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Single nucleotide polymorphisms and outcome risk in unrelated mismatched hematopoietic stem cell transplantation: an exploration study

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Genetic risk factors contribute to adverse outcome of hematopoietic stem cell transplantation (HSCT). Mismatching of the HLA complex most strongly determines outcomes, whereas non-HLA genetic polymorphisms are also having an impact. Although the majority of HSCTs are mismatched, only few studies have investigated the effects of non-HLA polymorphisms in the unrelated HSCT and HLA-mismatched setting. To understand these effects, we genotyped 41 previously studied single nucleotide polymorphisms (SNPs) in 2 independent, large cohorts of HSCT donor-recipient pairs (n = 460 and 462 pairs) from a homogeneous genetic background. The study population was chosen to pragmatically represent a large clinically homogeneous group (acute leukemia), allowing all degrees of HLA matching. The *TNF*-1031 donor-recipient genotype mismatch association with acute GVHD grade 4 was the only consistent association identified. Analysis of a subgroup of higher HLA matching showed consistent associations of the recipient *IL2*-330 GT genotype with risk of chronic GVHD, and the donor *CTLA4*-CT60 GG genotype with protection from acute GVHD. These associations are strong candidates for prediction of risk in a clinical setting. This study shows that non-HLA gene polymorphisms are of relevance for predicting HSCT outcome, even for HLA mismatched transplants. (*Blood.* 2012; 119(26):6365-6372)

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Introduction

It is thought that a large proportion of risk for adverse outcomes after hematopoietic stem cell transplantation (HSCT) is genetic, attributed to HLA matching,[1] killer-immunoglobulin-like receptor matching,[2,3] minor histocompatibility antigens,[4,5] and non-HLA gene polymorphisms.[6]

Whereas the degree of HLA mismatching exerts the strongest genetic effect on risks, such as acute and chronic GVHD, relapse, and survival, non-HLA polymorphisms in immune response genes, such as cytokines, at least modify these risks, as shown in studies that have shown light on the pathobiology of HSCT,[7,8] and the relation of cytokine gene polymorphisms,[6,9,10] with gene expression and biologic effects.[11-15]

Non-HLA gene polymorphisms have been widely studied (a systematic search conducted revealed 192 studies over the last 2 decades). Most of these studies used a candidate gene approach, and only one study was a genome-wide association study.[5] To minimize genetic confounding, most of these studies used either fully or largely HLA-matched related or unrelated HSCT cohorts. Limited availability of study subjects in the past made consideration of demographic or clinical risk factors in study cohort selection difficult, despite the existence of these risks being well established in the literature (eg, patient and donor age,[16,17] female donor to male recipient,[18] diagnosis and staging, prior chemotherapy, conditioning regimen,[19] concurrent infections). Although more than 100 genetic markers in more than 60 candidate genes have been studied, consistency of results has been poor across studies, which has been attributed to differences in HSCT setting or stem cell source, ethnicity of the population, marker genotype distribution, and study quality and power. Only a limited number of associations underwent replication studies, and very few of these showed some consistency in different settings, such as polymorphisms in *TNF, IL10, IL6, CTLA4*.[6]

HLA mismatching is common in daily unrelated donor HSCT practice, most commonly because of nonavailability of an HLA-matched donor. In the Japan Marrow Donor Program (JMDP), less than 10% of HSCT have a 12 of 12 allele HLA match, and approximately 30% have an 8 of 8 allele HLA match. Despite this, only a very small number of studies have deliberately used populations that represent the full spectrum of HLA matching.

It is an important clinical question whether non-HLA polymorphisms have an impact on HSCT outcome in an unrelated HSCT population despite the competing effects of HLA mismatching.

The aim of this study was to identify genetic polymorphisms influencing HSCT outcome in an unrelated donor, HLA-mismatched setting, pragmatically choosing a large diagnostic group (acute leukemia) with additional selection and correction for the most relevant confounding variables (see "Population"). We applied a study design aiming to comply with recommendations for more

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Table 1. Selected candidate SNP markers of this study

Target gene	SNP	Target gene	SNP
CCL4	rs2634508	*NOD2*	rs1077861
CD86	rs1129055		rs1861757
CTLA4	rs231777		rs1861759
	rs231775 (*CTLA4*–49)		rs6500328
	rs3087243 (*CTLA*-CT60)		rs2111234
FAS	rs1800682 (*FAS*-670)		rs2111235
FCGR2A	rs1801274		rs7203344
HLA-E	rs1264457 (*HLA-E* R128G)		rs17313265
	rs1800795	*TGFB1*	rs1800469 (*TGFB1*-509)
HSP70/hom	rs2075800		rs2241715
IFNg	rs2069705		rs2241716
IL1A	rs1800587 (*IL1A*-889)		rs4803455
IL1B	rs16944 (*IL1B*-511)	*TLR4*	rs12377632
IL2	rs2069762 (*IL2*-330)		rs1927907
IL10	rs1800896 (*IL10*-1082)	*TNF*	rs361525 (*TNF*-238)
	rs1800871 (*IL10*-819)		rs1799964 (*TNF*-1031)
	rs1800872 (*IL10*–592)		rs1800629 (*TNF*-308)
IL15RA	rs2228059 (*IL15RA* N182T)		rs1799724 (*TNF*-857)
IL23R	rs6687620	*TNFRSF1B*	rs1061622 (*TNFR2* codon 196)
MIF	rs755622	*VDR*	rs731236
MTHFR	rs1801133 (MTHFR C677T)		

stringent genetic association study designs,[20-24] testing a panel of strong candidate SNP markers from previous studies. Key features include significance as well as effect size testing on 2 large, independent, clinically homogeneous study cohorts stemming from a population of homogeneous ethnic background.

