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Histocompatibility

Analysis of Single Nucleotide Polymorphisms in the Gamma Block of the Major Histocompatibility Complex in Association with Clinical Outcomes of Hematopoietic Cell Transplantation: A Center for International Blood and Marrow Transplant Research Study

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A B S T R A C T

HLA haplotype mismatches have been associated with an elevated risk of acute graft-versus-host disease (aGVHD) in patients undergoing HLA-matched unrelated donor (URD) hematopoietic cell transplantation (HCT). The gamma block (GB) is located in the central MHC region between beta and delta blocks (encoding HLA-B and -C and HLA-DQ and -DR antigens, respectively) and contains numerous inflammatory and immune regulatory genes, including Bf, C2, and C4 genes. A single-center study showed that mismatches in SNPs c.2918+98G, c.3316C, and c.4385C in the GB block (C4 SNPs) were associated with higher risk of grade III-IV aGVHD. We investigated the association of GB SNP (GBS) mismatches with outcomes after 10/10 and 9/10 URD HCT (n = 714). The primary outcome was acute GVHD. Overall survival, disease-free survival, transplantation-related mortality, relapse, chronic GVHD, and engraftment were also analyzed. DNA samples were GBS genotyped by identifying 338 SNPs across 20 kb using the Illumina NGS platform. The overall 100-day incidence of aGVHD grade II-IV and II-IV were 41% and 17%, respectively. The overall incidence of matching at all GBSs tested and at the C4 SNPs were 23% and 81%, respectively. Neither being matched across all GB SNPs tested (versus mismatched) nor having a higher number of GBS mismatches was associated with transplantation outcomes. There was no association between C4 SNP mismatches and outcomes except for an unexpected significant association between having 2 C4 SNP mismatches and a higher hazard ratio (HR) for relapse (association seen in 15 patients only; HR, 3.38, 95% confidence interval, 1.75 to 6.53; $P = .0003$). These data do not support the hypothesis that mismatching at GB is associated with outcomes after HCT.

INTRODUCTION

HLA mismatches have the strongest impact on clinical outcomes following unrelated donor (URD) hematopoietic cell transplantation (HCT) [1,2]. In addition, in otherwise genotypically HLA-matched URD HCTs, major histocompatibility complex (MHC) haplotype mismatches have been associated with an elevated risk of graft-versus-host disease (GVHD) and a lower risk of disease relapse [3]. The MHC region spans approximately 3.8 Mb of the short arm of chromosome 6 at 6p21.3 and includes more than 269 loci [4,5]. Petersdorf et al

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[6] identified 12 single nucleotide polymorphisms (SNPs) as transplantation determinants. The risks associated with these SNPs were significant in a multivariate analysis that adjusted for HLA mismatching and nongenetic variables and were conferred by either donor or recipient SNP genotype or by donor-recipient SNP mismatch.

These SNPs may be in linkage disequilibrium (LD) with non-HLA transplant determinants. Similarly, a recent study using a combination of integrative computational approaches and random forest analysis of donor-recipient whole-exome sequencing data identified 65 non-HLA variants associated with a risk of antibody-mediated rejection in kidney transplant recipients [7].

These variants are functionally relevant to the rejection process in the kidney because they are related to genes enriched in genes expressed in kidney and vascular endothelium and underlie the immunobiology of graft rejection. However, the current standard of care in clinical HCT considers matching only at HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 loci [8]. Family pedigree analysis of HLA typing has shown that recombination occurs at specific locations within the MHC, leading to a block-like structure of 4 major genomic blocks, which Dawkins et al [9] termed the alpha, beta, gamma, and delta blocks. The alpha block contains HLA-A; the beta block contains HLA-C and HLA-B; the gamma block (GB) contains the complement proteins C2, C4, and factor B (Bf); and the delta block contains the HLA-DR and -DQ genes [10].

The GB is located in the central MHC region between the beta and delta blocks [11]. The clinical relevance of GB donor-recipient mismatches remains unclear. Investigating the clinical relevance of mismatches at GB in the clinical context of HCT is an important question because of its genomic location between the MHC class I and class II loci and the numerous inflammatory and immune regulatory genes that it contains, such as complement components C2, C4, and Bf genes [12-15]. In a multivariate analysis, C4 mismatch was identified as a significant risk factor associated with the development of acute GVHD (aGVHD) in matched URD HCT [16]. In addition, a single-center study has shown that mismatches in SNPs c.2918 +98G, c.3316C, and c.4385C (reference sequence C4A NG_011638.1) in the GB block (C4A SNP) were associated with grade III-IV aGVHD [17]. In this study, we tested the hypothesis that GB mismatch is associated with an increased risk of GVHD after URD HCT.

METHODS

Study Design and Population

The study population comprised 714 URD HCT recipients receiving an HLA-A-, -B-, -C-, -DRB1-, and -DQB1-matched (10/10) (n = 551) or 9/10 HLA-B mismatch only (n = 163) graft. We included adult and pediatric patients reported to the Center for International Blood and Marrow Transplant Research (CIBMTR) between 1999 and 2011 and had genomic DNA available at the CIBMTR research repository for GB SNP genotyping of recipients and their corresponding donors.

The study was limited to patients receiving a first myeloablative URD HCT for acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), or myelodysplastic syndrome (MDS). The 9/10 mismatched HCTs were limited to HLA-B mismatches, to maximize the frequency of GB SNP mismatches by virtue of the genomic location of GB between the HLA-B locus and class II HLA loci. All HLA typing was verified using DNA-based methods at high resolution, as described previously [18].

