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ORIGINAL REPORT

Patient HLA Germline Variation and Transplant Survivorship

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ABSTRACT

Purpose
HLA mismatching increases mortality after unrelated donor hematopoietic cell transplantation. The role of the patient's germline variation on survival is not known.

Patients and Methods
We previously identified 12 single nucleotide polymorphisms within the HLA region as markers of transplantation determinants and tested these in an independent cohort of 1,555 HLA-mismatched unrelated transplants. Linkage disequilibrium mapping across class II identified candidate susceptibility features. The candidate gene was confirmed in an independent cohort of 3,061 patients.

Results
Patient rs429916AA/AC was associated with increased transplantation-related mortality compared with rs429916CC (hazard ratio [HR], 1.39; 95% CI, 1.12 to 1.73; $P = .003$); rs429916A positivity was a proxy for DOA*01:01:05. Mortality increased with one (HR, 1.17; 95% CI, 1.0 to 1.36; $P = .05$) and two (HR, 2.51; 95% CI, 1.41 to 4.45; $P = .002$) DOA*01:01:05 alleles. HLA-DOA*01:01:05 was a proxy for HLA-DRB1 alleles encoding FEY ($P < 10E^{-15}$) and FDH ($P < 10E^{-15}$) amino acid substitutions at residues 26/28/30 that influence HLA-DRβ peptide repertoire. FEY- and FDH-positive alleles were positively associated with rs429916A ($P < 10E^{-15}$); FDY-positive alleles were negatively associated. Mortality was increased with FEY (HR, 1.66; 95% CI, 1.29 to 2.13; $P = .00008$) and FDH (HR, 1.40; 95% CI, 1.02 to 1.93; $P = .04$), whereas FDY was protective (HR, 0.88; 95% CI, 0.78 to 0.98; $P = .02$). Of the three candidate motifs, FEY was validated as the susceptibility determinant for mortality (HR, 1.29; 95% CI, 1.00 to 1.67; $P = .05$). Although FEY was found frequently among African and Hispanic Americans, it increased mortality independently of ancestry.

Conclusion
Patient germline HLA-DRB1 alleles that encode amino acid substitutions that influence the peptide repertoire of HLA-DRβ predispose to increased death after transplantation. Patient germline variation informs transplantation outcomes across US populations and may provide a means to reduce risks for high-risk patients through pretransplantation screening and evaluation.

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INTRODUCTION

Transplantation outcomes can be shaped by the clinical features of the patient, the donor, and the transplantation procedure itself. Efforts to improve the success of transplantation focus on optimizing modifiable factors, including better control of the patient's disease before transplantation to lower disease recurrence, complete donor matching of HLA genes to lower the risk of graft-versus-host disease, and the use of less-intense conditioning regimens to lower organ toxicity.[1-3]

A patient's inherited genome (germline) represents a nonmodifiable characteristic that is increasingly recognized as an important factor in shaping health outcomes. Germline variation may affect not only predisposition to disease but also host response to disease and therapeutic interventions.[4,5] The major histocompatibility complex (MHC) encodes the highest density of genes with immune-related function in the human genome and is a candidate region for genes that influence transplantation outcomes; however, a comprehensive and systematic analysis of the clinical importance of non-HLA sequence variation within the MHC is lacking.[6-8]

We tested the hypothesis that germline variation within the highly polymorphic MHC can impart risks to patients who undergo hematopoietic cell transplantation from HLA-mismatched donors that are not explained by patient-donor

ASSOCIATED CONTENT

Data Supplement
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HLA mismatching. We previously used MHC region single nucleotide polymorphisms (SNPs) and identified 12 candidate transplantation determinants.[9] The 12 SNPs gave rise to 14 associations—11 associations that involve 11 SNPs each with one clinical end point for either patient genotype, donor genotype, or patient-donor mismatch and three associations that involve one SNP with three clinical end points for patient genotype. In the current study, we conducted a multistage validation to confirm the susceptibility SNP and identify the true causative gene. Knowledge of clinically relevant germline variation may provide insight into a genetic component of transplantation survivorship and the possible mechanisms that shape an individual patient's outcome.

PATIENTS AND METHODS

Study Design and Population

Each of the 14 previously identified associations (cohort 1)[9] was tested in an independent cohort (cohort 2) of 1,555 transplantations with one HLA-A, -B, -C, -DRB1, or -DQB1 mismatch (Fig 1; Data Supplement). Of the 14 hypotheses, patient rs429916 genotype was validated as a marker of transplantation-related mortality. No associations were found between donor SNP genotype or patient-donor SNP mismatching (Data Supplement). Subsequently, fine-mapping of rs429916 was performed in 3,397 patients with residual DNA (2,052 from cohort 1 and 1,345 from cohort 2 [fine-mapping cohort]), and the candidate causative gene HLA-DRB1 was identified. An independent cohort of 3,061 transplantations with one HLA mismatch (cohort 3; Data Supplement) was then studied to confirm the HLA-DRB1 results (Fig 1). All patients provided informed consent for participation in this study. Clinical data were reported to the Center for International Blood and Marrow Transplant Research, and pretransplantation samples were provided from the Center for International Bone and Marrow Transplant Research Repository. Protocols were approved by the institutional review boards of the Fred Hutchinson Cancer Research Center and the National Marrow Donor Program.