Methods

Population

Donor and recipient HSCT pairs were selected from the JMDP registry of unrelated HSCT. This study was approved by the review boards of the JMDP and Tokai University Medical School, Isehara, Kanagawa, Japan. We chose pairs with a diagnosis of acute leukemia. These form the largest subgroup within HSCT. Cohorts represented 2 samplings of the same national pool, taken from 2 distinct timeframes (1993-2000, 2001-2005). Inclusion criteria were diagnosis (acute lymphoblastic leukemia; acute nonlymphoblastic leukemia), age (4-40 years), conditioning (myeloablative), and stem cell source (bone marrow). All transplants were T-cell replete and received GVHD prophylaxis with either cyclosporin A or tacrolimus with methotrexate and corticosteroids. Analysis of the source as well as the selected HSCT population showed that HLA mismatching, donor age, and GVHD prophylaxis regimen (cyclosporin A vs tacrolimus) were the only confounders remaining significant in multivariate analysis (data not shown here).

All donor-recipient pairs were HLA-typed retrospectively to allele level at 6 loci (HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQB1, and HLA-DPB1). The distribution of HLA matching of the confirmatory cohort was adjusted to that of the screening cohort by matching each sample of the screening cohort with a confirmatory cohort sample of the same HLA class or HLA class combination according to the previous literature[25,26] and our own analyses of risk matches/mismatches within this study population (data not shown). Supplemental Table 1 (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article) shows the demographic and clinical characteristics of the selected cohorts. There was no statistically significant difference between the cohorts in the baseline demographic criteria. Supplemental Table 2A and B specify the degree of HLA matching and mismatching. For reasons of comparison, we have used the National Marrow Donor Program/Center for International Blood and Marrow Transplant Research classification of HLA matching.[27] According to this classification, 357 HSCT pairs have an 8 of 8 (HLA A, B, C, DRB1) high-resolution allele match, 331 (35.9%) are partially matched (1 mismatch within these HLA loci), and 234 (25.4%) are mismatched (2 or more mismatches within these HLA loci). Considering the HLA DQ and DP loci also, only 78 HSCT pairs (8.5%) had a 12 of 12 allele match. In Japanese, HLA A, B, and C mismatches are associated with risk of acute GVHD. HLA C mismatches, however, have a protective effect on relapse (whereas HLA A, C, and B mismatches associate with a risk of death).[25,26,28] More recent research has focused on specific allele mismatches, rather than mismatches in loci, aiming to identify nonpermissive mismatches for acute GVHD[29] or protective mismatches against relapse,[30] as well as risk HLA haplotypes for GVHD.[31]

Gene and SNP marker selection

Selection of candidate markers was based on a search of the published literature on genetic associations with HSCT outcomes. As the TaqMan SNP genotyping platform was used, selection was limited to markers for which standard assays were available for this system.

For some genetic loci, the same markers that were associated in other populations were nonpolymorphic in Japanese (*NOD2, TGFB1*). The HapMap database (www.hapmap.org) was used to identify haploTag SNP for these loci.

The SNP markers included in this study are detailed in Table 1; the assay details are available in supplemental Methods.

Genotyping

TaqMan SNP genotyping assays (Applied Biosystems) were applied for 38 selected SNP according to the maker's instructions.

The *IL10* promoter SNPs rs1800872 (-592A/C), rs1800871 (-819T/C), and rs1800896 (-1082A/G) were genotyped by PCR-SSO using Luminex Multi-Analyte Profiling system (xMAP; Luminex). Details of both genotyping methods can be found in supplemental Methods.

Statistical analysis

Genotype results were imported into SPSS Statistics Version 17.0 (SPSS Inc). Because little is known about effects of non-HLA polymorphisms in HLA-mismatched populations, we used 3 analytic approaches to identify significant associations: 2-sided Fisher exact test (95% confidence intervals [CIs]) with Bonferroni correction for significance testing, odds ratio (OR; 95% CIs) as a measure of effect size, and independent testing in a confirmatory cohort (without application of multiple testing correction).

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Table 2. Results of SNP genotyping on all donor samples

Gene	Marker	Discovery cohort (genotype and association)	Confirmatory cohort (genotype and association)
CTLA4	rs231775	AA aGVHD* (P = .0043, OR = 0.049,* CI = 0.028-0.083)	NS
		GG aGVHD (P = .0071, OR = 1.90, CI = 1.19-3.03)	
CTLA4	rs3087243	GG aGVHD (P = .0086, OR = 1.81, CI = 1.18-2.78)	NS
CTLA4	Haplotype	CAA aGVHD (P = .0025, OR = 0.59, CI = 0.42-0.82)	NS
		CGG aGVHD* (P = .00057,* OR = 1.72, CI = 1.27-2.34)	
FAS	rs1800682	CC aGVHD4* (P = .023, OR = 0.21,* CI = 0.37-0.96)	NS
IFNg	rs2069705	CC ext cGVHD (P = .035, OR = 0.57, CI = 0.33-0.96)	NT
		CC relapse (P = .04, OR = 0.60, CI = 0.37-0.96)	
IL10	rs1800896	AA survival* (P = .001)* protective	NS
IL10	Haplotype	CCA survival (P = .032) protective	NT
MTHFR	rs1801133	CT cGVHD (P = .03, OR = 0.63, CI = 0.42-0.96)	NT
NOD2	rs17313265	CT survival (P = .012) risk	NT
		CC survival (P = .008) protective	NT
NOD2	rs2111235	TT aGVHD4* (P = .016, OR = 0.33,* CI = 0.14-0.80)	NS
NOD2	rs6500328	GG ext cGVHD* (P = .011, OR = 0.17,* CI = 0.023-0.78)	NS
TGFB1	rs1800469	CC aGVHD2-4 (P = .035, OR = 1.69, CI = 1.09-2.61)	NT
		CT aGVHD2-4 (P = .036, OR = 0.66, CI = 0.45-0.96)	NT
TGFB1	rs2241715	GG aGVHD2-4 (P = .047, OR = 1.64, CI = 1.06-2.53)	NT
		GT survival (P = .03) protective	NT
		GT ext cGVHD (P = .032, OR = 0.57, CI = 0.34-0.94)	NT
		GT aGVHD2-4 (P = .037, OR = 0.67, CI = 0.46-0.98)	NT
TNF	rs1799964	TT relapse (P = .041, OR = 1.71, CI = 1.04-2.82)	NT
TNF	rs1799724	CC survival (P = .014) protective	NT

P values (2-sided Fisher exact test; survival, log rank test, Kaplan-Meier). Marker rs231777 had no individual association and is therefore not included in this table, but it was included into the confirmatory cohort as part of the CTLA4 haplotype.

aGVHD indicates acute GVHD; aGVHD4, acute GVHD grade 4; aGVHD2-4, acute GVHD grade 2-4; cGVHD, chronic GVHD; ext cGVHD, extensive chronic GVHD; mismatch, genotype mismatch between donor and recipient; NS, not significant; and NT, not tested.