OUTCOME DEFINITIONS

The primary outcomes of interest in this study were aGVHD (grade II-IV, grade III-IV, and GI) and chronic GVHD (cGVHD; limited and extensive). Grade II-IV and III-IV aGVHD were defined by the Glucksberg scale, and limited and extensive cGVHD were defined according to the Seattle criteria [19,20]. Secondary outcomes analyzed included overall survival (OS), disease relapse, disease-free survival (DFS), treatment-related mortality (TRM), and neutrophil engraftment. OS was defined as the time from HCT to death from any cause. Relapse and DFS were defined according to CIBMTR criteria [21]. TRM was defined as death in continuous remission from the primary malignancy. Engraftment was defined as achievement of an absolute neutrophil count of 500/μL for 3 consecutive days.

GB SNP Genotyping and Analysis

GBS genotyping was determined using the next-generation sequencing platform and reagents from Illumina (San Diego, CA). DNA samples were obtained from the National marrow Donor Program Research Repository. The GB region was amplified in 4 long-range fragments (1.5 to 5.5 kbp long), using primers and PCR mix from Conexio Genomics (Perth, Australia). The resulting amplicons were purified using the Agencourt AMPure XP protocol (Beckman Coulter, Brea, CA) according to the manufacturer's instructions. The Illumina Nextera XT DNA library preparation kit and protocol were used for library creation before sequencing on the MiSeq sequencing platform. The resulting data was analyzed in Assign MPS (Conexio Genomics). The genomic locations of the SNPs tested and minor allele frequencies (MAFs) are shown in Supplementary Table S1. Among the 338 SNPs, 10 were excluded from the LD analysis owing to the presence of more than 2 alleles in the files. This ambiguity was produced by gene overlap at these particular SNPs. Although the kit used for testing can clearly distinguish whether donor-recipient pairs were matched or mismatched at these SNPs, it is not designed to determine copy number. Four of the analyzed SNPs were 4-bp insertions and deletions (indels) and were considered a single variant event. A total of 325 SNPs were included in the LD and MAF analyses. Input files were formatted in the Plink pedigree format, and Haploview software was used to generate the LD plots [22].

Statistical Analysis

Variables Analyzed

Sequence mismatches were considered across all SNPs tested (fully matched versus mismatched at 1+ SNPs), and the number of GB SNP mismatches (treating GB mismatches as a continuous variable) and mismatches at C4A SNP previously reported were analyzed for different endpoints. Patient-related variables included age at time of HCT, sex, and Karnofsky Performance Status score. Disease-related variables included disease (ALL, AML, or MDS) and disease status (early versus intermediate versus advanced versus other). Transplantation-related variables included hematopoietic cell source (bone marrow versus peripheral blood stem cells [PBSCs]), donor age, year of HCT, donor-recipient sex match (male-male versus male-female versus female-male versus female-female), donor-recipient cytomegalovirus serostatus (-/- versus -/+ versus +/- versus +/+ versus unknown), conditioning regimen (myeloablative versus reduced-intensity/nonmyeloablative) and GVHD prophylaxis (tacrolimus with or without others versus cyclosporine with or without others versus others). Variables considered in multivariate analyses included patient, disease, and transplantation characteristics, including recipient and donor age, race/ethnicity, and other factors.

GB SNP Analysis

SNP haplotypes were estimated using an accelerated EM algorithm similar to the partition/ligation method described elsewhere [22]. This creates highly accurate population

frequency estimates of the phased haplotypes based on the maximum likelihood as determined from the unphased input.

Descriptive Statistics, Univariable and Multivariable Analyses

Descriptive statistics included medians and ranges for continuous variables and frequencies for categorical variables. Multivariable models were built for OS, DFS, relapse, TRM, aGVHD (grade II-IV and III-IV), and cGVHD using the Cox proportional hazards model. All clinical variables were tested for affirmation of the proportional hazards assumption ($P < .01$). Factors violating the proportional hazards assumption were adjusted through stratification. All outcome events were defined based on competing risks, censored as appropriate and included in the Cox models. Fine and Gray models were used for modeling the subdistribution hazard. A stepwise variable selection procedure was then used to select adjusted clinical variables for each outcome, with a threshold of .05 for both entry and retention in the model. Then the association of GB SNP mismatches with clinical outcomes was tested with adjustments for the selected clinical variables. The center effect was also adjusted in all the models. A P value $<.01$ was considered significant to adjust for multiple testing. All statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC).

RESULTS

Study Population

A total of 714 patients were included in our analysis. The distributions of patient-, donor-, and transplantation-related variables among the cohort by GB SNP-matched versus GB SNP-mismatched pairs are summarized in Table 1. The majority of patients received PBSC grafts (75%) following myeloablative conditioning (70%) and were male (53%) and Caucasian (91%), with a median age of 49 years. AML (55%) was the most common indication for HCT, followed by MDS (29%) and ALL (16%), and most patients had early-stage disease (70%). All patients received calcineurin inhibitor-based GVHD prophylaxis, most commonly with a tacrolimus-based regimen (76%).

Characterization of the GB SNPs

Supplementary Table S1 presents the genomic positions and MAFs at each SNP. Figure 1 shows a histogram of the MAF distribution of the analyzed SNPs. Supplementary Table S2 lists the pairwise LD of each SNP that reached $r^2 < .8$. Figure 2 shows the LD for each SNPs studied and for the C4 SNPs. Figure 3A shows each haplotype in a block with its population haplotype frequency and connections from one block to the next. Figure 3B shows similar analysis of haplotype blocks estimated for the 25 SNPs analyzed in the Cleveland Clinic cohort [17].

Donor-Recipient SNP Mismatches

GB SNP mismatches were identified in 551 of the 714 pairs (77%), of which 404 (73%) were 10/10 and 147 (27%) were 9/10. GB mismatches were significantly less frequent in the 10/10 HLA-matched pairs (n = 551) than in the 9/10 HLA-matched pairs (n = 163) (73% versus 90%). The distribution of C4 SNP mismatches in different HLA/SNP matching groups is shown in Table 2.

