transplant Research (CIBMTR) and received a non–T-cell–depleted, myeloablative allogeneic SCT from an HLA-identical sibling who was also ≥18 years old, between 1995 and 1999, for acute lymphoblastic or myelogenous leukemia or chronic myelogenous leukemia. Characteristics of the population are listed in Tables 2 and 3.

Donors and recipients were categorized as male, nulliparous female, or parous (≥1 pregnancy) female. Covariables of interest are listed in Table 4. Of note, "bidirectional" ABO incompatibility (eg, type A donor → type B recipient) was classified as minor, rather than major, incompatibility. This approach is consistent with previous studies of ABO incompatibility in SCT [30-32] and has the effect of maximizing the contribution of ABO incompatibility to GVHD. We felt that this was the most conservative approach.

Study Endpoints

The primary endpoints of this retrospective cohort study included overall survival and acute and chronic GVHD. Secondary endpoints were relapse and transplant-related mortality. Acute GVHD was present if graded II-IV according to criteria of Glucksberg et al [33]; cumulative incidence is reported at 100 days after transplantation. Chronic GVHD included limited and extensive disease; only patients surviving past day 100 were considered at risk for this endpoint. Cumulative incidence is reported at 2 years after transplantation. Transplant-related mortality was defined as any death within 28 days of transplantation or death in continuous remission.

Statistical Analysis

Analyses were performed with STATA 7.0 (STATA Corporation, College Station, Tex) and SAS 8.2 (SAS Institute, Cary, NC).

All covariables were compared across the 3 donor types (male, nulliparous female, and parous female; Table 2) and across the 3 groups of recipients (data not shown). Categorical variables were compared with chi-square test. Continuous variables (patient and donor age) were compared with the Kruskal-Wallis test.

Univariable analysis. First, the effect of donor sex/parity was estimated for each primary endpoint (survival and acute and chronic GVHD), with male donors serving as the reference group. Overall survival was evaluated with the Kaplan-Meier method, and groups were compared by using the Tarone-Ware modification of the log-rank test. For acute and chronic GVHD, cumulative incidence (with death as a competing risk [34,35]) was compared across the 3 donor groups. A separate set of models was then created in similar fashion to estimate independently the effect of recipient sex/parity on these 3 primary endpoints. We estimated univariable hazard ratios for each covariable (Table 2) for all endpoints. Only those covariables that were statistically significantly associated with an endpoint ($P < .05$) were included in multivariable analysis of that endpoint.

Multivariable analysis. For multivariable analyses, the main effect was the combination of donor and recipient sex/parity as presented in Table 3 (9 groups in total). We used Cox proportional hazards regression models to estimate the risk of each outcome for each donor/recipient combination, with the male donor/male recipient (M-D → M-R) group serving as the reference. A model was built for each endpoint as a dependent variable and the main effect term for the