*Withstanding Bonferroni multiple testing corrections or have OR $\leq$ 0.5 or $\geq$ 2.

Variables were the 3 individual genotypes, and mismatch between donor and recipient genotypes. Outcomes were acute GVHD (0-4), acute GVHD grades 2 to 4, acute GVHD grades 3 to 4, acute GVHD grade 4, chronic GVHD, extensive chronic GVHD, relapse, death (overall, at 100 d/1 y/3 y), and survival (as log-rank test in Kaplan-Meier analysis). For the screening cohort, we considered as significant a *P* value of .05 with Bonferroni correction for the number of SNP markers tested. As the *P* value is not a good surrogate marker for effect size, and often small in HSCT-outcome association studies, we decided to separately include associations showing ORs of less than or equal to 0.5 and $\geq$ 2.0 (this follows observations of ORs of significant markers in previous studies).

Screening and confirmatory cohort data were analyzed on the overall cohort in the first instance. To reduce confounding by HLA mismatching, we conducted identical analyses on a subgroup with a higher degree of HLA matching (8 of 8 allele matching at the HLA A, B, C, DRB1 loci, with additional exclusion of combined HLA-DQB1 and DPB1 mismatches; allowing for either a HLA-DQB1 or a HLA-DPB1 mismatch only), similar to previous reports from JMDP,[5] resulting in cohorts of 160 (discovery) and 166 (confirmatory) pairs.

For the screening cohort, we would genotype all 41 chosen SNP markers (Table 1) on both donor and recipient cohorts and conduct overall and subgroup analyses. Markers only that show a corrected *P* value of less than .05 and/or an OR of less than or equal to 0.5 and more than or equal to 2.0 in either the overall or the subgroup analyses would be selected for confirmatory typing. If a marker showed an association that was persisting when applying Bonferroni correction, we tested other associations of the same marker in the confirmatory cohort, even if these would not reach the multiple testing thresholds, to capture borderline significance or effect size of genotypes, building on the strength of testing in an independent confirmatory cohort.

Given the high degree of linkage between the *CTLA4* as well as the *IL10* SNPs in the study, unambiguous haplotypes could be determined directly without recourse to computational methods.

As the distribution of acute GVHD degrees of severity was significantly different between the screening and confirmation cohort, all associations with acute GVHD as outcome were reanalyzed after randomizing the study population into 2 different cohorts (using an online based tool for random assignment: http://www1.assumption.edu/users/avadum/applets/RandAssign/GroupGen.html).

Multivariate analysis was performed on the combined cohorts using STATA Version 11.0. OR of acute GVHD for the selected SNP in multivariate analysis was estimated by a multivariate logistic regression analysis with the adjustment for recipient and donor ages, underlying diagnosis, the use of total body irradiation, antithymoglobulin, female donor into male transplant, GVHD prophylaxis (tacrolimus vs cyclosporin A), relapse, and HLA mismatch to address possible confounding.

Results

Screening cohort

All transplants (n = 460 pairs). In the screening cohort, involving 460 bone marrow transplants performed between 1993 and 2000, 41 single nucleotide SNP markers were typed in both patient and donor cohorts. Of these, 6 markers were excluded from analysis, for technical (multiple clusters: rs1927907, rs4803455) and statistical reasons (minor allele frequency $<$ 5%: rs1800795, rs6687620, rs361525, rs1800629). All 35 markers included in the analysis were in Hardy-Weinberg equilibrium (defined as $P > .05$, with statistical correction for the number of tested markers).

Thirteen markers, plus the *IL10* and *CTLA4* haplotypes, showed an association with an HSCT outcome in the donor screening cohort (Table 2). By significance testing applying Bonferroni correction, only the marker *IL10*-1082 and the *CTLA4* haplotype showed significant association, whereas 3 further markers were selected for confirmatory typing by their effect size (marker *CTLA4* rs231775 also shows relevant effect size individually; marker *CTLA4* rs231777, which showed no individual association, was

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Table 3. Significant results of SNP genotyping on all recipient samples