Associations between Donor-Recipient GB SNP Mismatches and Clinical Outcomes

The overall 100-day incidences of grade II-IV and III-IV aGVHD in the entire cohort were 41% and 17%, respectively. The overall incidences of matching at all GB SNPs tested and at the C4A SNPs were 23% and 81%, respectively. Neither being matched across all GB SNPs tested (versus mismatched at 1+ SNPs) nor having a higher number of GB SNP mismatches (treating GB mismatches as a continuous variable) was associated with any of the analyzed outcomes (Figure 4). Table 3 presents the results of the multivariable analysis of the association between GB SNPs and various study outcomes. There were no associations between mismatches at the C4A SNPs and any of the analyzed outcomes except an unexpected significant association between having 2 SNP mismatches (n = 15 pairs) with a higher incidence of relapse (hazard ratio [HR], 3.38; 95% confidence interval [CI], 1.75 to 6.53; $P = .0003$) and lower DFS (HR, 2.76; 95% CI, 1.60 to 4.78; $P = .0003$). Paradoxically, in a subset analysis of the 10/10 HLA-matched cohort that received PBSC grafts (n = 412), the C4 SNPs previously reported to be associated with a higher cumulative incidence of grade III-IV aGVHD were found to be associated with a lower incidence of aGVHD grade II-IV (HR, .52; 95% CI, .32 to .82; $P = .0053$) and a higher incidence of relapse (HR, 1.65; 95% CI, 1.12 to 2.44; $P = .01$), but no association with grade III-IV aGVHD (p = .12). Competing-risks analysis did not change these results.

DISCUSSION

In numerous studies, HLA matching has been shown to significantly impact clinical outcomes of HCT [1,2,23,24]. HCT with HLA-matched sibling donors is associated with lower rates of life-threatening GVHD compared with HCT with matched URDs [25]. One possible explanation for this may be that unlike matched siblings, who share HLA haplotype by descent, URDs despite being HLA-matched, could be haplotype-mismatched and thus mismatched at other genetic variants within the MHC, such as SNPs, indels, and copy number variations (CNVs). It is hypothesized that mismatches at non-HLA MHC sequences contribute to the inflammatory processes seen in post-transplantation complications. Petersdorf et al [3] put this hypothesis to the test by physically separating DNA from alternate haplotypes and showing that HLA-matched URDs could either be haplotype-matched or -mismatched. Being haplotype-matched meant that the matched HLA alleles of the donor and patient at different loci were physically linked on the same haplotype, whereas being haplotype-mismatched meant that matched HLA alleles were linked to different alleles at the other loci. In this study, patients who were haplotype-matched with their donors had a reduced risk of severe aGVHD. If these findings can be reproduced, they may aid in donor selection. However, the techniques used to separate haplotypes are not technically feasible for routine HLA testing laboratories; therefore, alternative techniques are needed to determine the likelihood of haplotype matching. Developing these techniques requires an understanding of the MHC structure. Mismatches in non-HLA loci located in between HLA class I and class II loci, such as MHC class I chain-related gene A (MICA), has been associated with worse HCT outcomes [26–28]. This effect has been postulated to reflect MHC haplotype heterogeneity or to be related to regulatory elements of inflammatory processes [26,29].

In this study, we investigated the associations between GB SNP mismatch and HCT outcomes. The results of our analysis do not show significant differences in any transplantation outcomes among GB SNP-matched and -mismatched counterparts irrespective of whether mismatches were evaluated as a dichotomous “yes” versus “no” or quantitatively as number of mismatches. These results are consistent with those of a recent single-institution study in which gamma-type matching status was not associated with outcomes of adult matched URD HCT

Table 1
Summary of SNP Demographics by All SNPs Matched versus SNPs Mismatched

Variable	SNP-Matched	SNP-Mismatched	*P* Value*
Number of patients	163	551	
Number of centers	59	98	
Recipient age at HCT, yr			.78
10-19, n (%)	4 (02)	16 (03)	
20-29, n (%)	31 (19)	90 (16)	
30-39, n (%)	17 (10)	67 (12)	
40-49, n (%)	37 (23)	109 (20)	
50-59, n (%)	74 (45)	269 (49)	
Median (range)	47 (18-74)	50 (18-75)	.63
Recipient race/ethnicity, n (%)			.49
Caucasian, non-Hispanic	152 (95)	498 (92)	
African-American, non-Hispanic	0	10 (02)	
Asian, non-Hispanic	2 (01)	5 (01)	
Native American, non-Hispanic	0	2 (<1)	
Hispanic, Caucasian	5 (03)	24 (04)	
Hispanic, African-American	0	1 (<1)	
Hispanic, race unknown	1 (01)	1 (<1)	
Unknown	3 (N/A)	10 (N/A)	
Recipient sex, n (%)			.76
Male	88 (54)	290 (53)	
Female	75 (46)	261 (47)	
Karnofsky Performance Status score, n (%)			.61
10-80	50 (34)	187 (36)	
90-100	97 (66)	328 (64)	
Unknown	16 (N/A)	36 (N/A)	
High-resolution HLA matches, n (%)			<.001
9/10	16 (10)	147 (27)	
10/10	147 (90)	404 (73)	
Stem cell source, n (%)			.74
Bone marrow	39 (24)	139 (25)	
PBSCs	124 (76)	412 (75)	
Conditioning regimen, n (%)			.63
Myeloablative	117 (72)	383 (70)	
Reduced intensity	36 (22)	116 (21)	
Nonmyeloablative	6 (04)	32 (06)	
Other	4 (02)	20 (04)	
Donor age at donation, yr			.004
10-19, n (%)	7 (04)	17 (03)	
20-29, n (%)	68 (42)	168 (30)	
30-39, n (%)	56 (34)	175 (32)	
40-49, n (%)	25 (15)	137 (25)	
50+, n (%)	7 (04)	54 (10)	
Median (range)	31 (18-56)	35 (18-61)	<.001
Disease at HCT, n (%)			.89
AML	91 (56)	307 (56)	
ALL	27 (17)	84 (15)	
MDS	45 (28)	160 (29)	
Disease status at HCT, n (%)			.68
Early	114 (70)	385 (70)	
Intermediate	1 (01)	5 (01)	
Advanced	44 (27)	137 (25)	
Other	4 (02)	24 (04)	
Donor/recipient CMV serostatus, n (%)			.80
Negative/negative	51 (31)	150 (27)	
Negative/positive	55 (34)	188 (34)	
Positive/negative	17 (10)	59 (11)	
Positive/positive	36 (22)	143 (26)	
Unknown	4 (02)	11 (02)	
GVHD prophylaxis, n (%)			.68
Tacrolimus + MMF ± others	33 (20)	89 (16)	
Tacrolimus + MTX ± others (except MMF)	92 (56)	329 (60)	
CSA + MMF ± others (except tacrolimus)	12 (07)	44 (08)	
CSA + MTX ± others (except tacrolimus and MMF)	26 (16)	89 (16)	
Donor-recipient sex match, n (%)			.14
Male-male	62 (38)	200 (36)	
Male-female	54 (33)	149 (27)	
Female-male	26 (16)	90 (16)	
Female-female	21 (13)	112 (20)	
Year of HCT, n (%)			.11
2000	4 (02)	8 (01)	
2001	2 (01)	12 (02)	
2002	7 (04)	15 (03)	