Genotyping and Expression Studies

HLA genes and SNPs were genotyped and quantitative polymerase chain reaction and RNA sequencing performed as previously described[2,9,10] (Data Supplement). Linkage disequilibrium (LD; D′, r, and r^2) between markers was estimated with Haploview, PLINK version 1.07, and R Package genetics version 1.3.8.1, as previously described.[9]

Statistical Analysis

Criteria for SNP validation in cohort 2 required association with the same end point in the same genetic variant form as previously observed in cohort 1.[9] Outcomes were assessed using Cox proportional hazards

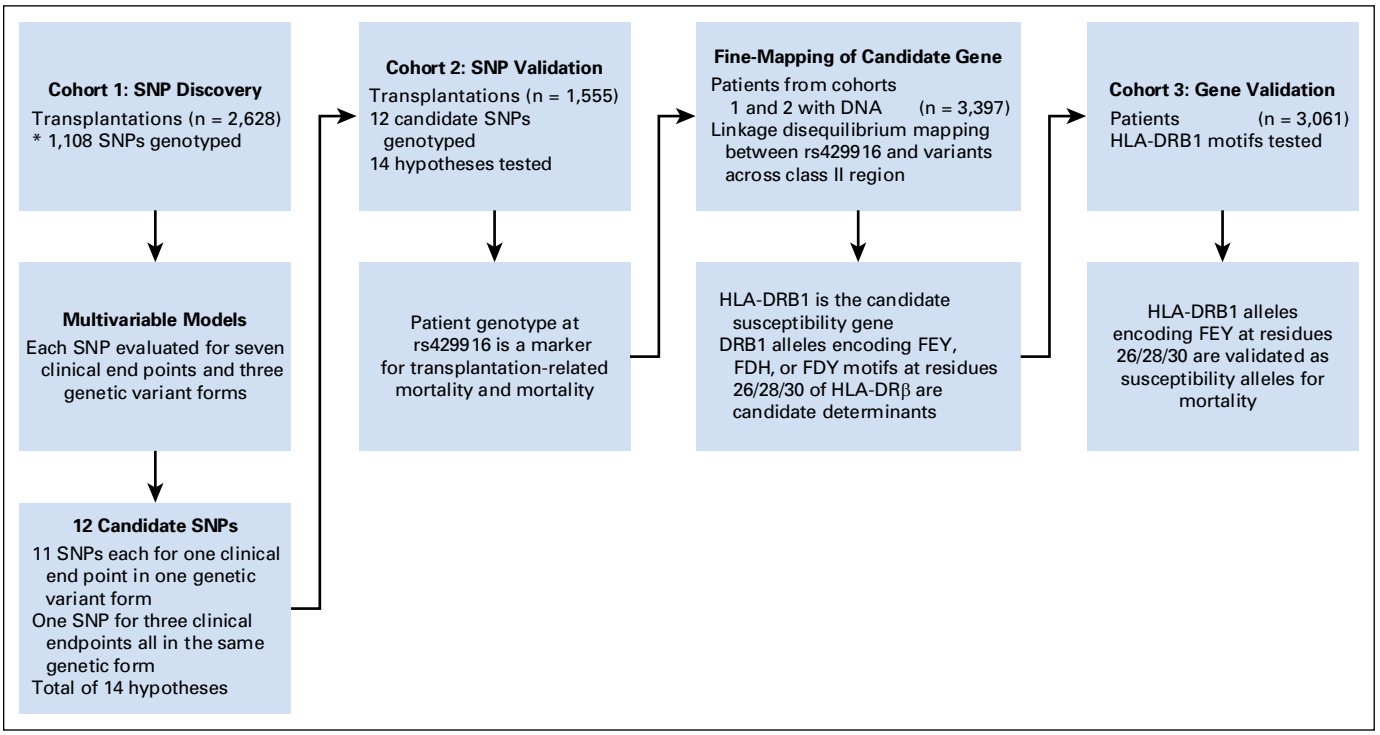

Fig 1. Study design. A previous survey of 1,108 single nucleotide polymorphisms (SNPs) within the major histocompatibility complex (MHC) was conducted in 2,628 patients to identify markers that correlate with clinical outcome after transplantation from unrelated donors with one HLA mismatch (cohort 1, first column).[9] Of the 1,108 SNPs, 11 were associated with one clinical end point (acute graft-versus-host disease [grades 2 to 4 or 3 to 4], chronic graft-versus-host disease, relapse, transplantation-related mortality, disease-free survival, or mortality) in one genetic form (patient genotype, donor genotype, or patient-donor mismatch). One of the 12 SNPs was associated with mortality, disease-free survival, and transplantation-related mortality, all in the patient genotype model. In total, the discovery phase identified 14 associations or hypotheses worthy of validation. The current study was designed to identify the true susceptibility gene responsible for clinical outcome. To that end, the same 14 hypotheses were tested in an independent cohort of 1,555 patients who received a transplant from an unrelated donor with one HLA-A, -B, -C, -DRB1, or -DQB1 mismatch for a blood disorder (cohort 2; second column). Each SNP was tested for the original clinical end point and genetic form that was identified in cohort 1. Of the 14 hypotheses tested, patient genotype at rs429916 was validated for transplantation-related mortality. To identify the gene responsible for increased mortality, linkage disequilibrium mapping across 500,000 base pairs of the class II region of the MHC was performed in cohort 1 and 2 patients with residual DNA (third column). HLA-DRB1 was identified as the candidate susceptibility locus, and alleles that encode amino acid substitutions that influence the peptide-binding region of HLA-DRβ were identified as candidate alleles worthy of validation. Risks associated with HLA-DRB1 residues were evaluated in an independent cohort of 3,061 patients who received a transplant from an unrelated donor with one HLA mismatch (cohort 3; fourth column). HLA-DRB1 alleles encoding the FEY motif at residues 26/28/30 of HLA-DRβ were validated as the susceptibility alleles for mortality.

regression models, which model the cause-specific hazards with competing events treated as censored. For each adjusted covariate, unknown data were excluded from regression models if there were fewer than 10 cases; otherwise, missing data were treated as a separate group. Proportional hazards were examined for all clinical covariates using a time-dependent covariate approach.