Gene	Marker	Discovery cohort (genotype and association)	Confirmatory cohort (genotype and association)
CTLA4	rs231775	AA cGVHD (*P* = .046, OR = 1.83, CI = 1.02-3.28)	NS
CTLA4	rs231777	Mismatch aGVHD (*P* = .004, OR = 1.91, CI = 1.24-2.96)	NS
CTLA4	haplotype	CAA cGVHD (*P* = .011, OR = 1.5, CI = 1.11-2.03)	NS
		CGG cGVHD* (*P* = .0013,* OR = 0.62, CI = 0.47-0.83)	NS
		CGG aGVHD2-4 (*P* = .019, OR = 0.70, CI = 0.52-0.94)	NS
		TAG aGVHD4* (*P* = .0071, OR = 3.71,* CI = 1.56-8.86)	NS
FAS	rs1800682	CC relapse (*P* = .017, OR = 1.68, CI = 1.03-2.74)	NS
		CT relapse* (*P* = .0025, OR = 0.50,* CI = 0.33-0.78)	NS
		CT aGVHD (*P* = .009, OR = 1.79, CI = 1.15-2.77)	NS
		TT cGVHD (*P* = .024, OR = 1.75, CI = 1.03-2.82)	NS
		TT ext cGVHD (*P* = .014. OR = 1.74, CI = 1.03-2.94)	NS
HLA-E	rs1264457	Mismatch survival (*P* = .023) risk	NT
IL1A	rs1800578	Mismatch aGVHD2-4 (*P* = .026, OR = 1.69, CI = 1.11-2.56)	NT
IL1B	rs16944	AA aGVHD (*P* = .048, OR = 0.63, CI = 0.39-0.99)	NT
		GG aGVHD (*P* = .032, OR = 1.75, CI = 1.08-2.82)	NT
IL15RA	rs2228059	AC survival (*P* = .024) risk	NT
IL2	rs2069762	GG aGVHD4* (*P* = .0014,* OR = 4.51,* CI = 1.91-10.6)	NS
		GT survival (*P* = .0021) protective	NS
		TT survival (*P* = .0061) risk	NS
NOD2	rs17313265	CC aGVHD2-4 (*P* = .036, OR = 2.15, CI = 1.06-4.37)	NS
TGFB1	rs1800469	Mismatch aGVHD2-4 (*P* = .02, OR = 1.63, CI = 1.1-6.4)	NT
TGFB1	rs2241715	Mismatch aGVHD2-4 (*P* = .015, OR = 1.61, CI = 1.09-2.39)	NT
		Mismatch cGVHD (*P* = .035, OR = 1.58, CI = 1.04-2.41)	NT
TGFB1	rs2241716	AA ext cGVHD* (*P* = .0041, OR = 2.58,* CI = 1.36-4.87)	NS
TNF	rs1799964	Mismatch aGVHD4*† (*P* = .022, OR = 2.53,*† CI = 1.16-5.53)	Mismatch aGVHD4*† (*P* = .0053, OR = 3.40,*† CI = 1.48-7.81)
		CC aGVHD4* (*P* = .041, OR = 4.92,* CI = 1.27-19.02)	CC aGVHD4 trend (*P* = .06)
TNF	rs1799724	CC survival (*P* = .02) protective,	NT
		CT survival (*P* = .02) risk	NT
TNFRSF1B	rs1061622	TT aGVHD4* (*P* = .023, OR = 4.69,* CI = 1.1-20.11)	NS

The marker rs3087243 was not associated individually with chronic GVHD (cGVHD) or acute GVHD (aGVHD) and is not listed here, but it was included in the confirmatory cohort forming part of the CTLA4 haplotype.

NS indicates not significant; and NT, not tested. For other abbreviations please see Table 2.

*Withstanding Bonferroni multiple testing corrections or have OR ≤ 0.5 or ≥ 2.

†Consistent associations.

included in the confirmatory cohort as part of the *CTLA4* haplotype, not listed in Table 2). The recipient cohort (Table 3) revealed 15 markers, plus the *CTLA4* haplotype, that were associated with a HSCT outcome. The *IL2*-330 SNP and the *CTLA4* haplotype revealed significant associations above the multiple testing thresholds, whereas 5 SNP markers had ORs ≤ 0.5 and ≥ 2.0.

HLA-matched subgroup (n = 160 pairs). When analyzing the HLA-matched subgroups of these cohorts, 7 markers and the *CTLA4* and *IL10* haplotypes in the donor cohort (Table 4) showed outcome associations, of which 5 markers and the *CTLA4* haplotype were included for confirmatory typing. Only the *CTLA4* haplotype had a *P* value significant when multiple testing correction was applied. In the HLA matched recipient subgroup, 3 markers showed an association with HSCT outcome, of which one was selected for the confirmation cohort by strength of OR (Table 5).

Confirmatory cohort

All transplants (n = 462 pairs). Seven markers for the donor cohort (*CTLA4*: rs231775, rs231777, rs3087243 [included for forming the *CTLA4* haplotype, only rs231775 and rs3087243 showed an association in the screening cohort]; *FAS*: rs1800682; *IL10*: rs1800896; *NOD2*: rs2111235, rs6500328) and 10 markers for the recipient cohort (*CTLA4*: rs231775, rs231777, rs3087243

Table 4. Results of SNP genotyping on HLA-matched donor samples

Gene	Marker	Discovery cohort (genotype and association)	Confirmatory cohort (genotype and association)
CTLA4	rs231775	GG aGVHD* (*P* = .026, OR = 2.02,* CI = 1.09-3.75)	NS
CTLA4	rs3087243	GG aGVHD (*P* = .021, OR = 1.97, CI = 1.11-3.50)	NS
CTLA4	Haplotype	CAA aGVHD (*P* = .012, OR = 0.55, CI = 0.35-0.87)	NS
		CGG aGVHD* (*P* = .00097,* OR = 2.06,* CI = 1.22-5.94)	NS
IFNg	rs2069705	CC ext cGVHD* (*P* = .036, OR = 0.42,* CI = 0.20-0.93)	NS
		CT ext cGVHD* (*P* = .017, OR = 2.69,* CI = 1.22-5.94)	NS
IL10	rs1800896	AA aGVHD* (*P* = .038, OR = 0.21,* CI = 0.04-0.96)	NS
IL10	Haplotype	CCG aGVHD* (*P* = .027, OR = 4.70, CI = 1.08-20.54)	NS
MTHFR	rs1801133	TT aGVHD (*P* = .0016, OR = 12.13,* CI = 2.73-53.90)	NT
NOD2	rs17313265	CT relapse* (*P* = .013, OR = 2.68,* CI = 1.02-7.09)	NS
TNF	rs1799724	CC survival (*P* = .006) protective	NT

NS indicates not significant; and NT, not tested. Explanation of other abbreviations found in Table 2.

*Withstanding Bonferroni multiple testing corrections or have OR ≤ 0.5 or ≥ 2.

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Table 5. Results of SNP genotyping on HLA-matched recipient samples

Gene	Marker	Discovery cohort (genotype and association)	Confirmatory cohort (genotype and association)
FAS	rs1800682	CT aGVHD* (P = .0024, OR = 0.39,* CI = 0.22-0.71)	NS
IL1B	rs16944	AA aGVHD (P = .043, OR = 0.51, CI = 0.27-0.97)	NT
IL2	rs2069762	GT survival (P = .037) protective	NS
		GT cGVHD (P = .039, OR = 1.97, CI = 1.05-3.71)	GT cGVHD*† (P = .00041,*† OR = 3.24,*† CI = 1.69-6.20)
		TT survival (P = .039) risk	NS

NS indicates not significant; and NT, not tested.
*Withstanding Bonferroni multiple testing corrections or have OR ≤ 0.5 or ≥ 2.
†Consistent associations.