(*continued*)

Table 1 (*Continued*)

Variable	SNP-Matched	SNP-Mismatched	*P* Value*
2003	7 (04)	35 (06)	
2004	8 (05)	45 (08)	
2005	18 (11)	80 (15)	
2006	21 (13)	76 (14)	
2007	31 (19)	75 (14)	
2008	26 (16)	66 (12)	
2009	21 (13)	63 (11)	
2010	16 (10)	43 (08)	
2011	2 (01)	33 (06)	
Follow-up of survivors			
Evaluated, n	68	214	
Duration, mo, median (range)	48.5 (12.4-140.1)	60.0 (3.3-147.6)	.19

MMF indicates mycophenolate mofetil; MTX, methotrexate; CSA, cyclosporine A.

* The Pearson χ^2 test was used for comparing discrete variables, and the Kruskal-Wallis test was used for comparing continuous variables.

recipients [30]. Likewise, there was no significant association between GB mismatch or C4 SNP mismatch and the study outcomes. The finding of an association between C4 SNP mismatches and relapse of malignancy was unexpected. We hypothesized that these mismatches would lead to higher rates of GVHD, correlating with better relapse control due to an enhanced graft-versus-leukemia effect. We report this finding cautiously due to the small sample size in the subgroup with this end point (n=15). Nevertheless, a related finding of a significant association between higher relapse and MICA mismatches was reported before in the same cohort [31]. Further investigation of these related findings in an independent cohort could be a subject of future studies.

Our results are consistent with the results of a recent single center study that reported no significant associations between GBSP and HCT outcomes [30]. That study included 66 adult recipients of allogeneic HCT, and GB mismatches were considered dichotomously, with donor-recipient pairs classified as either matched or mismatched. However, our results differed from those observed in a single-center study that reported a significant association between GB SNP and severe aGVHD [17]. Possible explanations for the discordant results include the limited diagnoses (ALL, AML, and MDS) in our cohort compared with the previous study that included all diagnoses. The effect of GB SNPs, particularly C4 SNP mismatching, may be more pronounced in the diagnoses not included in the present study. Another possible explanation is the heterogeneity introduced by inclusion of many centers in the present study compared with single-center studies, particularly in the scoring of aGVHD among different centers compared with more consistent scoring at a single center. Overestimation of GB SNP-matched pairs is a possibility. We performed a qualitative analysis of C4 SNPs. The unexpectedly lower cumulative incidence of severe aGVHD in our study cohort compared with the previous single-center study might have rendered our study underpowered to detect statistically significant associations.

The lack of significant association between GB SNP mismatches and HCT outcomes and the unexpected findings in our study do not necessarily disprove a potential clinical relevance of GB composition. Petersdorf et al [6] identified 12 SNPs with significant associations with OS, RFS, TRM, aGVHD (grade II-IV and III-IV), and cGVHD. Only 6 of those SNPs conferred risk by donor-recipient mismatch (rs2242656, rs209130, rs2523957, rs3830076, rs2071479, and rs107822). The remaining SNPs conferred risk by a given recipient genotype (rs429916, rs915654, and rs2075800) or a given donor genotype (rs2244546, rs986522, and rs394657) regardless of donor-recipient matching at these SNPs. In the present study, we did not consider individual donor and recipient SNP genotypes, because we did not have sufficient power for these analyses. In addition, because GB polymorphism involves both SNPs and CNVs, and because CNV was not interrogated in this study, it is conceivable that in some cases, CNV might have impacted clinical outcomes and were unaccounted for in our analysis. At positions where 2 SNPs were identified, we were not able to determine and compare the relative copy numbers