Factors that violated proportional hazards were adjusted through stratification. Stepwise forward-backward model selection was used to identify clinical prognostic risk factors as well as HLA allele or antigen mismatches at a 5% significance level. To assess whether a particular HLA allele may influence a clinical end point, comparison of genotypes with zero, one, or two copies of the allele in patients and donors were made at a 1% significance level. Finally, each SNP was tested separately by forcing the SNP into the multivariable model with an adjustment for the identified variables (Data Supplement). To adjust for multiple testing of 14 hypotheses for the 12 SNPs, the Bonferroni's threshold of 0.0036 was used to indicate statistical significance. Fine-mapping and validation of the at-risk gene used Cox regression models to compare the hazards of failure with the possession of the number of copies of HLA-DOA allele, HLA-DRB1 alleles, and HLA-DRβ residues. *P* values from Cox proportional hazards regression modeling were obtained from the Wald test. All *P* values are two sided. Data analyses were performed using SAS 9.3 statistical software (SAS Institute, Cary, NC).

RESULTS

Patient rs429916AA Genotype and Mortality

Of the 14 hypotheses, the association of patient rs429916 genotype with transplantation-related mortality was validated (Fig 1; Table 1; Data Supplement). Patients with rs429916AA and -AC genotypes had a higher 1- and 2-year transplantation-related mortality (45% and 50%, respectively) than those with rs429916CC (31% and 37%, respectively) and higher hazards of transplantation-related mortality (hazard ratio [HR], 1.39; 95% CI, 1.12 to 1.73; $P = .003$). In the fine-mapping cohort, the risks of both transplantation-related mortality and mortality increased with increasing numbers of rs429916A, consistent with a biologic step-up of risks (Table 1).

The rs429916 genotype resides in a haplotype block that includes only HLA-DOA (Fig 2). HLA-DOA encodes the DOα heterodimer of HLA-DO, a natural inhibitor of HLA-DM involved in peptide loading of class II molecules.[12-15] Three DOA proteins, DOA*01:01, DOA*01:02, and DOA*01:03, are recognized.[16] DOA*01:01 is encoded by DOA*01:01:01 to 01:01:06 distinguished by silent substitutions. Among known DOA alleles, only DOA*01:01:05 is in strong positive LD with rs429916A (D′, 0.80; r, +0.60; $P < 10E^{-15}$), which suggests that rs429916A and DOA*01:01:05 are proxies for each other (Data Supplement). Among 292 HLA-DOA*01:01:05-positive patients, 249 (85%) had rs429916A; among 3,105 HLA-DOA*01:01:05-negative patients, only 255 (8%) had rs429916A ($P < 2.2E^{-16}$). In contrast, rs429916A positivity was observed in only 8% of DOA*01:01:01/03-, 14% of DOA*01:01:02/04-, 4% of DOA*01:01:06-, and 11% of DOA*01:02-positive patients (Data Supplement).

In 1000 Genomes populations with rs429916 genotype and data that enable assignment of WHO-recognized HLA-DOA alleles, DOA*01:01:05 is in strong positive LD with rs429916A (D′, 0.96; r, +0.60; $P < 10E^{-15}$), whereas all other

Table 1. Multivariable Models for Transplantation-Related Mortality and Mortality According to Patient rs429916 Genotype in Cohort 1, Cohort 2, and the Fine-Mapping Cohort

Cohort	Clinical End Point	Overall *P*	Patient rs429916 Genotype	No.	HR (95% CI)	*P*
Cohort 1†	Overall mortality	< .001	CC	1,909	1	
			AC	296	1.11 (0.94 to 1.31)	.230
			AA	19	3.47 (1.95 to 6.16)	< .001
	Disease-free survival		CC	1,708	1	
			AC	268	1.06 (0.88 to 1.26)	.560
			AA	19	3.75 (2.10 to 6.68)	< .001
	Transplantation-related mortality	< .001	CC	1,696	1	
			AC	267	1.20 (0.97 to 1.49)	.10
			AA	19	4.52 (2.31 to 8.86)	< .001
Cohort 2	Transplantation-related mortality	.003	CC	1,107	1	
			AC + AA‡	196	1.39 (1.12 to 1.73)	.003
Fine-mapping cohort 1 and 2	Overall mortality	< .001	CC	2,882	1	
			AC	474	1.16 (1.03 to 1.31)	.010
			AA	29	2.12 (1.40 to 3.22)	< .001
	Transplantation-related mortality	< .001	CC	2,882	1	
			AC	474	1.24 (1.07 to 1.43)	.004
			AA	29	2.13 (1.26 to 3.86)	.005