Table 2. *Patient Characteristics*

	Donor, n (%)				
	Male n = 1637 (62)	Nulliparous Female n = 277 (11)	Parous Female n = 712 (27)	Total n = 2626	*P*
Recipient					
Male	1007 (62)	153 (55)	398 (56)	1558 (59)	<.001
Nulliparous female	145 (9)	53 (19)	62 (9)	260 (10)	
Parous female	485 (30)	71 (26)	252 (35)	808 (31)	
Recipient age (y), median (range)	38.3 (18.1-71.5)	31.2 (18.1-58.8)	39.3 (18.7-62.1)	38.0 (18.1-71.5)	.0001
Donor age (y), median (range)	37.0 (18.0-74.1)	27.4 (18.0-68.6)	39.0 (19.0-72.1)	37.0 (18.0-74.1)	.0001
Race					
Caucasian	1194 (73)	200 (72)	529 (74)	1923 (73)	.93
Other	403 (25)	71 (26)	168 (24)	642 (24)	
Missing	40 (2)	6 (2)	15 (2)	61 (2)	
Disease type*					
AML	658 (40)	124 (45)	273 (38)	1055 (40)	.03
ALL	273 (17)	50 (18)	99 (14)	422 (16)	
CML	706 (43)	103 (37)	340 (48)	1149 (44)	
Disease stage†					
Low	1102 (67)	185 (67)	467 (66)	1754 (67)	.26
Intermediate	155 (9)	38 (14)	76 (11)	269 (10)	
High	350 (21)	47 (17)	156 (22)	553 (21)	
Not evaluable	30 (2)	7 (3)	13 (2)	50 (2)	
Interval from diagnosis to transplantation					
≤1 y	1163 (71)	207 (75)	498 (70)	1868 (71)	.33
>1 y	474 (29)	70 (25)	214 (30)	758 (29)	
GVHD prophylaxis‡					
CSA + MTX ± Other	1338 (82)	225 (81)	572 (80)	2135 (81)	.73
Other	299 (18)	52 (19)	140 (20)	491 (19)	
Total body irradiation					
No	1021 (62)	166 (60)	450 (63)	1637 (62)	.63
Yes	616 (38)	111 (40)	262 (37)	989 (38)	
Stem cell source					
Bone marrow	1083 (66)	199 (72)	472 (66)	1754 (67)	.17
Peripheral blood stem cells	554 (34)	78 (28)	240 (34)	872 (33)	
ABO compatibility					
Compatible	827 (51)	143 (52)	354 (50)	1324 (50)	.39
Minor incompatibility	218 (13)	45 (16)	83 (12)	346 (13)	
Major incompatibility	196 (12)	26 (9)	91 (13)	313 (12)	
Donor or recipient unknown	396 (24)	63 (23)	184 (26)	643 (24)	
History of donor transfusion					
No	1030 (63)	211 (76)	441 (62)	1682 (64)	<.001
Yes	23 (1)	1 (0.3)	36 (5)	60 (2)	
Unknown	584 (36)	65 (23)	235 (33)	884 (34)	
Donor/recipient CMV status					
Negative/negative	404 (25)	83 (30)	160 (22)	647 (25)	<.001
Negative/positive	259 (16)	43 (16)	71 (10)	373 (14)	
Positive/negative	158 (10)	34 (12)	66 (9)	258 (10)	
Positive/positive	699 (43)	103 (37)	373 (52)	1175 (45)	
Donor or recipient unknown	117 (7)	14 (5)	42 (6)	173 (7)	

*AML indicates acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia.

†Low-risk disease: first remission or first chronic phase; intermediate-risk disease: at least second remission or second chronic phase; high-risk disease: relapse, primary induction failure, accelerated phase, or blast crisis.

‡GVHD indicates graft-versus-host disease; CSA, cyclosporine; MTX, methotrexate.

sex/parity of donor/recipient pairs and all relevant covariables (Table 4) as explanatory variables. The proportional hazards assumption for each variable and each endpoint were examined with time-varying covariables. For relapse and transplant-related mortality, nonproportional hazards were identified for disease type (acute myelogenous leukemia, acute lymphoblastic leukemia, and chronic myelogenous leukemia) and disease stage (low, intermediate, high, and not evaluable). Thus, Cox regression models were stratified by these variables for those models. A P value $\leq .05$ was considered statistically significant for the main effect of donor/recipient sex/parity and for the univariable effects of each confounding covariable. We also ex-

Table 3. *Variables Examined in Multivariable Analysis: Main Effect of Donor/Recipient Pregnancy Status**

	Donor, n (%)			
	Male	Nulliparous Female	Parous Female	Total
Recipient				
Male	M-D → M-R† 1007 (65)	NF-D → M-R 153 (10)	PF-D → M-R 398 (25)	1558 (59)
Nulliparous female	M-D → NF-R 145 (58)	NF-D → NF-R 53 (20)	PF-D → NF-R 62 (24)	260 (10)
Parous female	M-D → PF-R 485 (60)	NF-D → PF-R 71 (9)	PF-D → PF-R 252 (31)	808 (31)
Total	1637 (62)	277 (11)	712 (27)	2626