[part of *CTLA4* haplotype, only rs231775 and rs231777 were associated in the screening cohort]; *FAS*: rs1800682; *IL2*: rs2069762; *NOD2*: 17313265; *TGFB1*: rs2241716; *TNF*: rs1799964; *TNFRSF1B*: rs1061622) were selected for typing in the confirmatory cohort. First, we were seeking to confirm associations from the screening cohorts that had significant P values after multiple testing correction (high significance); then, associations that had ORs ≤ 0.5 or ≥ 2.0 (large effect size); and third, associations within these selected markers that were consistent in both screening and confirmatory cohort (independent cohort confirmation), regardless of multiple testing correction or effect size.

There were no consistent findings in the overall donor confirmatory cohort (Table 2). In the overall recipient confirmatory cohort (Table 3), the donor-recipient genotype mismatch of the *TNF*-1031 SNP (rs1799964) was consistently associated in both screening and confirmatory cohorts with a higher risk of severe acute GVHD (grade 4). The CC genotype of the same marker was associated with acute GVHD grade 4 in the screening cohort and just escaped significance level in the confirmatory cohort (P = .06).

HLA-matched subgroups (166 pairs). In the donor HLA-matched subgroup (Table 4), none of the markers typed in the confirmatory cohort showed any association. The HLA-matched recipient cohort (Table 5) revealed a consistent association between risk of chronic GVHD and the GT genotype of rs2069762 (*IL2*-330).

Table 6 summarizes the consistent associations of this study, composed of the *IL2*-330 and *TNF*-1031 SNP.

Further analyses

To understand the mechanism of the associated genotype, we extended the analysis to all *IL2*-330 genotypes and chronic GVHD outcomes in the confirmatory cohort and found that GT also associated with extensive chronic GVHD (P = .00022, OR = 5.18, 95% CI, 2.37-11.39). The TT genotype exerts a protective effect against extensive chronic GVHD (P = .0029, OR = 0.3, 95% CI, 0.13-0.67). This finding is replicated when combining screening and confirmatory cohorts (GT and extensive chronic GVHD: P = .00055, OR = 2.90, 95% CI, 1.74-5.08; TT and extensive chronic GVHD: P = .001, OR = 0.40, 95% CI, 0.23-0.71), suggesting that the GG genotype is probably the higher risk genotype. We did not find a significant association with the GG genotype, which is probably because of limited statistical power of this low frequency genotype. Mirroring the analysis by MacMillan et al[32] in our combined cohorts, the G allele showed a trend with risk of extensive chronic GVHD (P = .07), but not with acute GVHD.

The extended analysis of the *TNF*-1031 CC genotype in the confirmatory cohort showed that it was also associated with acute GVHD grade 2 to 4 (P = .029, OR = 3.41, 95% CI, 1.99-5.82). The *TNF*-1031 donor-recipient genotype mismatch was found to be a risk factor for acute GVHD grade 2 to 4 (P = .003, OR = 1.93, 95% CI, 1.13-3.30) and grade 3 or 4 (P = .002, OR = 2.21, 95% CI, 1.13-3.80) in the confirmatory cohort.

The stratification we applied in "matching" the degree of HLA mismatch of the confirmatory cohort to that of the screening cohort may have introduced bias (significantly different distribution of acute GVHD grades; supplemental Table 1). To address this, we randomly assigned samples to 2 cohorts, resolving any significant difference between time frames, and acute GVHD as an outcome measure. Reanalysis of the data for acute GVHD outcomes showed that the genotype mismatch of the *TNF*-1031 SNP as a risk factor for acute GVHD grade 4 would still hold up as significant (P = .005, OR = 3.26, 95% CI, 1.91-5.58; P = .021, OR = 2.60, 95% CI, 1.52-4.45). The *CTLA4*-CT60 (rs3087243) SNP showed a consistent association of the GG genotype as protective against acute GVHD (P = .022, OR = 0.46, 95% CI, 0.27-0.78; P = .045, OR = 0.49, 95% CI, 0.29-0.83) in the random cohort analysis of the HLA-matched subgroup.

Multivariate analyses

Multivariate analyses (Tables 7-9) were performed on the combined (screening and confirmatory) cohorts and showed that the *TNF*-1031 donor-recipient genotype mismatch (acute GVHD grade 4), the CC genotype (acute GVHD grade 4), and the *IL2*-330 GT genotype (chronic GVHD) are independent risk factors, whereas the *CTLA4*-CT60 GG genotype is independently protective against acute GVHD.

Table 6. SNP markers showing significant association in recipient screening and cohorts

Marker	Genotype	Cohort	Outcome	P	Total	Cases, all	Controls, all	Cases positive	Cases negative	Controls positive	Controls negative	OR	OR (95% CI)
TNF-1031	Mismatch	Screening	aGVHD4	.022	448	28	420	12	16	96	324	2.53	1.16-5.53
rs1799964, recipients (all)	Mismatch	Confirmation	aGVHD4	.0053	460	24	436	12	12	99	337	3.40	1.48-7.81
IL2-330	GT	Screening	cGVHD	.039	160	72	88	39	33	33	55	1.97	1.05-3.71
rs2069762, recipients (HLA matched)	GT	Confirmation	cGVHD	.00041	166	75	92	40	35	23	68	3.24	1.70-6.20
CTLA4-CT60	GG	Random 1	aGVHD	.022	159	58	101	20	38	54	47	0.46	0.27-0.78
rs3087243, donors (HLA matched)	GG	Random 2	aGVHD	.045	166	53	11	22	31	67	46	0.49	0.29-0.83

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Table 7. Multivariate analysis of the IL2–330 GT genotype as risk factor for chronic GVHD in the HLA-matched subgroup