NOTE. As previously described, patient rs429916 genotype correlates with the risks of overall mortality, disease-free survival, and transplantation-related mortality in the discovery cohort (cohort 1).[9] In the current study, the association between patient rs429916 genotype and transplantation-related mortality and mortality was confirmed in cohort 2. Fine-mapping to identify the causative gene was performed in cohorts 1 and 2 with sufficient residual DNA. Adjusted variables used for cohort 1 models were previously described.[9] For cohort 2, the transplantation-related mortality model was adjusted for patient age; disease stage; graft-versus-host disease prophylaxis; Karnofsky performance score; copy number of patient's DRB1*13; and patient-donor allele mismatching at HLA-A, -B, and -C and stratified by graft type. For the combined cohorts 1 and 2, the overall mortality model was adjusted for patient-donor cytomegalovirus serostatus, patient age, disease type, disease stage, Karnofsky performance score, patient ancestry, patient-donor allele mismatching at HLA-DRB1 or HLA-DQB1, and time from diagnosis to transplantation and stratified by graft type and year of transplantation; the transplantation-related mortality model was adjusted for patient-donor cytomegalovirus serostatus, patient age, disease type, disease stage, graft-versus-host disease prophylaxis, Karnofsky performance score, patient ancestry, patient-donor allele mismatching at HLA-DRB1 or -DQB1, T-cell depletion, and time from diagnosis to transplantation and stratified by graft type and year of transplantation.

Abbreviation: HR, hazard ratio.

†Previously reported by Petersdorf et al.[9]

‡Only 10 rs429916AA patients in cohort 2; therefore, AA and AC genotype patients were combined.