*M-D indicates male donor; M-R, male recipient; NF-R, nulliparous female recipient; PF-R, parous female recipient; NF-D, nulliparous female donor; PF-D, parous female donor.
†Reference group.

plored interactions between donor sex/parity and recipient sex/parity and between the main effect (donor/recipient pairs) and other significant explanatory variables. There were no statistically significant interactions for any endpoint.

The multivariable analysis described above permitted direct comparisons across the 3 donor types (male, nulliparous female, or parous female) for male recipients only. Because one of our goals was to enable a transplantation clinician to optimize donor selection for any patient, male or female, we used pairwise analysis to create an additional model that would facilitate direct comparison of the risks associated with each donor type for nulliparous female recipients and for parous female recipients. Using this analysis, every combination of donor/recipient sex/parity was compared with every other possible donor/recipient combination (36 comparisons in all). For clarity, only the relevant comparisons are reported (ie, risk of chronic GVHD in parous female donor/male recipient pairs vs nulliparous female donor/nulliparous female recipient pairs is not reported). With 36 subgroups in this pairwise analysis, there is a clear need to adjust for multiple comparisons. However, the Bonferroni method, which is the usual procedure to control the overall error rate, is notoriously stringent and risks erroneously accepting the null hypothesis. Thus, we set the threshold for statistical significance at ≤.01 and reported 99% confidence intervals (CIs). As a confirmatory analysis, we used the Benjamini-Hochberg procedure [36], a method that decreases the chance of incorrectly accepting the null hypothesis and is more forgiving than the Bonferroni procedure. This analysis confirmed our findings (Appendix).

RESULTS

Patient Characteristics

There were 2626 patients in the CIBMTR who met the inclusion criteria and had complete information on donor/recipient sex/parity. In general, very few other data were missing (range of missing values, 2%-5%). Two exceptions were donor transfusion history in 884 cases (34%) and ABO compatibility in 643 (24%) donor/recipient pairs.

Race categories are reported to the CIBMTR as Caucasian, Asian, Black, Native American, Hispanic, or Other/Missing. There were small numbers of Asian (n = 394), Black (n = 73), Native American (n = 6), and Hispanic (n = 169) patients, and their effects on each endpoint were similar (data not shown). We therefore decided to pool these groups into a single, non-Caucasian group.

Recipient and donor pregnancy statuses were strongly associated with one another ($P < .001$; Table 2), and donor and patient age were also correlated (Spearman $\rho = .82$, $P < .001$). Patient and donor age, CMV status, and donor transfusion history also dif-

Table 4. *Covariables Examined in Multivariate Analysis**

Variable	Reference	Comparator(s)
Patient age	—	—
Donor age	—	—
Patient race	Caucasian	Others
Disease type	AML	ALL CML
Disease risk	Low	Intermediate High
Interval from diagnosis to transplant	≤1 y	>1 y
GVHD prophylaxis	CSA + MTX ± other	Others
TBI in conditioning regimen	No	Yes
Stem cell source	Bone marrow	Peripheral blood
ABO compatibility†	Compatible	Minor incompatibility Major incompatibility
Donor history of transfusion	No	Yes
Donor/recipient CMV status	Negative/negative	Negative/positive Positive/negative Positive/positive

*GVHD, indicates graft-versus-host disease; TBI, total body irradiation; CMV, cytomegalovirus; AML, acute myelogenous leukemia; CSA, cyclosporine; MTX, methotrexate; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia.
†"Bidirectional" ABO incompatibility was classified as minor incompatibility (see text).