	Univariate		Multivariate	
Variable	OR (95% CI)	P	OR (95% CI)	P
Recipient age	1.008 (0.99-1.03)	.481	1.008 (0.98-1.03)	.528
Donor age	1.024 (0.99-1.05)	.106	1.020 (0.99-1.05)	.195
Female to male transplant	0.900 (0.52-1.57)	.71	0.876 (0.48-1.60)	.664
Diagnosis ANLL vs ALL	1.087 (0.70-1.69)	.711	1.022 (0.63-1.67)	.929
Total body irradiation	1.419 (0.72-2.80)	.313	1.284 (0.62-2.67)	.502
Cyclosporine vs tacrolimus	1.024 (0.66-1.59)	.916	0.996 (0.61-1.62)	.987
Relapse	0.526 (0.32-0.86)	.011	0.573 (0.34-0.96)	.033
Genotype GT	2.507 (1.60-3.93)	.000066	2.273 (1.42-3.63)	.0006

The genotype is an independent risk factor.

Discussion

This study has identified 3 consistent non-HLA SNP associations with HSCT outcome: the *TNF*-1031 donor-recipient genotype mismatch with severe GVHD (grade 4, in the overall cohort), the recipient *IL2*-330 GT genotype with risk of chronic GVHD, and the *CTLA4*-CT60 GG genotype protective against acute GVHD (grade 1-4; the latter 2 associations were found in the HLA-matched subgroup only).

TNF-α is a cytokine that has been associated with severity of acute GVHD in several previous genetic, gene expression, and animal model studies. Teshima et al have demonstrated in an animal model that TNF is essential in the development of acute GVHD.[13] Previous data from a Japanese population have shown that the *TNF* haplotype, including *TNF*-1031, was associated with severe GVHD,[33] and the *TNF*-1031C allele was associated with higher TNF expression.[34] A more recent study[35] describes the C allele as a risk factor for grade 3 or 4 acute GVHD. Therefore, an association of the *TNF*-1031 CC genotype with severe acute GVHD, as seen in this study, albeit showing only a trend in the confirmation cohort, would be biologically meaningful and replicate previous findings. However, the *TNF*-1031 CC genotype displays strong linkage disequilibrium with HLA, in particular with HLA-B61.[34] This may explain our finding of the strong association between donor-recipient genotype mismatch and acute GVHD grade 4 in the overall cohort only, but not in the HLA matched subgroup. Our study did not have the power to elucidate whether any particular *TNF*-1031 genotype mismatch combinations carry a higher risk. As the group affected with acute GVHD grade 4 is small (just $> 5\%$), further studies should confirm this result independently. The finding that genotype mismatch was also associated with grade 2 to 4 as well as grade 3 or 4 acute GVHD (which are larger groups) in the confirmatory cohort gives further indication that the genotype mismatch is probably a risk factor for acute GVHD. Nevertheless, the strength and consistency of this association mean that it is potentially a strong discriminator for prediction of the most severe form of acute GVHD (grade 4), which could be exploited in clinical practice.

The *IL2*-330 (rs2069762) SNP has an almost identical genotype distribution between white and Japanese populations (white: TT, 0.536; GT, 0.464; GG, 0; Japanese [this study]: TT, 0.450; GT, 0.440; GG, 0.110). The G allele is the known high-expressing allele, and high levels of IL2 have been described to correlate with severity of acute GVHD.[32,36] A previous study from North America on a cohort of similar time frame to our screening cohort[32] reported an association between the recipient *IL2*-330 G allele and acute GVHD as well as a trend toward risk of chronic GVHD. In our study, we found an association of the GT genotype with risk of chronic GVHD. More detailed analysis showed that the low-frequency GG genotype is probably the highest risk genotype for chronic GVHD, whereas GT associated with risk, and TT with protection. Our findings therefore confirm those of the previous study, even across different ethnic populations, qualifying this marker as a predictor of chronic GVHD risk.

The effect of the *CTLA4*-CT60 polymorphism on HSCT outcomes was studied previously, in settings of HLA matched sibling donors[37,38] and matched unrelated donors[39] in white populations. In HLA-matched sibling transplants, the donor G allele was associated with increase of relapse and worse survival, whereas the AA genotype was linked to risk of acute GVHD. The findings in matched unrelated donor HSCT were similar, with the donor AA genotype associating with severe acute GVHD (grade 3 or 4), but risk of G allele or GG genotype with relapse or survival was not observed. Our findings are in accordance with these results, identifying the GG genotype as protective against acute GVHD (remarkably, the screening cohort result indicated a risk of the GG genotype with acute GVHD [Table 4], a finding completely reversed by the randomization). We could not establish any risk of the GG genotype with relapse or survival, or the AA genotype with acute GVHD. This may be explained by the fact that, in the

Table 8. Multivariate analysis of the CTLA4-CT60 GG genotype for acute GVHD (grade 1-4 vs no GVHD) in the HLA-matched subgroup, confirming this genotype as an independent risk factor

	Univariate		Multivariate	
Variable	OR (95% CI)	P	OR (95% CI)	P
Recipient age	1.017 (0.99-1.04)	.146	1.020 (0.99-1.05)	.121
Donor age	0.995 (0.97-1.03)	.763	0.997 (0.97-1.03)	.854
Female to male transplant	1.644 (0.93-2.89)	.085	1.630 (0.89-2.97)	.111
Diagnosis ANLL vs ALL	1.280 (0.81-2.03)	.296	1.129 (0.69-1.85)	.631
Total body irradiation	0.847 (0.43-1.68)	.634	0.916 (0.45-1.86)	.809
Relapse	1.255 (0.77-2.06)	.369	1.330 (0.80-2.24)	.273
Genotype GG	0.468 (0.29-0.75)	.002	0.497 (0.31-0.80)	.004

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Table 9. Multivariate analysis of TNF-1031 genotype mismatch and CC genotype as a risk factors* for acute GVHD grade 4 in the overall (HLA matched and mismatched) cohort