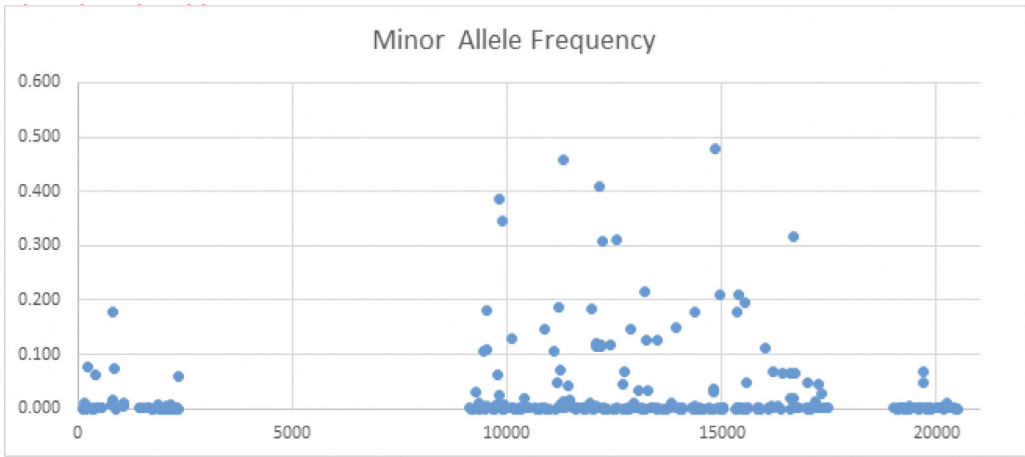

Figure 1. Minor allele distribution of the C4 SNPs. SNP positions are based on reference sequence NG_011638.1 (reference sequence used in this study: agaaggtagc agacagacag acggatctaa).

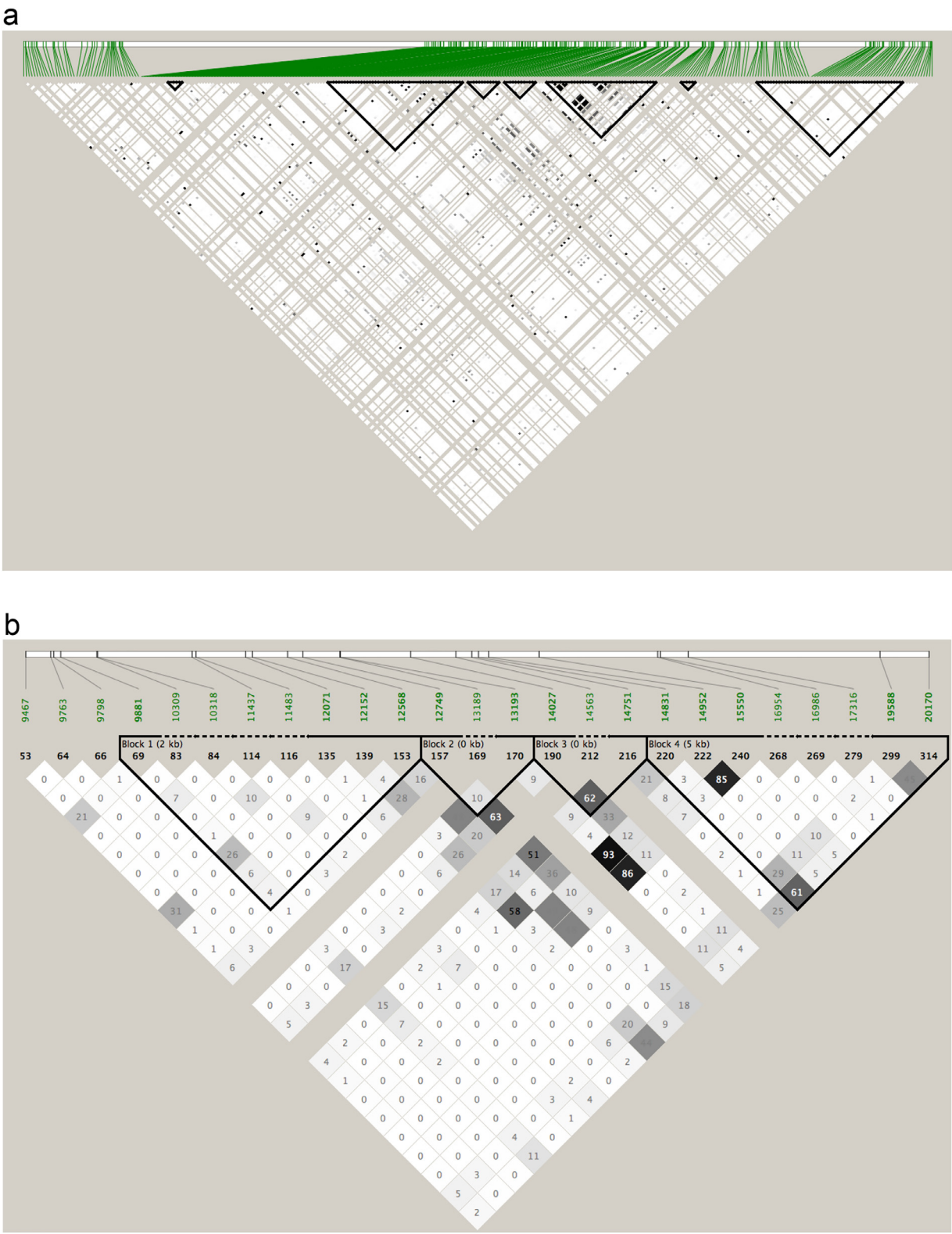

Figure 2. (A) LD map of analyzed GB SNPs based on r^2 values. Colors are coded as follows: $r^2 = 0$ is in white, $0 < r^2 < 1$ is in shades of gray, and $r^2 = 1$ is in black. Triangles outlined in black represent haplotype blocks. (B) LD map of the 25 GB SNPs tested previously in the Cleveland Clinic cohort based on r^2 values. SNP positions are listed in the top row. Colors are coded as follows: $r^2 = 0$ is in white, $0 < r^2 < 1$ is in shades of gray, and $r^2 = 1$ is in black. Triangles outlined in black represent haplotype blocks.

of each SNP. It is noteworthy that CNV has not been universally accounted for in previous studies and could be included in future studies.

In conclusion, our study results show that neither GB SNP nor C4 SNP mismatches were significantly associated with HCT outcomes.