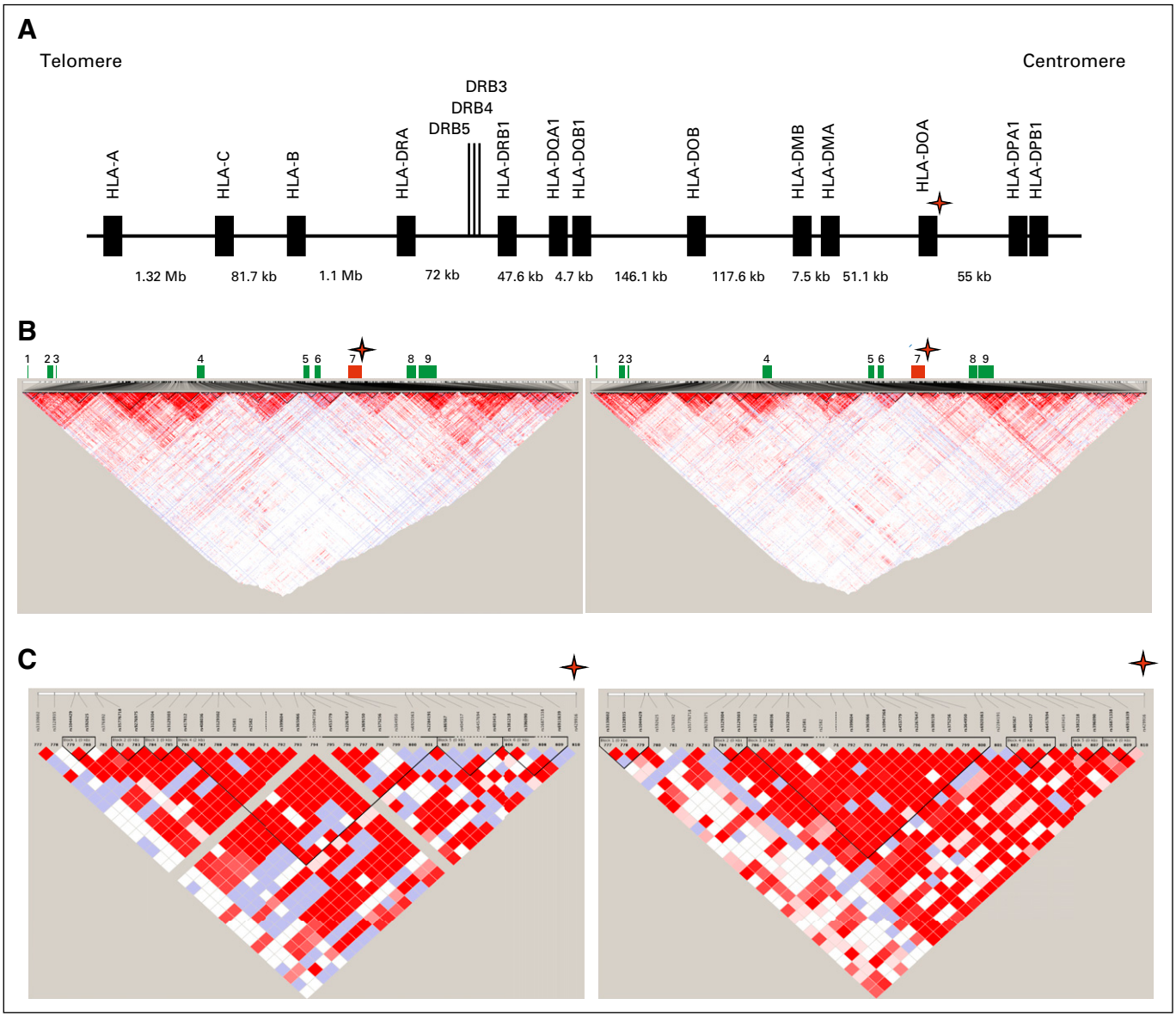

Fig 2. Linkage disequilibrium (LD) within the HLA class II genetic region. (A) The location of HLA class II genes is shown relative to the rs429916 marker for mortality (indicated by a star). (B) Long-range positive LD exists between rs429916 and variation across the region in both white and African American populations from HapMap Phase II.[11] Data on the left are for 267 white individuals (Utah residents with northern and western European ancestry from the Centre d'Etude du Polymorphism Humain collection [n = 165] and Toscani from Italy [n = 102]). Data on the right include 584 African American individuals (African ancestry from southwestern United States [n = 87]; Maasai from Kinyawa, Kenya [n = 184]; Yoruba from Ibadan, Nigeria [n = 203]; and Luhya from Webuye, Kenya [n = 110]). Class II genes are noted on the LD plots by green bars and represent (1) HLA-DRB1, (2) HLA-DQA1, (3) HLA-DQB1, (4) HLA-DOB, (5) HLA-DMB, (6) HLA-DMA, (7) HLA-DOA (red), (8) HLA-DPA1, and (9) HLA-DPB1. (C) LD within the HLA-DOA genetic region is illustrated for white individuals on the left and African American individuals on the right. Specific LD measurements (D') are provided in the Data Supplement.

DOA alleles are negatively associated (Data Supplement). Finally, we characterized HLA-DOA in HLA homozygous reference cells (Data Supplement). The reference cells KAS116 and COX were homozygous DOA*01:01:05 and rs429916AA. Taken together, these data strongly support rs429916A as a marker for HLA-DOA*01:01:05.

*DOA*01:01:05 and Mortality*

We tested the hypothesis that DOA*01:01:05 affects outcome. Increasing numbers of DOA*01:01:05 alleles were associated with higher risks of mortality and transplantation-related mortality, even after adjusting for HLA mismatching (Table 2).

The silent substitutions that define HLA-DOA*01:01:01 to 01:01:06 occur at sites that putatively influence methylation.[17] We observed that HLA-DOA expression values for DOA*01:01:05-homozygous patients are consistent with mean expression among DOA*01:01:05-negative patients (Data Supplement). In summary, DOA*01:01:05 and rs429916A define a haplotype, but neither are causative of mortality. We hypothesized that the true susceptibility gene is carried on DOA*01:01:05- and rs429916A-positive haplotypes.

Table 2. Multivariable Models of Mortality

Model† and Variation	Group	Fine-Mapping Cohort (n = 3,397) No.	Mortality HR (95% CI)	P	Cohort 3 (n = 3,061) No.	Mortality HR (95% CI)	P
HLA-DOA							
rs364950 TT	No DOA*01:01:05	3,105	1.0		NT‡	NT	
rs364950 CT	One DOA*01:01:05	278	1.17 (1.00 to 1.36)	.050	NT	NT	
rs364950 CC	Two DOA*01:01:05	14	2.51 (1.41 to 4.45)	.002	NT	NT	
HLA-DRB1							
Residues 26/28/30§	No FEY	3,314	1.0		2,966	1.0	
	Any FEY	83	1.66 (1.29 to 2.13)	< .001	95	1.29 (1.00 to 1.67)	.05
	No FDH	3,343	1.0		2,992	1.0	
	Any FDH	54	1.40 (1.02 to 1.93)	.040	69	0.96 (0.70 to 1.33)	.82
	No FDY	566	1.0		451	1.0	
	Any FDY	2,831	0.88 (0.78 to 0.98)	.020	2,610	1.08 (0.95 to 1.23)	.24

NOTE. rs429916A is a proxy for DOA*01:01:05. The presence of two DOA*01:01:05 alleles is associated with higher risks of mortality than the absence of DOA*01:01:05. Fine-mapping of DOA*01:01:05-positive patients elucidated HLA-DRB1 as the candidate susceptibility locus, with alleles encoding the FEY or FDH motifs at residues 26/28/30 associated with higher mortality risk and alleles that encoded FDY with lower mortality risk. The three candidate motifs each was subsequently evaluated in an independent cohort of 3,061 patients (cohort 3), which validated HLA-DRB1 alleles that encoded FEY at residues 26/28/30 to be the susceptibility alleles. The location of residues 26/28/30 and their putative effect on the peptide-binding repertoire of HLA-DRβ is illustrated in Figure 3. The association of HLA-DRβ residues did not depend on HLA mismatching; patients with the FEY motif had similar rates of HLA-DR mismatching as those with no FEY (6.7% *v* 2.4%, respectively, for the fine-mapping cohort). Similarly, the effect of DRβ FEY residues did not depend on patient ancestry (Table 3).

Abbreviations: HR, hazard ratio; NT, not tested.

†The table lists the results for four independent models: number of HLA-DOA*01:01:05 alleles, presence of FEY relative to absence of FEY, presence of FDH relative to absence of FDH, and presence of FDY relative to absence of FDY. All models were adjusted for the following variables: year of transplantation; total body irradiation; patient age; donor age; patient-donor cytomegalovirus serostatus; patient-donor sex; disease status; graft type; disease type and severity; conditioning regimen and intensity; T-cell depletion; and mismatched HLA-A, -B, -C, -DRB1, -DQB1, or -DPB1 locus. Adjustment for patient ancestry did not qualitatively change any association or improve the appropriate regression model.