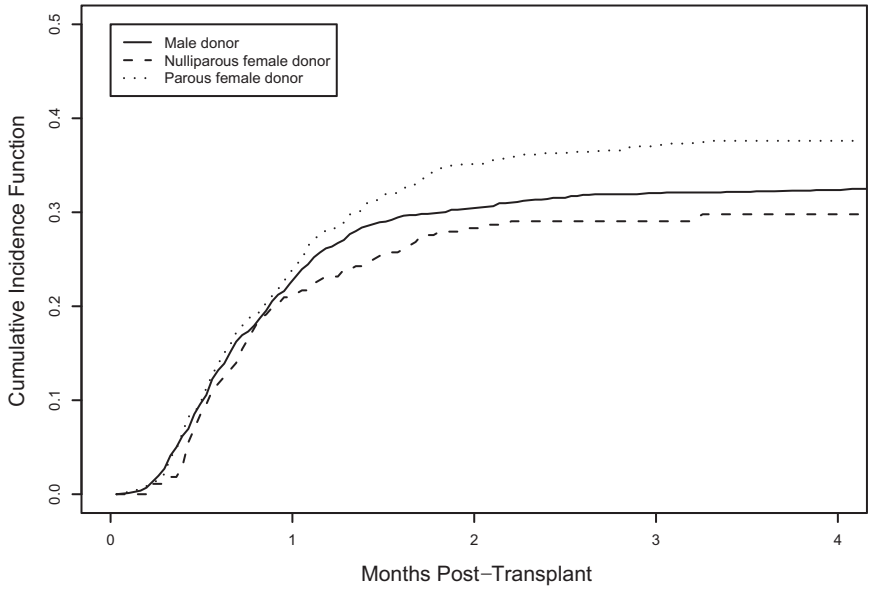

Figure 1. Cumulative incidence of acute GVHD by donor sex/parity.

fered across sex/parity groups (Table 2). Nulliparous women were younger than men and parous women, whereas parous women were more likely to be CMV-seropositive and to have undergone transfusion. Other covariables did not differ significantly across sex/parity groups. Sixty-two percent of all recipients received hematopoietic stem cell grafts from male donors.

The univariable hazard ratios (HRs) for the relation of each covariable to each endpoint were similar to those reported in the literature (data not shown), suggesting that our cohort of patients was similar to other patient populations.

Survival

Univariable models. Nulliparous female recipients had more favorable overall survival (unadjusted HR, .79; 95% CI, .64-.96; $P = .02$). Donor parity had no effect on overall survival.

Multivariable model. Disease type and risk, donor and patient age, use of total body irradiation, and type of GVHD prophylaxis were independently associated with survival. After adjusting for these variables, there was no effect of donor/recipient sex/parity on overall survival ($P = .68$, 8 *df* test).

Acute GVHD

Univariable models. There was an increased risk of acute GVHD in patients who received grafts from parous female donors (unadjusted HR, 1.16; 95% CI, 1.00-1.34; $P = .04$). Cumulative incidence of acute GVHD at 100 days (Figure 1) was 38% for patients receiving grafts from parous women compared with 29% for those receiving grafts from nulliparous women ($P = .02$) and 31% for recipients of male grafts ($P = .04$). There was no difference between recipients of male versus nulliparous female donor grafts ($P = .29$).

Recipient parity had no effect on the risk of acute GVHD (data not shown).

Multivariable model. In addition to donor sex/parity, disease type and risk, patient age, and use of total body irradiation were associated with risk of acute GVHD. After adjusting for these factors in multivariable analysis, donor/recipient sex/parity had no effect on risk of acute GVHD ($P = .64$, 8 *df* test; Table 5).