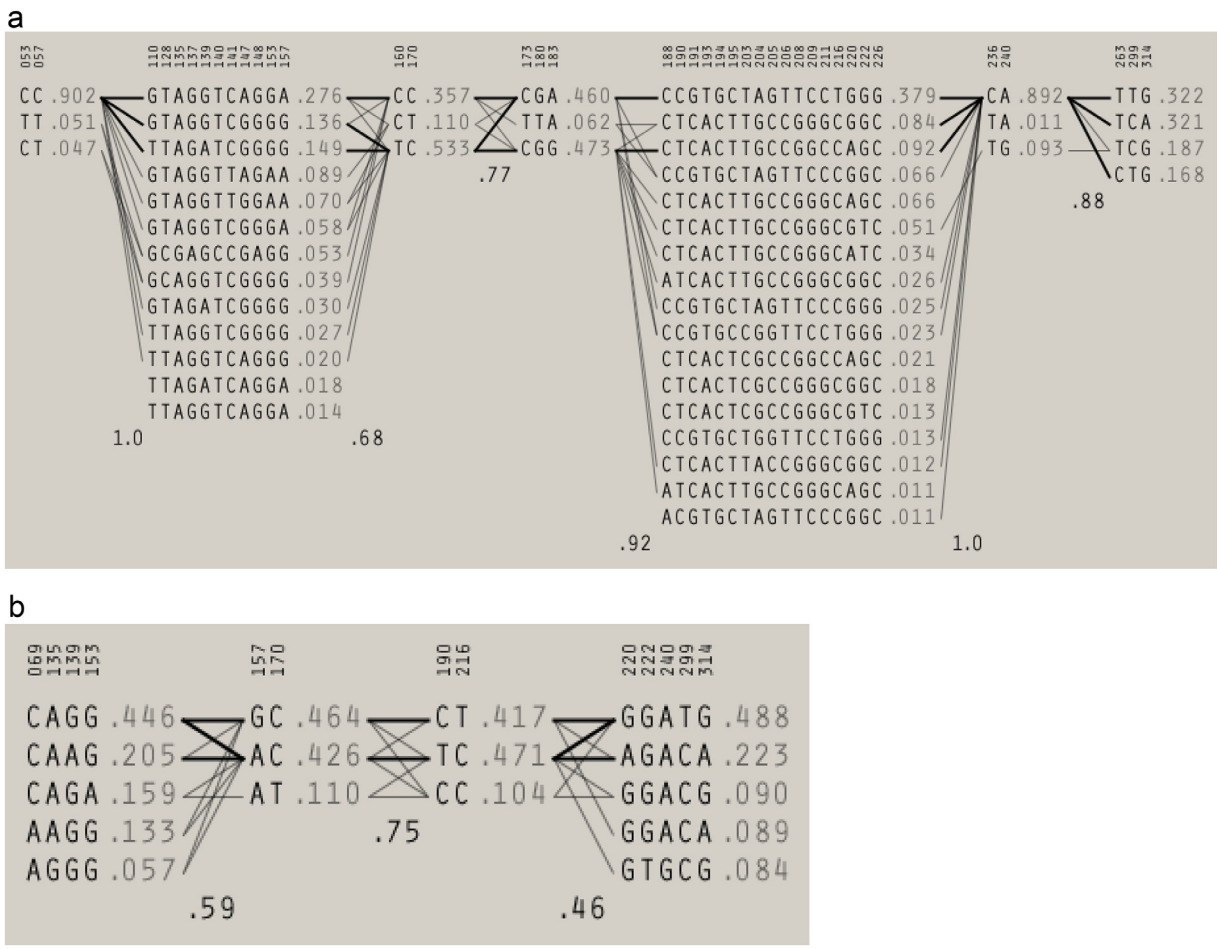

Figure 3. (A) Details of the 7 haplotype blocks outlined in Figure 2A. The top row lists the SNP indices (as listed in Supplementary Table S1). The haplotype display shows each haplotype in a block with its population haplotype frequency and connections from one block to the next. Lines show the most common crossings from one block to the next, with thicker lines indicating more common crossings than thinner lines. Shown beneath the crossing lines is multilocus D′. This represents the level of recombination between the 2 blocks. (B) Haplotype blocks estimated for the 25 CC SNPs as highlighted by the black triangles in Figure 2B.

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*Corporate members.

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Table 2
Distribution of C4 SNP Mismatches in HLA/SNP-Matched and -Mismatched Groups