	Univariate		Multivariate	
Variable	OR (95% CI)	P	OR (95% CI)	P
Recipient age	0.978 (0.95-1.01)	.109	0.975 (0.94-1.01)	.112
Donor age	1.038 (1.00-1.08)	.044	1.033 (0.99-1.07)	.105
Female to male transplant	0.610 (0.27-1.38)	.235	0.582 (0.24-1.42)	.236
Diagnosis ANLL vs ALL	1.001 (0.57-1.76)	.996	1.148 (0.60-2.18)	.673
Total body irradiation	0.909 (0.40-2.07)	.819	0.992 (0.39-2.51)	.987
Antithymoglobulin	3.562 (0.99-12.73)	.051	2.246 (0.45-11.15)	.322
Cyclosporine vs tacrolimus	1.336 (0.75-2.37)	.321	1.516 (0.80-2.86)	.198
Relapse	0.115 (0.03-0.48)	.003	0.154 (0.04-0.65)	.011
HLA match	0.465 (0.24-0.92)	.027	0.765 (0.35-1.67)	.502
Genotype CC	4.336 (1.7-11.1)	.002	3.888 (1.39-10.90)	.010
Genotype mismatch	2.905 (1.65-5.1)	.00023	2.307 (1.18-4.52)	.015

*Both are independent risk factors, with competing effects from HLA matching and relapse.

Japanese population, the GG genotype is more prominent than in whites, whereas the AA genotype is more rare (HapMap data of genotypes: whites: AA, 0.208; AG, 0.513; GG, 0.283; Japanese: AA, 0.047; AG, 0.389; GG, 0.542). The risk of acute GVHD, relapse, or survival associated with this marker may therefore be lower in the Japanese population, compared with whites.

The results raise also some methodologic questions which are beyond the scope of this study: (1) By incorporating a measure of effect size into the statistical analysis, this study extends beyond previous approaches focusing on significance and correction for multiple testing. Our results suggest that this approach may be more sensitive; but because of limited power and small number of identified associations, no conclusions could be made about the impact on sensitivity and specificity, and statistical multiple testing burden. (2) Despite the effort to control variability of study population characteristics, reproducibility of associations remains low and appeared to be dependent on distribution of these characteristics among the cohorts. This may be the result of the overall small effect size of the associations, confounders in the study cohort, or both. A more comprehensive typing (full typing of all markers on both screening and confirmation cohort) and analysis would be required.

Clinical and population characteristics of study cohorts may explain some of the contradictory results observed in previous studies; therefore, careful design of study cohorts and control of confounders should receive more attention. The growing number of HSCTs may facilitate in the future the availability of larger, genetically and clinically more homogeneous study cohorts; however, the changing and expanding indications of HSCT are likely to prove a challenge.

In conclusion, this study demonstrates that non-HLA genetic association with HSCT outcomes does exist and can be detected, even in the HLA-mismatched setting. Such associations could be useful for application in future clinical practice in this clinically highly relevant population. These findings should be verified by larger studies also on populations of different ethnicities.

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Authorship

Contribution: C.H. designed and coordinated the project, carried out the experiments and univariate data analyses, and wrote the manuscript; A.O. designed the study and the experiment and provided technical advice; M.O., H.I., A.R.G., and K.A. designed the study; P.G.M. designed the study and experiment and inferred the CTLA4 haplotypes; K.K., K.H., and T.Y. performed the IL-10 SNP genotyping and haplotype inference; H.N. gave statistical advice and performed multivariate analyses; and Y.M. designed the study and acted as liaison to JMDP, providing clinical datasets and DNA samples.

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References

1. Hansen JA, Petersdorf EW, Lin MT, et al. Genetics of allogeneic hematopoietic cell transplantation: role of HLA matching, functional variation in immune response genes. *Immunol Res.* 2008; 41(1):56-78.
2. Hsu KC, Chida S, Geraghty DE, Dupont B. The killer cell immunoglobulin-like receptor (KIR) genomic region: gene-order, haplotypes and allelic polymorphism. *Immunol Rev.* 2002;190:40-52.
3. Yabe T, Matsuo K, Hirayasu K, et al. Donor killer immunoglobulin-like receptor (KIR) genotype-patient cognate KIR ligand combination and antithymocyte globulin preadministration are critical factors in outcome of HLA-C-KIR ligand-mismatched T cell-replete unrelated bone marrow transplantation. *Biol Blood Marrow Transplant.* 2008;14(1):75-87.
4. Kawase T, Nanya Y, Torikai H, et al. Identification of human minor histocompatibility antigens based on genetic association with highly parallel genotyping of pooled DNA. *Blood.* 2008;110(6):3286-3294.
5. Ogawa S, Matsubara A, Onizuka M, et al. Exploration of the genetic basis of GVHD by genetic

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association studies. *Biol Blood Marrow Transplant.* 2008;15(1 Suppl):39-41.