Chronic GVHD

Univariable models. Donor sex/parity was an important influence on risk of developing chronic

Table 5. *Acute GVHD (n = 2626)**

Recipient	Donor		
	Male (n = 1637)	Nulliparous Female (n = 277)	Parous Female (n = 712)
Male (n = 1558)	1.00 (reference), n = 1007	.99 (.74-1.35), P = .99, n = 153	1.17 (.97-1.42), P = .11, n = 398
Nulliparous female (n = 260)	1.15 (.85-1.54), P = .36, n = 145	.96 (.57-1.62), P = .88, n = 53	1.18 (.77-1.80), P = .45, n = 62
Parous female (n = 808)	1.01 (.84-1.22), P = .90, n = 485	.79 (.50-1.24), P = .31, n = 71	1.14 (.91-1.43), P = .26, n = 252

*Multivariable hazard ratios (95% confidence intervals) with male donor/male recipient pairs as reference group. Overall *P* value for donor/recipient parity is 0.64 (8 *df* test).

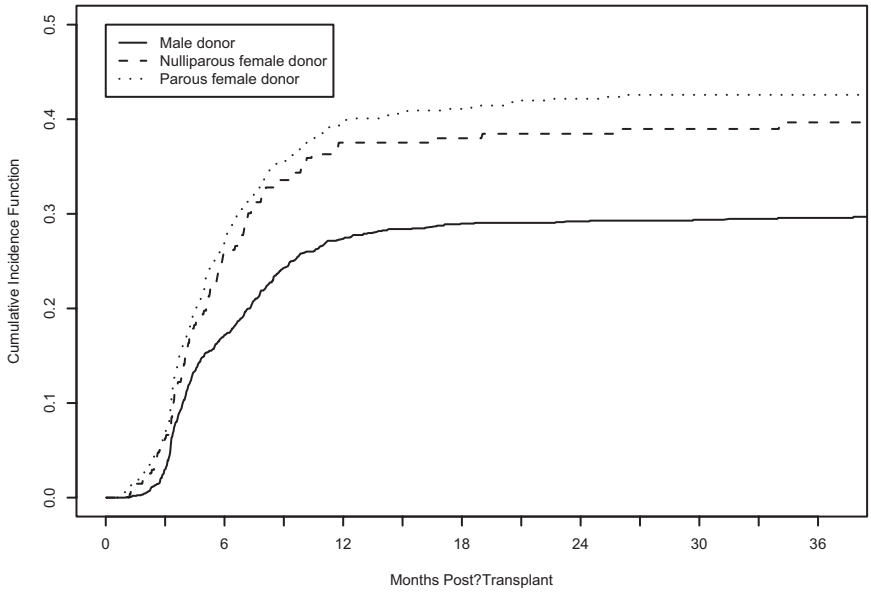

Figure 2. Cumulative incidence of chronic GVHD by donor sex/parity.

GVHD. Compared with male donors, parous female donors imparted a significantly greater risk of chronic GVHD (unadjusted HR, 1.62; 95% CI, 1.40-1.89; $P < .01$), as did nulliparous female donors (unadjusted HR, 1.38; 95% CI, 1.12-1.71; $P = .003$). Cumulative incidences of chronic GVHD were 30% for patients receiving transplants from male donors, 39% for recipients of nulliparous female donor grafts, and 41% for recipients of parous female donor grafts (Figure 2). The incidence of chronic GVHD in patients receiving transplants from nulliparous female donors was not statistically significantly different from those receiving parous female donor grafts ($P = .16$).

There was no relation between recipient sex/parity and chronic GVHD (data not shown).

Multivariable model. Sex/parity remained significantly associated with chronic GVHD in multivariable analysis. After accounting for donor and recipient sex/parity, the only other covariable that reached statistical significance was patient age (HR, 1.19 for each additional year of age; 95% CI, 1.01-1.39; $P = .03$).

For male recipients, nulliparous and parous female donors conferred an increased risk of chronic GVHD, and this increase was of similar magnitude (Table 6). Compared with male donors, the HRs for chronic GVHD were 1.56 for parous female donors ($P < .001$) and 1.44 for nulliparous female donors ($P = .02$).