‡Residual DNA was not available for any patient in cohort 3.

§Any FEY, any FDH, and any FDY each includes patients with one or two copies of the motif of interest relative to patients with no motif.

HLA Class II Alleles Among High-Risk Patients

We leveraged the strong long-range LD between rs429916 and variants within the class II region to identify the causal gene (Fig 2). Because HLA-DO and -DM interact to modulate antigen presentation,[12-15] we defined HLA-DOB, -DMA, and -DMB in HLA homozygous reference cells. Together with published data,[18] no specific HLA-DOB, -DMA, or -DMB alleles are linked exclusively with DOA*01:01:05, which suggests that variation at these loci cannot explain mortality risk (Data Supplement). However, three striking features were found for HLA-DRB1. First, HLA-DRB1 alleles differed according to zero, one, and two DOA*01:01:05 alleles, notably DRB1*03:02 (0.2%, 5.4%, and 28.6%, respectively) and DRB1*15:03 (0.5%, 3.1%, and 21.4%, respectively); in contrast, DRB1*07:01 was negatively correlated (12.3%, 24.1%, and 7.1%, respectively; Data Supplement). Second, DRB1*03:02 and DRB1*15:03 were only observed among nonwhite patients, whereas DRB1*03:01 and DRB1*15:01 were found in multiple ancestries: DRB1*03:01 (white American) versus DRB1*03:02 (African American; $P = 1.3E^{-57}$) and DRB1*15:01 (white American) versus DRB1*15:03 (African American; $P = 5.2E^{-114}$; Data Supplement). Among the 28 haplotypes represented in the 14 DOA*01:01:05-homozygous patients, 15 (54%) carried DRB1*03:02 and/or DRB1*15:03. The 14 patients were African American (n = 10), white American (n = 3), and Hispanic American (n = 1). In sharp contrast, DOA*01:01:05-negative patients encoded DRB1*03:01 and/or DRB1*15:01. These results indicate that DRB1 alleles were skewed across the entire study population and that two alleles, DRB1*03:02 and DRB1*15:03, were enriched among DOA*01:01:05-rs429916A–positive patients.

HLA nomenclature provides information on the HLA-DRB1 allele sequence (structure), which defines the constituent amino acid residues that influence peptides accommodated by the HLA-DRβ groove (function; Fig 3). The third striking feature was the strong LD between HLA-DRβ residues 26/28/30 and DOA*01:01:05 (Data Supplement). DRB1*15:01 encodes FDY, DRB1*15:03 FDH, and DRB1*03:02 FEY. The frequency of FEY and FDH increased with zero, one, and two DOA*01:01:05 alleles, whereas FDY was inversely proportional. Formal LD analysis (Data Supplement) demonstrates the strongest LD association between FEY and DOA*01:01:05 ($P < 10E^{-15}$) followed by FDH ($P < 10E^{-15}$); FDY has the strongest negative LD ($P < 10E^{-15}$). Furthermore, rs429916A is in positive LD with FDH ($P < 1.0E^{-15}$) and FEY ($P = 3.3E^{-12}$) and in negative LD with FDY ($P < 1.0E^{-15}$; Data Supplement). Finally, the estimated three-locus haplotype frequency of FEY-DOA*01:01:05-rs429916A in the fine-mapping cohort (0.00146) is greater than the expected frequency on the basis of the product of the three allele frequencies (0.000354), which supports a > 400-kb-long haplotype. In summary, rs429916A-positive patients encode different HLA-DRβ residues than rs429916A-negative patients. Evaluation of residues shared among distinct HLA-DRB1 alleles may shed light on common features that are biologically important in transplantation survivorship.

HLA-DRB1 and Mortality

In the fine-mapping cohort, we tested the hypothesis that mortality depends on the presence of specific amino acid substitutions in the peptide-binding region of HLA-DRβ. The FEY, FDY, and FDH motifs at residues 26/28/30 of HLA-DRβ were evaluated because of their strong positive (FEY and FDH) and negative (FDY) LD with rs429916A and HLA-DOA*01:01:05, the proxies for mortality. We hypothesized that presence of FEY and FDH increases the risk of mortality, whereas FDY is protective. Compared with patients