Group	HLA 10/10	HLA 9/10
GB SNP-matched, n	147	16
GB SNP-mismatched, n	404	147

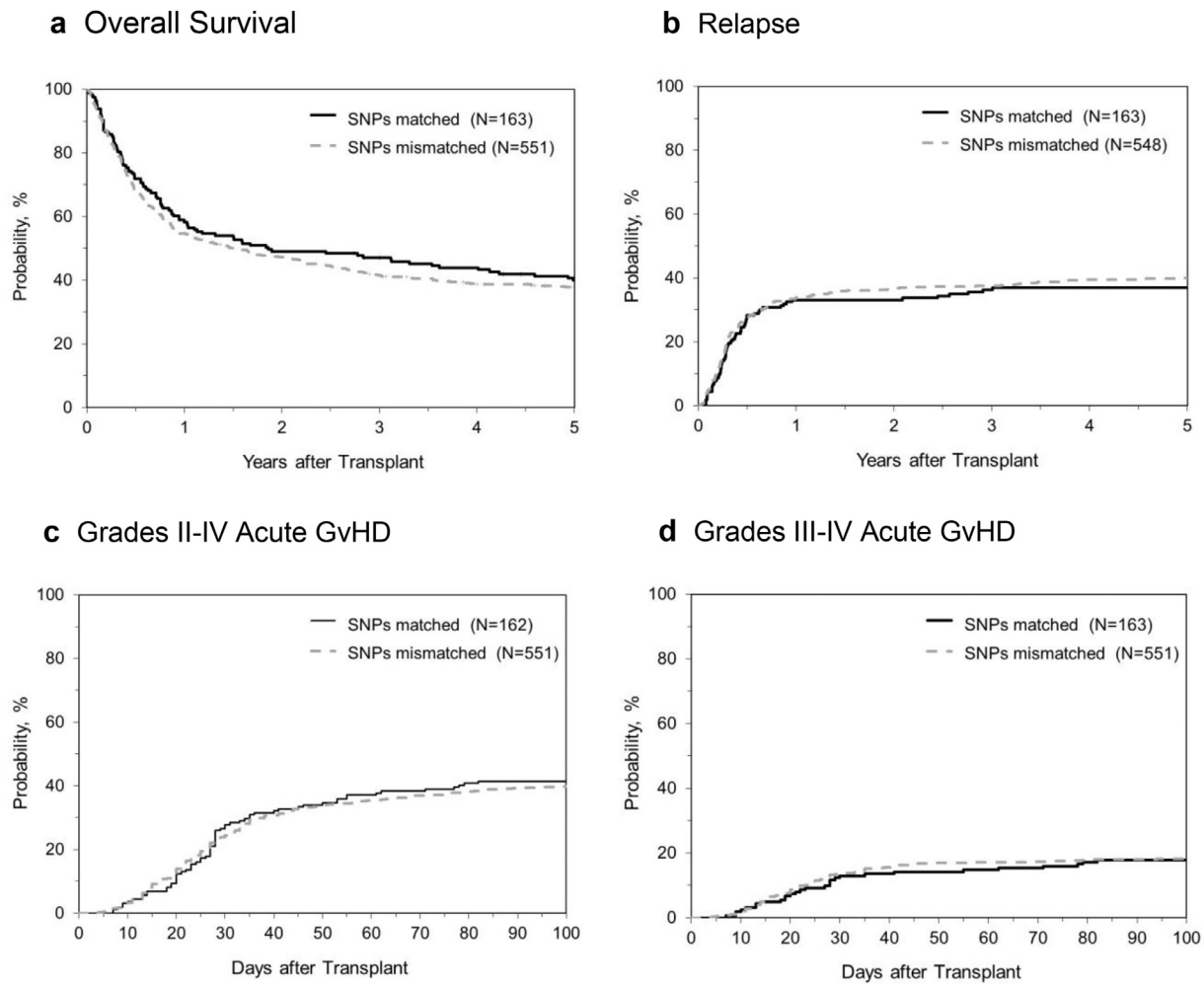

Figure 4. Associations between SNP mismatches and clinical outcomes. (A) OS. (B) Relapse. (C) Grade II-IV aGVHD. (D) Grade III-IV aGVHD.

Table 3
Multivariate Analysis of the GB SNP-Matched and -Mismatched Groups

	GB SNP-Matched (Reference)		GB SNP-Mismatched		
Outcome	Number	HR (Reference)	Number	HR (95% CI)	*P* Value
aGVHD grade II-IV	162	1.00	551	.94 (.71-1.25)	.676
aGVHD III-IV	163	1.00	551	.92 (.60-1.40)	.689
cGVHD	159	1.00	545	.88 (.68-1.15)	.349
OS	162	1.00	546	1.00 (.79-1.26)	.991
DFS	162	1.00	543	1.09 (.88-1.35)	.444
Relapse	162	1.00	543	1.19 (.89-1.59)	.245
TRM	163	1.00	548	.85 (.61-1.20)	.359
Neutrophil engraftment	162	1.00	544	.96 (.79-1.16)	.646

aGVHD grade II-IV adjusted for conditioning intensity, donor race/ethnicity, graft type, HLA match, center effects, and year of HCT; aGVHD grade III-IV adjusted for HLA match, center effects and use of TBI; cGVHD adjusted for graft type, use of ATG/Campath, center effects, and year of HCT; OS adjusted for ABO match, CMV serostatus match, disease status, graft type, Karnofsky Performance Status (KPS) score, HLA match, center effects, and recipient age, with data stratified by year of HCT; DFS adjusted for CMV match, conditioning intensity, disease status, KPS score, center effects, and year of HCT, stratified by sex match; relapse adjusted for CMV serostatus match, disease status, center effects, and use of ATG/Campath, stratified by sex match and year of HCT; TRM adjusted for ABO match, disease, HLA match and recipient age, stratified by sex match, center effects, and year of HCT; neutrophil engraftment adjusted for ABO match, disease status, center effects, and GVHD prophylaxis, stratified by conditioning regimen and graft type.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.bbmt.2018.12.008.

REFERENCES

1. Lee SJ, Klein J, Haagenson M, et al. High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. *Blood*. 2007;110:4576–4583.
2. Pidala J, Lee SJ, Ahn KW, et al. Nonpermissive HLA-DPB1 mismatch increases mortality after myeloablative unrelated allogeneic hematopoietic cell transplantation. *Blood*. 2014;124:2596–2606.
3. Petersdorf EW, Malkki M, Gooley TA, Martin PJ, Guo Z. MHC haplotype matching for unrelated hematopoietic cell transplantation. *PLoS Med*. 2007;4:e8.
4. Complete sequence and gene map of a human major histocompatibility complex. The MHC sequencing consortium. *Nature*. 1999;401:921–923.
5. Stewart CA, Horton R, Allcock RJ, et al. Complete MHC haplotype sequencing for common disease gene mapping. *Genome Res*. 2004;14:1176–1187.