For female recipients, pairwise analysis was required to directly compare the risk of chronic GVHD imparted by each type of donor (see Methods). Parous female donors significantly increased the risk of chronic GVHD in nulliparous and parous female recipients (Table 7). Table 7 presents the relative risk of chronic GVHD in nulliparous female recipients. The reference group is composed of male donor/nulliparous female recipient pairs. In nulliparous female recipients, risk of chronic GVHD from parous female donors compared with male donors is 2.10 (99% CI, 1.01-4.35; $P = .009$). Table 7 also presents the risk of chronic GVHD in parous female recipients, with male donor/parous female recipient pairs as the reference group. In parous female recipients, the HR for chronic GVHD from parous female donors versus male donors is 1.49 (99% CI, 1.01-2.19; $P = .008$).

Table 6. *Chronic GVHD (n = 2112)**

	Donor		
Recipient	**Male (n = 1304)**	**Nulliparous Female (n = 234)**	**Parous Female (n = 574)**
Male (n = 1254)	**1.00 (reference), n = 805**	**1.44 (1.07-1.95), P = .02, n = 133**	**1.56 (1.26-1.94), P < .001, n = 316**
Nulliparous female (n = 213)	**.75 (.51-1.11), P = .15, n = 117**	**1.02 (.57-1.82), P = .95, n = 42**	**1.58 (1.02-2.45), P = .04, n = 54**
Parous female (n = 645)	**.84 (.67-1.06), P = 0.15, n = 382**	**1.33 (.85-2.08), P = .21, n = 59**	**1.26 (.97-1.64), P = .08, n = 204**

*Multivariable hazard ratios (95% confidence intervals) with male donor/male recipient pairs as reference group for patients surviving >100 days. Overall *P* value for donor/recipient parity is <.001 (8 *df* test).

Table 7. *Chronic GVHD Pairwise Comparisons**

	Donor		
	Male	Nulliparous Female	Parous Female
Nulliparous female recipients (n = 213)	1.00 (reference), n = 117	1.35 (.58-3.27), P = .38, n = 42	2.10 (1.01-4.35), P = .009, n = 54
Parous female recipients (n = 645)	1.00 (reference), n = 382	1.57 (.84-2.91), P = .059, n = 59	1.49 (1.01-2.19), P = .008, n = 204

*To account for multiple comparisons, only *P* values < .01 were considered significant, and 99% confidence intervals are shown. Male recipients are presented in the top row.

Secondary Endpoints

Univariable analyses were not performed for secondary endpoints.

Relapse. There was no relation between sex/parity and relapse risk ($P = .39$, 8 *df*; Table 8). As an exploratory analysis, we proceeded to estimate the HRs for each of the 9 donor/recipient combinations, with male donor/male recipient pairs as the reference group. The only group that demonstrated a decreased relapse risk was male recipients of parous female donor grafts (HR, .75; 95% CI, .58-.97; $P = .03$). There was no such decreased risk in any other donor/recipient sex/parity combination, including those who had an increased risk of chronic GVHD.

Transplant-related mortality. Important covariables, after stratifying models by disease type and disease stage due to nonproportional hazards, included patient and donor age, use of total body irradiation in the conditioning regimen, >1 year from diagnosis to transplantation, type of GVHD prophylaxis, and donor/recipient CMV status. After controlling for these variables, donor/recipient sex/parity had no effect on transplant-related mortality ($P = .38$, 8 *df*).

DISCUSSION

Parous female donors imparted an increased risk of chronic, but not acute, GVHD in all recipients. Further, all female donors (parous and nulliparous) resulted in an increased risk of chronic GVHD in male recipients. The relation between sex/parity and chronic GVHD remained strong despite adjusting for other important covariables. After accounting for sex/parity, the only other variable that remained associated with chronic GVHD was patient age. Somewhat surprisingly, this increased risk of chronic GVHD did not translate into increased transplant-related mortality or decreased risk of relapse.