6. Hansen JA, Chien JW, Warren EH, Zhao LP, Martin PJ. Defining genetic risk for graft-versus-host disease and mortality following allogeneic hematopoietic stem cell transplantation. *Curr Opin Hematol.* 2010;17(6):483-492.
7. Choi SW, Levine JE, Ferrara JL. Pathogenesis and management of graft-versus-host disease. *Immunol Allergy Clin North Am.* 2010;30(1):75-101.
8. Billingham RE. The biology of graft-versus-host reactions. *Harvey Lect.* 1966;62:21-78.
9. Martín-Antonio B, Granell M, Urbano-Ispizua Á. Genomic polymorphisms of the innate immune system and allogeneic stem cell transplantation. *Expert Rev Hematol.* 2010;3(4):411-427.
10. Weissinger EM, Dickinson AM. Immunogenomics and proteomics in hematopoietic stem cell transplantation: predicting post-hematopoietic stem cell transplant complications. *Cancer Treat Res.* 2009;144:95-129.
11. Ferrara JL, Krenger W. Graft-versus-host disease: the influence of type 1 and type 2 T cell cytokines. *Transfus Med Rev.* 1998;12(1):1-17.
12. Socié G. Graft-versus-host disease: proteomics comes of age. *Blood.* 2009;113(2):271-272.
13. Teshima T, Ordemann R, Reddy P, et al. Acute graft-versus-host disease does not require alloantigen expression on host epithelium. *Nat Med.* 2002;8(6):575-581.
14. Buzzeo MP, Yang J, Casella G, Reddy V. A preliminary gene expression profile of acute graft-versus-host disease. *Cell Transplant.* 2008;17(5):489-494.
15. Paczesny S, Krijanovski OI, Braun TM, et al. A biomarker panel for acute graft-versus-host disease. *Blood.* 2009;113(2):273-278.
16. Kollman C, Howe CW, Anasetti C, et al. Donor characteristics as risk factors in recipients after transplantation of bone marrow from unrelated donors: the effect of donor age. *Blood.* 2001;98(7):2043-2051.
17. Loren AW, Bunin GR, Boudreau C, et al. Impact of donor and recipient sex and parity on outcomes of HLA-identical sibling allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant.* 2006;12(7):758-769.
18. Randolph SS, Gooley TA, Warren EH, Appelbaum FR, Riddell SR. Female donors contribute to a selective graft-versus-leukemia effect in male recipients of HLA-matched, related hematopoietic stem cell transplants. *Blood.* 2004;103(1):347-352.
19. Pérez-Simón JA, Diez-Campelo M, Martino R, et al. Influence of the intensity of the conditioning regimen on the characteristics of acute and chronic graft-versus-host disease after allogeneic transplantation. *Br J Haematol.* 2005;130(3):394-403.
20. Colhoun HM, McKeigue PM, Davey Smith G. Problems of reporting genetic associations with complex outcomes. *Lancet.* 2003;361(9360):865-872.
21. Gambaro G, Anglani F, D'Angelo A. Association studies of genetic polymorphisms and complex disease. *Lancet.* 2000;355(9200):308-311.
22. Hirschhorn JN, Lohmueller K, Byrne E, Hirschhorn K. A comprehensive review of genetic association studies. *Genet Med.* 2002;4(2):45-61.
23. Lander ES, Schork NJ. Genetic dissection of complex traits. *Science.* 1994;265(5181):2037-2048.
24. Schork NJ. Genetics of complex disease: approaches, problems, and solutions. *Am J Respir Crit Care Med.* 1997;156(4):S103-S109.
25. Sasazuki T, Juji T, Morishima Y, et al. Effect of matching of class I HLA alleles on clinical outcome after transplantation of hematopoietic stem cells from an unrelated donor: Japan Marrow Donor Program. *N Engl J Med.* 1998;339(17):1177-1185.
26. Morishima Y, Sasazuki T, Inoko H, et al. The clinical significance of human leukocyte antigen (HLA) allele compatibility in patients receiving a marrow transplant from serologically HLA-A, HLA-B, and HLA-DR matched unrelated donors. *Blood.* 2002;99(11):4200-4206.
27. Weisdorf D, Spellman S, Haagenson M, et al. Classification of HLA-matching for retrospective analysis of unrelated donor transplantation: revised definitions to predict survival. *Biol Blood Marrow Transplant.* 2008;14(7):748-758.
28. Morishima Y, Yabe T, Matsuo K, et al. Effects of HLA allele and killer immunoglobulin-like receptor ligand matching on clinical outcome in leukemia patients undergoing transplantation with T-cell-replete marrow from an unrelated donor. *Biol Blood Marrow Transplant.* 2007;13(3):315-328.
29. Kawase T, Morishima Y, Matsuo K, et al. High-risk HLA allele mismatch combinations responsible for severe acute graft versus host disease and implication for its molecular mechanism. *Blood.* 2007;110(7):2235-2241.
30. Kawase T, Matsuo K, Kashiwase K, et al. HLA mismatch combinations associated with decreased risk of relapse: implications for the molecular mechanism. *Blood.* 2009;113(12):2851-2858.
31. Morishima S, Ogawa S, Matsubara A, et al. Impact of highly conserved HLA haplotype on acute graft-versus-host disease. *Blood.* 2010;115(23):4664-4670.
32. MacMillan ML, Radloff GA, Kiffmeyer WR, DeFor TE, Weisdorf DJ, Davies SM. High-producer interleukin-2 genotype increases risk for acute graft-versus-host disease after unrelated donor bone marrow transplantation. *Transplantation.* 2003;76(12):1758-1762.
33. Ishikawa Y, Kashiwase K, Akaza T, et al. Polymorphisms in TNFA and TNFR2 affect outcome of unrelated bone marrow transplantation. *Bone Marrow Transplant.* 2002;29(7):569-575.
34. Higuchi T, Seki N, Kamizono S, et al. Polymorphism of the 5′-flanking region of the human tumor necrosis factor (TNF)-alpha gene in Japanese. *Tissue Antigens.* 1998;51(6):605-612.
35. Goyal RK, Lin Y, Schultz KR, et al. Tumor necrosis factor-alpha gene polymorphisms are associated with severity of acute graft-versus-host disease following matched unrelated donor bone marrow transplantation in children: a Pediatric Blood and Marrow Transplant Consortium study. *Biol Blood Marrow Transplant.* 2010;16(7):927-936.
36. Das H, Imoto S, Murayama T, et al. Kinetic analysis of cytokine gene expression in patients with GVHD after donor lymphocyte infusion. *Bone Marrow Transplant.* 2001;27(4):373-380.
37. Pérez-Garcia A, De la Camara R, Roman-Gomez J, et al. CTLA-4 polymorphisms and clinical outcome after allogeneic stem cell transplantation from HLA-identical sibling donors. *Blood.* 2007;110(1):461-467.
38. Murase M, Nishida T, Onizuka M, et al. Cytotoxic T-lymphocyte antigen 4 haplotype correlates with relapse and survival after allogeneic hematopoietic SCT. *Bone Marrow Transplant.* 2011;46(11):1444-1449.
39. Vannucchi AM, Guidi S, Guglielmelli P, et al. Significance of CTLA-4 and CD14 genetic polymorphisms in clinical outcome after allogeneic stem cell transplantation. *Bone Marrow Transplant.* 2007;40(10):1001-1002.

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