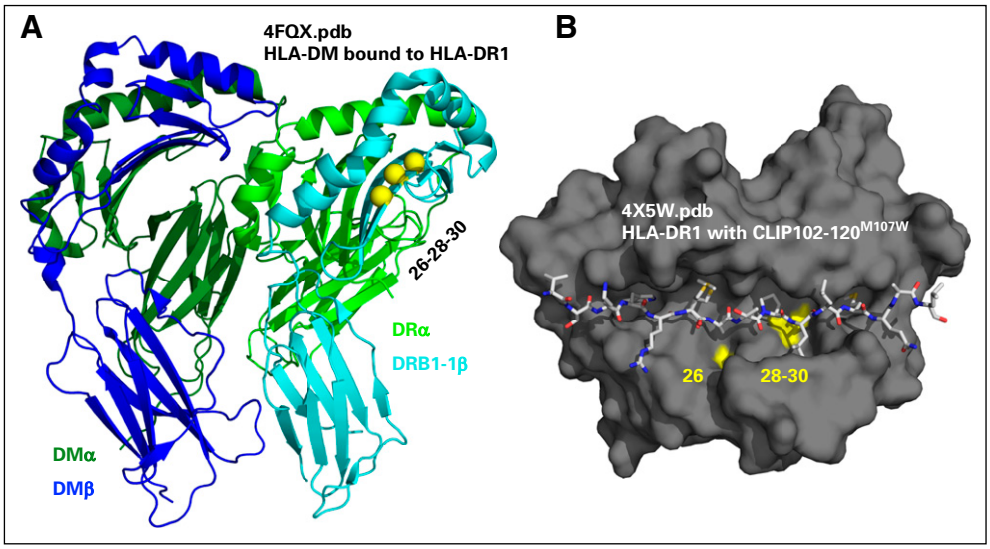

Fig 3. Effect of amino acid variation at residues 26/28/30 on the HLA-DRβ peptide-binding groove. Positions 26/28/30 HLA-DRβ form the constituent residues that influence the P7 position of peptides accommodated by the HLA-DR binding groove and to a lesser extent the P5 position. (A) Illustration of the crystal structure complex between HLA-DM (dark green and dark blue) and HLA-DR1 (light green and light blue).[19,20] The α-carbon positions of key residues evaluated in the study are represented by yellow spheres and numbered by sequence position. The side-chains of these residues all point inward toward the bound peptide in the groove and do not mediate direct interactions with HLA-DM, T-cell receptor, CD4, or any other cognate receptor/coreceptor. (B) The crystal structure of HLA-DR1 is shown in a molecular surface representation (gray) that highlights the peptide-binding groove.[21,22] The bound peptide (residues 102 to 120 of CLIP, with a methionine-to-tryptophan mutation at residue 107) is shown in a licorice-stick representation colored by atom type. This view is the perspective from an incoming T-cell receptor. Contributions to the molecular surface from side-chains from residues at positions 26/28/30 are shown in yellow. The multivariable models that demonstrate risks of mortality associated with substitutions at residues 26/28/30 are presented in Table 2. Residues are numbered according to the IPD-IMGT/HLA Database.[16]

without FEY, those with FEY had a significantly increased risk of mortality; risks also were increased among patients with FDH compared with those without the motif (Table 2). Compared with patients without FDY, those with FDY had a significantly lower risk of mortality, which suggests a protective effect of the motif. Little evidence was found of a statistical interaction between FEY and FDH (P = .94), FEY and FDY (P = .44), or FDH and FDY (P = .75), which suggests that the effects of one motif did not vary according to the presence of another motif and vice versa. Furthermore, no evidence was found of an interaction between motifs and ancestry (P = .58 for FEY; P = .34 for FDH; P = .44 for FDY), which suggests that the effect of each motif on the risk of mortality was approximately the same across ancestries. In summary, FEY, FDH, and FDY are each candidate determinants worthy of validation.

To validate which, if any, of the three motifs is a marker for mortality, we tested FEY, FDH, and FDY separately in an independent cohort of 3,061 patients (cohort 3; Data Supplement). A similar step-up of mortality hazard was observed for the presence of FEY compared with the absence of FEY (HR, 1.29); however, no protective effect of FDY (HR, 1.08) or at-risk effect of FDH (HR, 0.96) was observed (Table 2). In summary, HLA-DRB1 alleles that encode the FEY motif at residues 26/28/30 of HLA-DRβ in patients increase the risk of mortality after HLA-mismatched unrelated donor transplantation.

HLA-DRB1 alleles that encode the FEY motif are found at strikingly different frequencies across diverse ancestries[23] (Data Supplement). To evaluate whether the detrimental effect of FEY depends on ancestry, we examined risks associated with the presence of FEY compared with absence of FEY in African, Hispanic, and white American patients from cohorts 1, 2, and 3 combined; only two patients of Asian American ancestry encoded alleles with the FEY motif and were not analyzed (Table 3). Among African, Hispanic, and white American patients, little evidence was found that the effect of FEY presence on outcome was different across these ancestries (interaction P = .63). In each ancestry, an increased hazard of mortality was found among patients who possessed FEY compared with those who did not (HR, 1.60 for African Americans, 1.35 for Hispanic Americans, and 1.27 for white Americans). Therefore, the deleterious FEY effect was consistent across ancestries. Because African and Hispanic American patients may encode HLA-DRB1 alleles without the FEY motif (Data Supplement), HLA-DRB1 allele typing provides important prognostic information for transplantation patients.

The clinical events before the patients' deaths were similar between patients with and without FEY motifs (Data Supplement). Taken together, the risk of mortality after transplantation is influenced by HLA-DRB1 alleles with substitutions that influence the peptide-binding region of HLA-DR molecules. HLA-DRB1 alleles that confer increased mortality risk are observed more commonly among patients of African and Hispanic American ancestry than patients of other backgrounds. The patient's HLA germline influences disparities in survivorship among US continental populations.