This study was the first to evaluate the role of recipient pregnancy status in outcomes of SCT. For the endpoints studied, there was no independent effect of recipient sex/parity. However, the effects of recipient sex/parity on other endpoints such as time to engraftment or graft failure are issues that will be addressed in future work.

Further, this is the only multicenter study in the modern transplantation era to evaluate the effect of sex mismatching. We confirmed that female-to-male transplants result in a higher rate of chronic GVHD. The finding that nulliparous female donors confer a risk of chronic GVHD similar to that of parous female donors may reflect the development after transplantation of cytotoxic T cells reactive against H-Y antigens, several of which have been identified as T-cell targets [37-39]. Anti–H-Y alloimmunization could also be mediated by antibodies. Antibodies to Y-encoded peptides have been detected in male recipients of female stem cells after transplantation [25,26], and normal women (pregnancy status unknown) harbor antibodies to H-Y antigens [26]. An alternative explanation is that women who reported nulliparity may in fact have been pregnant in the past, a pregnancy of which they may or may not have been aware.

It is notable that 62% of all donors were male, which is more than one would expect by chance and may reflect many clinicians' preference to choose donors who are male or sex matched (59% of recipients were male). Importantly, however, the increased

Table 8. *Relapse (n = 2626)**

	Donor		
Recipient	Male (n = 1637)	Nulliparous Female (n = 277)	Parous Female (n = 712)
Male (n = 1558)	1.00 (reference), n = 1007	1.02 (.73-1.42), P = .90, n = 153	.75 (.58-0.97), P = .03, n = 398
Nulliparous female (n = 260)	0.91 (.64-1.30), P = .60, n = 145	1.06 (.64-1.76), P = .83, n = 53	1.03 (.63-1.68), P = .91, n = 62
Parous female (n = 808)	.84 (.67-1.06), P = .14, n = 485	.66 (0.38-1.16), P = .15, n = 71	1.00 (.76-1.30), P = .99, n = 252

*Multivariable hazard ratios (95% confidence intervals) with male donor/male recipient pairs as reference group. Overall *P* value for donor/recipient parity is 0.39 (8 *df* test).

risk of chronic GVHD in female recipients supports the hypothesis that autosomal minor histocompatibility antigens also play an important role in GVHD pathophysiology.

We attempted to account for other mechanisms of alloimmunization, such as previous blood transfusions, by creating an "alloimmunized" group of donors that included parous women plus all male and nulliparous female donors who reported previous blood transfusion. A sensitivity analysis duplicating all of the statistical models for the 3 primary endpoints (survival and acute and chronic GVHD) compared alloimmunized with non-alloimmunized donors. Although limited by the very small number of these additional alloimmunized donors (n = 24), including them did not significantly alter the results.

Despite its large sample of 2626, this study may have been underpowered to answer some important questions. There were 4 areas in particular that may have been at a disadvantage from inadequate sample size. (1) The nulliparous female groups, recipient and donor, were quite small, making it difficult to draw decisive conclusions from comparisons involving these groups. (2) Pairwise analysis also had lack of power, which was exacerbated by the more conservative *P* value chosen to indicate statistical significance. (3) There were insufficient data to explore the hypothesis that there may be a "dose-response" relation with pregnancy (ie, that a larger number of donor pregnancies confers an increasingly higher risk of GVHD in recipients). (4) Although the overall *P* value for the effect of sex/parity on relapse risk was not statistically significant, an exploratory evaluation of the HRs and 95% CIs for each donor/recipient pair suggested that male recipients of parous female donor grafts may have a lower risk of relapse. If confirmed, this could have important implications on targeting immunotherapy to H-Y antigens.