6. Petersdorf EW, Malkki M, Horowitz MM, Spellman SR, Haagenson MD, Wang T. Mapping MHC haplotype effects in unrelated donor hematopoietic cell transplantation. *Blood*. 2013;121:1896–1905.
7. Pineda S, Sigdel TK, Chen J, Jackson AM, Sirota M, Sarwal MM. Novel non-histocompatibility antigen-mismatched variants improve the ability to predict antibody-mediated rejection risk in kidney transplant. *Front Immunol*. 2017;8:1687.
8. Spellman SR, Eapen M, Logan BR, et al. National Marrow Donor Program; Center for International Blood and Marrow Transplant Research. A perspective on the selection of unrelated donors and cord blood units for transplantation. *Blood*. 2012;120:259–265.
9. Gaudieri S, Leelayuwat C, Tay GK, Townend DC, Dawkins RL. The major histocompatibility complex (MHC) contains conserved polymorphic genomic sequences that are shuffled by recombination to form ethnic-specific haplotypes. *J Mol Evol*. 1997;45:17–23.
10. Gabriel SB, Schaffner SF, Nguyen H, et al. The structure of haplotype blocks in the human genome. *Science*. 2002;296:2225–2229.
11. Milner CM, Campbell RD. Genetic organization of the human MHC class III region. *Front Biosci*. 2001;6:D914–D926.
12. Blanchong CA, Chung EK, Rupert KL, et al. Genetic, structural and functional diversities of human complement components C4A and C4B and their mouse homologues, Slp and C4. *Int Immunopharmacol*. 2001;1:365–392.
13. Kitamura K, Andoh A, Inoue T, et al. Sodium butyrate blocks interferon-gamma (IFN-gamma)-induced biosynthesis of MHC class III gene products (complement C4 and factor B) in human fetal intestinal epithelial cells. *Clin Exp Immunol*. 1999;118:16–22.
14. Yu CY. The complete exon-intron structure of a human complement component C4A gene. DNA sequences, polymorphism, and linkage to the 21-hydroxylase gene. *J Immunol*. 1991;146:1057–1066.
15. Cunliffe V, Trowsdale J. The molecular genetics of human chromosome 6. *J Med Genet*. 1987;24:649–658.
16. Park Y, Cheong JW, Park MH, Kim MS, Kim JS, Kim HS. Effect of major histocompatibility complex haplotype matching by C4 and MICA genotyping on acute graft versus host disease in unrelated hematopoietic stem cell transplantation. *Hum Immunol*. 2016;77:176–183.
17. Askar M, Majhail NS, Rybicki L, et al. Single nucleotide gene polymorphisms in the gamma block of the major histocompatibility complex are independent risk factors for severe acute graft-versus-host disease in unrelated donor hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2015;21:S326–S327.
18. Spellman S, Setterholm M, Maiers M, et al. Advances in the selection of HLA-compatible donors: refinements in HLA typing and matching over the first 20 years of the National Marrow Donor Program Registry. *Biol Blood Marrow Transplant*. 2008;14(9 suppl):37–44.
19. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation*. 1974;18:295–304.
20. Shulman HM, Sullivan KM, Weiden PL, et al. Chronic graft-versus-host syndrome in man: a long-term clinicopathologic study of 20 Seattle patients. *Am J Med*. 1980;69:204–217.
21. Flomenberg N, Baxter-Lowe LA, Confer D, et al. Impact of HLA class I and class II high-resolution matching on outcomes of unrelated donor bone marrow transplantation: HLA-C mismatching is associated with a strong adverse effect on transplantation outcome. *Blood*. 2004;104:1923–1930.
22. Qin ZS, Niu T, Liu JS. Partition-ligation-expectation-maximization algorithm for haplotype inference with single-nucleotide polymorphisms. *Am J Hum Genet*. 2002;71:1242–1247.
23. Ciurea SO, Saliba RM, Rondon G, et al. Outcomes of patients with myeloid malignancies treated with allogeneic hematopoietic stem cell transplantation from matched unrelated donors compared with one human leukocyte antigen mismatched related donors using HLA typing at 10 loci. *Biol Blood Marrow Transplant*. 2011;17:923–929.
24. Fleischhauer K, Shaw BE, Gooley T, et al. Effect of T-cell-epitope matching at HLA-DPB1 in recipients of unrelated-donor haemopoietic-cell transplantation: a retrospective study. *Lancet Oncol*. 2012;13:366–374.
25. Lee C, Haneuse S, Wang HL, et al. Prediction of absolute risk of acute graft-versus-host disease following hematopoietic cell transplantation. *PLoS One*. 2018;13 e0190610.
26. Askar M, Sun Y, Rybicki L, et al. Synergistic effect of major histocompatibility complex class I-related chain a and human leukocyte antigen-DPB1 mismatches in association with acute graft-versus-host disease after unrelated donor hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2014;20:1835–1840.
27. Parmar S, Del Lima M, Zou Y, et al. Donor-recipient mismatches in MHC class I chain-related gene A in unrelated donor transplantation lead to increased incidence of acute graft-versus-host disease. *Blood*. 2009;114:2884–2887.
28. Carapito R, Jung N, Kwemou M, et al. Matching for the nonconventional MHC-I MICA gene significantly reduces the incidence of acute and chronic GVHD. *Blood*. 2016;128:1979–1986.
29. Gam R, Shah P, Crossland RE, Norden J, Dickinson AM, Dressel R. Genetic association of hematopoietic stem cell transplantation outcome beyond histocompatibility genes. *Front Immunol*. 2017;8:380.
30. Moyer AM, Hashmi SK, Kroning C, et al. Does matching for SNPs in the MHC gamma block in 10/10 HLA-matched unrelated donor-recipient pairs undergoing allogeneic stem cell transplant improve outcomes? *Hum Immunol*. 2018;79:532-536.
31. Askar M, Sobecks R, Wang T, et al. MHC Class I Chain-Related Gene A (MICA) donor-recipient mismatches and MICA-129 polymorphism in unrelated donor hematopoietic cell transplantations has no impact on outcomes in acute lymphoblastic leukemia, acute myeloid leukemia, or myelodysplastic syndrome: a Center for International Blood and Marrow Transplant Research study. *Biol Blood Marrow Transplant*. 2017;23:436–444.