BIOLOGY

Polymorphisms in CCR6 Are Associated with Chronic Graft-versus-Host Disease and Invasive Fungal Disease in Matched-Related Hematopoietic Stem Cell Transplantation

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Graft-versus-host disease (GVHD) and fungal infections are frequent complications after allogeneic hematopoietic stem cell transplantation (HSCT). Single nucleotide polymorphisms (SNPs) in genes of the immune system can influence the inflammatory cascade and T cell–driven alloimmune reactions after HSCT, and thus increasing the incidence of GVHD and infectious complications. Here, we investigated the effect of SNPs in IL-23R and CCR6 on posttransplantation outcome in 161 recipients of partially T cell–depleted HSCT. Remarkably, IL-23R SNPs were not associated with clinical outcome, but we found that disparities in the CCR6 tagSNP rs2301436 and SNP rs3093023 are independently associated with the occurrence of chronic GVHD (cGVHD) and invasive fungal disease. In multivariate analysis, patients receiving a transplant from a homozygous rs2301436 G allele donor showed less cGVHD (odds ratio [OR]: 0.16; $P = .002$), as was the case for a homozygous donor rs3093023 G allele (OR: 0.24; $P = .005$). In parallel, the GG genotype at rs2301436 in donors was associated with a higher incidence of invasive fungal disease at day 100 after HSCT (OR: 3.59; $P = .008$). This study shows that CCR6 SNPs can be used to predict clinical outcome, and that polymorphisms in the CCR6 gene may influence T cell–mediated immune reactions after HSCT.

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INTRODUCTION

Graft-versus-host disease (GVHD) and fungal infections remain major causes of morbidity and mortality in allogeneic hematopoietic stem cell transplantation (HSCT) [1]. The pathophysiology of acute GVHD (aGVHD) is a multistep process involving tissue damage and cytokine release induced by pretransplantation conditioning followed by alloreactive T cell expansion, trafficking, and destruction of target tissues by effector T cells [2]. The pathogenesis of chronic GVHD (cGVHD) is even more complex and has not yet been fully defined [3,4]. Recent insights have challenged the paradigm of GVHD being almost exclusively a Th1-mediated immune disorder, given that studies have shown a role for Th17 responses in both aGVHD and cGVHD. Similarly, new insights have emerged in the field of antifungal immunity showing a pronounced role of Th17 responses, including release of the cytokines interleukin (IL)-17 and IL-22, in fungal defenses complementing Th1 responses.

The IL-23/Th17 pathway is a new player at the crossroads of innate and adaptive immunity [5]. Th17 cells are induced by the release of IL-1β, IL-6, and transforming growth factor β from antigen-presenting cells (APCs), and further expansion is enhanced by IL-23, although controversy exists because of the considerable differences seen in animal and human studies [6]. Th17 $CD4^+$ T cells are characterized by expression of retinoid-related orphan receptor and the chemokine receptors CCR4 and CCR6 [7]. CCR6 expression recently has been shown to be pivotal for Th17 migration to the gut in inflammatory bowel disease and to the joints in rheumatoid arthritis

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[8]. Th17 responses seem to be involved predominantly in host barrier defenses of the skin and mucosa [9], enhancing the innate immunity against extracellular microorganisms, including fungi, through increased phagocyte recruitment and production of antimicrobial peptides [10]. However, this pathway is also reportedly involved in the occurrence of various autoinflammatory and autoimmune diseases [5,11]. Therefore, the IL-23/Th17 axis is now being studied with regard to transplantation complications, with an emphasis on GVHD and infections, although the existence of alloreactive Th17 cells also suggests a possible role in graft-versus-tumor immunity [12-14]. However, most studies on the IL-23/Th17 axis in GVHD have been performed in animal models [15,16], and the role of this axis in human GVHD remains controversial.

Currently, the strongest evidence for a role of the IL-23/Th17 pathway in human HSCT comes from the association between a nonsynonymous *IL-23* receptor (*IL-23R*) single nucleotide polymorphism (SNP), rs11209026 (Arg381Gln), in transplant donors and the incidence of aGVHD [17-19]. This SNP is one of the now many so-called non-HLA polymorphisms that have been associated with HSCT outcome [20]. Previously, we reported that performing partial T cell depletion of donor transplants did not reduce the impact of nucleotide-binding oligomerization domain SNPs on GVHD and treatment-related mortality (TRM) in the setting of HLA-matched sibling HSCT [21]. To investigate whether SNPs in genes more directly involved in T cell–mediated immunity, including those involved in the IL-23/Th17 pathway, are more influenced by the T cell depletion strategy, we performed a retrospective analysis on the impact of *IL-23R* polymorphisms (rs11209026 and rs11805303) in a homogenous cohort of Dutch patients (n = 161) undergoing partially T cell–depleted HSCT. In addition, we studied the impact of a tagSNP for *CCR6* (rs2301436) and the *CCR6* SNP rs3093023, given that these SNPs have been associated with the occurrence of Crohn's disease and rheumatoid arthritis [22,23].