This is the largest study to examine the effect of donor parity, and the only study to create a comprehensive model evaluating the effects of recipient/donor sex/parity on outcomes of HLA-identical sibling allogeneic SCT. We have established that donor parity is an important risk factor for the development of chronic GVHD, not just in male recipients but also in female recipients. The pathophysiology, traditionally attributed to pregnancy-induced alloimmunization against minor histocompatibility antigens, in particular those encoded by the Y-chromosome, requires elucidation. A major question that cannot be addressed by this study is the biologic mechanism underlying these observations. Parity is at best a "surrogate exposure." The true immunologic implications of previous pregnancies are unclear. Although alloimmunization clearly occurs in some women, others become tolerant to fetal antigens. Up to 75% of parous women may harbor cells of fetal origin for decades after pregnancy, thus creating a long-term state of microchimerism [40-42]. Although this observation was first made in the 1970s, recent work has highlighted the frequency with which this phenomenon is observed [43]. The activity and importance of these chimeric cells have never been evaluated directly in the setting of SCT, but several groups have begun to exploit maternal tolerance to fetal antigens by demonstrating that non–T-cell–depleted haploidentical transplants between children and their microchimeric mothers (both child to mother and mother to child) have lower-than-expected rates of graft rejection and GVHD [44-46]. Conversely, fetal exposure to maternal antigens also occurs, as evidenced by the detection of maternal cells in umbilical cord blood [47-50] and in breast milk [51]. A retrospective registry-based study [52] and a prospective small trial [53] demonstrated that haploidentical sibling transplants from donors who are mismatched at the maternal allele (ie, the allele shared by the sibling pair is paternally derived) also carry a relatively lower risk of GVHD than other haploidentical transplants. This observation supports the hypothesis that offspring are tolerant to noninherited maternal antigens.

Cells of fetal origin are pluripotent and can differentiate into several different cell types, including T cells [54], granulocytes [12], hepatocytes [55], and thyroid tissue [56]. They have thus been dubbed "pregnancy-associated progenitor cells" [57]. It is clear that fetal microchimerism is not immunologically silent. These cells may have an important role in the initiation and propagation of disease [42,58,59] and seem to target and proliferate in areas of tissue damage and inflammation [60]. Further, they are detectable in peripheral blood stem cell products [61]. These cells might serve as mediators of GVHD if they are transplanted from a parous woman into a patient. Thus, the different possible effects of pregnancy—alloimmunization, microchimerism as a reflection (or a source) of tolerance, or microchimerism as a reservoir of haploidentical stem cells infused with the donor's HLA-identical stem cells—would theoretically have very different effects on the outcomes of transplantation. Simply labeling a donor as *parous* may mask a very heterogeneous population of women.

Future studies to evaluate the effects of pregnancy on the maternal immune system will offer additional insight into the physiology underlying the observations from this retrospective registry-based study. Until these mechanisms are better understood, it is appropriate to avoid parous female donors and preferentially to choose male donors for male recipients whenever possible for HLA-identical sibling SCT.

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APPENDIX. A

B-H Procedure to Test for Statistical Significance of Hazard Ratios for Chronic GVHD in Pairwise Analysis*

Comparison	*P*	B-H Threshold
MD → MR† vs PFD → MR†	<.0001	.0056
MD → PFR† vs PFD → PFR†	.0083	.0111
MD → NFR† vs PFD → NFR†	.0088	.0167
MD → MR† vs NFD → MR†	.0161	.0222
MD → PFR vs NFD → PFR	.0591	.0278
NFD → NFR vs PFD → NFR	.2207	.0333
MD → NFR vs NFD → NFR	.3809	.0389
NFD → MR vs PFD → MR	.6327	.0444
NFD → PFR vs PFD → PFR	.828	.0500

*B-H indicates Benjamini-Hochberg; MD, male donor; MR, male recipient; PFR, parous female recipient; NFR, nulliparous female recipient; PFD, parous female donor; NFD, nulliparous female donor. Comparisons are listed according to magnitude of *P* value (in ascending order).

†According to the B-H procedure, if the *P* value is lower than the B-H threshold, the comparison is statistically significant [35].

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