DISCUSSION

We identified inherited MHC genetic variation in the patient to be a risk factor for mortality after HLA-mismatched unrelated donor transplantation. High-risk HLA haplotypes are defined by the presence of amino acid substitutions at key positions of the HLA-DRβ peptide-binding groove. The susceptibility variants are linked on patient haplotypes that span > 500,000 base pairs and involve genes responsible for antigen processing (HLA-DOA) and

Table 3. Effect of the HLA-DRB1 FEY Motif According to Ancestry

Ancestry	Group	No.	Mortality HR (95% CI)	*P*
African American (n = 412)	No FEY	342	1.0	
	Any FEY	70	1.60 (1.18 to 2.17)	.002
Hispanic American (n = 582)	No FEY	506	1.0	
	Any FEY	76	1.35 (0.99 to 1.84)	.060
White American (n = 5,036)	No FEY	5,021	1.0	
	Any FEY	15	1.27 (0.70 to 2.30)	.430

NOTE. To address whether mortality was solely due to the increased fraction of Hispanic and African Americans who possess FEY motifs relative to whites, we evaluated the effect of FEY among patients of African, Hispanic, and white American ancestry in cohorts 1, 2, and 3 combined. The models adjusted for year of transplantation; use of total body irradiation, age; cytomegalovirus serostatus; disease risk; graft type; HLA-A, -B, -C, -DRB1, -DQB1, -DPB1 mismatch; and donor age. Two Asian Americans in cohorts 1, 2, and 3 encoded HLA-DRB1 alleles with the FEY motif and were not analyzed.
Abbreviation: HR, hazard ratio.

presentation (HLA-DR). The clustering of genes with coordinated function in immune regulation highlights the sentinel role of class II genes in health and disease.[24] The results of the current study suggest that identification of patients with high-risk HLA-DRB1 alleles will enhance risk assessment.

Donor mismatching of HLA genes is a well-known risk factor for transplantation-related complications.[1] When HLA-matched donors are not available, the judicious use of HLA-mismatched donors can provide life-saving transplants. The identification of clinically important heritable variants in transplantation patients extends the understanding of the genetics of transplantation beyond that of donor mismatching. In the current study, all transplantations had the same degree of donor HLA mismatching; furthermore, the risk of mortality associated with HLA-DR/DOA did not depend on the kind of HLA mismatch, nor was it a consequence of more HLA-DRB1 mismatching among African or Hispanic American patients. In contrast to previous studies in which risks are defined for pairwise patient-donor differences,[25] the mapping of susceptibility residues in the patient's germline was facilitated by the association of disease (ie, mortality) with HLA-DRB1, an approach used in classic disease-association mapping.[26] Translation of the HLA-DRB1 sequence to HLA-DRβ amino acid motifs enabled us to evaluate shared structural properties of HLA-DR molecules that correlate with mortality risk across ethnically diverse patients. Future studies of the effect of high-risk residues after HLA-matched transplantation are warranted when a larger US transplantation experience is available to rigorously test this hypothesis in patients of diverse ancestries.

Considerable clinical experience highlights marked disparities in cancer mortality in the United States, particularly for African Americans, who have the highest mortality of all populations.[27-30] The major goal of the current study was to identify clinically relevant genetic variants beyond the known HLA mismatch to better understand the total burden of genetic variation on outcome after HLA-mismatched transplantation. Although we were agnostic to ancestry in the design of our study (in that ancestry was not used to partition patients into comparison groups to identify susceptibility variants), the marked skewing of HLA alleles among patients at highest risk of mortality was an important feature that facilitated the mapping of the HLA-DRβ FEY susceptibility motif found at higher frequencies in African and Hispanic Americans compared with other US populations.

Throughout human evolution, MHC alleles have diversified at key positions of the peptide-binding groove, which likely reflects selective pressures posed by antigenic challenges of infection. Although patients with and without FEY motifs experienced similar clinical events before death, future studies are warranted to fully assess the specific reasons for transplantation failure. Nonetheless, variation at critical residues of the peptide-binding region represents functional constructs. The structural role of substitutions at residues 26/28/30 is to alter the interaction with the peptide at the P7 position through a combination of specificity, affinity, and half-life and not through direct effects on interactions with T-cell receptors or coreceptors. Hence, variation that alters P7 may have major consequences on the peptide repertoire of HLA-DRβ molecules.

An individual's germline variation provides a biologic paradigm for understanding health outcomes disparities.[31,32] The MHC has long served as a model for understanding population differences where HLA alleles found uniquely in a population serve as ancestral markers.[23,33] Although the likelihood of any given patient carrying high-risk HLA-DRβ residues is higher for certain US continental populations than others, possession of high-risk residues confers risks regardless of ancestry. As such, high-risk HLA-DRβ residues might be a close proxy for ancestry, but ancestry is not a robust proxy for disease-causative DNA variation and cannot be relied on to choose which patient will benefit from pretransplantation risk assessment.

Transplantation is optimal when the benefits of curative therapy outweigh the risks associated with the procedure itself. The current data suggest that knowledge of patients' HLA-DRβ residues may supplement prognostication measures of transplantation outcomes to enhance counseling,[34] may guide clinical decision making about the optimal timing of transplantation and tailored selection of drugs and regimens,[3] and may enable high-risk patients to benefit from increased surveillance of post-transplantation complications. The current study sheds light on a role for the patient's germline HLA haplotype in outcome after unrelated donor transplantation. Future studies of the importance of DRβ residues in haploidentical and cord blood transplantation will further understanding of the HLA barrier in transplantation for these modalities. Knowledge of patients with high-risk HLA-DRβ residues has utility in the design of future clinical trials[35] and in the interpretation of health outcomes data.[4,5] In these ways, the patient's unique genetic make-up can be leveraged to improve his or her survival.

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Patient HLA Germline Variation and Transplant Survivorship

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