We found that the *IL-23R* SNPs were not associated with the occurrence of GVHD, but that *CCR6* tagSNP rs2301436 and SNP rs3093023 were significantly associated with less cGVHD. In addition, *CCR6* tagSNP rs2301436 also was associated with a higher incidence of invasive fungal disease (IFD) after partially T cell–depleted HSCT.

PATIENTS AND METHODS

Patients and Donors

A total of 161 Dutch patients and their donors were included in the study. All of the patients had been admitted to our transplantation unit between 1996 and 2009 for an HLA-matched sibling, partially T cell–depleted allogeneic HSCT. To obtain the most homogenous cohort possible, we selected only those patients who received myeloablative conditioning. Of the 161 patients, 124 had received a conditioning regimen containing idarubicin. The characteristics of patients, donors, and HSCT procedures are summarized in Table 1. All patients and donors provided informed consent for the prospective collection of data and DNA samples for investigational use. The study was approved by the Radboud University Nijmegen Medical Centre's Institutional Review Board.

Table 1. Patient, Donor, and Transplant Characteristics

Characteristic	
Age at transplantation, years, median (range)	48 (19-64)
Male sex, n (%)	104 (65)
Sex of patient–donor pair, n (%)	
Male–female	42 (26)
Other	119 (74)
Underlying disease, n (%)	
AML/MDS	88 (55)
ALL	18 (11)
CML/MPS	29 (18)
NHL/CLL	26 (16)
Advanced disease stage, n (%)	46 (29)
Conditioning regimen, n (%)	
Cy-Bus	8 (5)
Cy-TBI	29 (18)
Ida-Cy-Bus	16 (10)
Ida-Cy-TBI	108 (67)
Stem cell source, n (%)	
Bone marrow	62 (39)
Peripheral blood	99 (61)
T cell depletion method, n (%)	
Elutriation	44 (27)
CD34 selection	85 (53)
CD3/CD19 depletion	32 (20)
aGVHD, n (%)	
Grade II-IV	57 (35)
Grade III-IV	21 (13)
cGVHD, n (%)	n = 142
Limited	16 (10)
Extensive	25 (16)
Invasive fungal disease at day 100, n (%)	
Candidemia	16 (10)
Invasive mold disease*	12 (7.5)
Probable/proven	8 (5)

ALL indicates acute lymphocytic leukemia; AML, acute myeloid leukemia; Bus, busulfan; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; Cy, cyclophosphamide; Ida, idarubicin; MDS, myelodysplastic syndrome; MPS, myeloproliferative syndrome; NHL, non-Hodgkin lymphoma; TBI, total body irradiation.

*Based on European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and National Institute of Allergy and Infectious Diseases Mycoses Study Group consensus criteria [27].

Treatment Protocol

All patients had been treated according to the same protocol, as described previously [21]. In brief, the conditioning regimen consisted of cyclophosphamide (60 mg/kg for 2 days) in combination with either total body irradiation (4.5 Gy for 2 days) or busulfan (4 mg/kg for 4 days). Idarubicin (42 mg/m^2 in 48 hours) was commonly added to these conditioning regimens

Table 2. Primer Sequences for KASPar Genotyping Assays

SNP	Gene	Allele	Forward Primer (5′ to 3′)	Reverse Primer (5′ to 3′)
rs11209026	*IL-23R*	a	gaaggtgaccaagttcatgctttgattgggatatttaacagatcattcc	gtctaaatcagaaaacagaaattctgcaaa
		g	gaaggtcggagtcaacggattgggatatttaacagatcattccg	
rs11805303	*IL-23R*	c	gaaggtgaccaagttcatgcttgcaaacagagaactgtttcctc	gtcggagctttgtctatttagcaactaat
		t	gaaggtcggagtcaacggattgcttgcaaacagagaactgtttcctt	
rs2301436	*tagSNP CCR6*	t	gaaggtgaccaagttcatgctctgggtaatggaaaagggcttct	tctgtttgatataaactaaattgacctctt
		c	gaaggtcggagtcaacggattctgggtaatggaaaagggcttcc	
rs3093023	*CCR6*	a	tattgaaacttcctcaaatttaaaatcacat	tttatgcacctcacagtgtctatgcaaat
		g	attgaaacttcctcaaatttaaaatcacac	

to reduce the risk of relapse in the setting of partially T cell–depleted HSCT [24]. On day 0, all patients received a stem cell graft containing a median of 3.4×10^6 $CD34^+$ cells/kg (range, 0.8-11.6 $\times$ 10^6; bone marrow or peripheral blood stem cells) and a median of 0.5×10^6 $CD3^+$ T cells/kg (range, 0.3-0.8 $\times$ 10^6) achieved by dosed T cell add-back. GVHD prophylaxis consisted of cyclosporine A only. Antimicrobial prophylaxis consisted of ciprofloxacin 500 mg twice daily and valaciclovir 500 mg 3 times daily. Fluconazole (200 mg/day) was given orally only to those patients considered colonized by *Candida albicans*. No mold-active prophylaxis was provided.

Definition of Outcome Variables

aGVHD was graded according to the criteria of Przepiorka et al. [25], and cGVHD was classified according to the revised Seattle criteria of Lee et al. [3]. IFD was scored according to the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group consensus guidelines [26], but only probable and proven cases were included in the analysis. Blood cultures were considered positive if a microorganism was recovered from 1 or more bottles, with the exception of coagulase-negative staphylococci, for which 2 separate positive blood cultures with the same strain were required [27]. Early Gram-positive bacteremia was defined as bacteremia with streptococci or staphylococci occurring between the start of conditioning up to engraftment.

TRM, disease-free survival (DFS), and overall survival (OS) were defined according to standard criteria.

SNP Genotyping Using the KASPar System

Genotyping was performed for SNPs rs11209026 (Arg381Gln) and rs11805303, which are polymorphisms in the *IL-23R* gene in linkage disequilibrium with each other [28]. In addition, a tagSNP for *CCR6*, rs2301436, was genotyped. This SNP is located in the *FGFR1OP* gene, adjacent to *CCR6*, and has been associated with Crohn's disease [23]. SNP rs3093023 is located within the *CCR6* gene and has been associated with rheumatoid arthritis [22]. The HLA-matched HSCT donor–recipient pairs were genotyped by specific KASPar assays (KBioscience, Hoddesdon, United Kingdom), which are fluorescence-based competitive allele-specific polymerase chain reaction assays using nonlabeled primers. Details of the methodology are available at http://www.kbioscience.co.uk. Primer sequences are listed in Table 2.

Statistical Analysis

Associations between polymorphisms in recipient and donor on the one hand and occurrence of aGVHD, cGVHD, relapse, and IFD before day 100 after HSCT on the other hand were tested using the chi-square test and the Fisher exact test, as appropriate, and by univariate logistic regression analysis. To control for possible confounders (eg, aGVHD and sex combination), multivariate logistic regression analyses was performed. TRM, OS, and time of occurrence of IFD were analyzed using Kaplan-Meier curves and the log-rank test.

All statistical analyses were performed using the cmprsk package of open-source language R version 2.6.2 (www.r-project.org, R Foundation, Vienna, Austria). A P value $<.05$ was considered statistically significant.

RESULTS

SNP Genotype Frequencies

The genotype frequency of the rs11209026, rs11805303, rs2301436, and rs3093023 polymorphisms in both patients and donors are presented in Table 3. For the *IL-23R* SNP rs11209026 (ie, Arg381Gln), virtually no difference was seen between the GA and GG genotype frequency in donors and patients. A homogenous AA gene variant of rs11209026 was not detected in any of the donors or patients. Genotype frequencies for *IL-23R* SNP rs11805303, *CCR6* tagSNP rs2301436, and *CCR6* SNP rs3093023 showed equal distribution in donors and patients.

SNPs in IL-23R and CCR6 Are Not Associated with the Incidence of aGVHD

The cumulative incidence of aGVHD grade II-IV was 35% (57 of 161), with grade III-IV occurring in 13% (21 of 161) (Table 1). *IL-23R* SNPs rs11209026 and rs11805303 and *CCR6* SNPs rs2301436 and rs3093023 were not associated with the incidence of

Table 3. SNP Genotype Frequencies in Patients and Donors

Variable	Number Evaluable		n (%)	
	Patient	Donor	Patient	Donor
rs11209026 (IL-23R)	160	158		
GG			140 (87.5)	140 (88.5)
GA			20 (12.5)	18 (11.5)
rs11805303 (IL-23R)	160	159		
CC			79 (49.5)	79 (50)
CT			59 (37)	63 (39.5)
TT			22 (13.5)	17 (10.5)
rs2301436 (tagSNP CCR6)	159	156		
AA			32 (20)	29 (18.5)
AG			79 (50)	81 (52)
GG			48 (30)	46 (29.5)
rs3093023 (CCR6)	153	153		
AA			24 (16)	30 (20)
AG			72 (47)	68 (44)
GG			57 (37)	55 (36)

aGVHD (data not shown). Importantly, in contrast to previous studies [17], patients who received a transplant from a donor with a GA genotype at the *IL-23R* SNP rs11209026 (Arg381Gln) did not experience less aGVHD.

Associations between the CCR6 Polymorphisms and cGVHD

Of the 142 patients at risk for cGVHD, 10% developed limited cGVHD and 26% developed extensive cGVHD (Table 1). In univariate analysis, the donor genotypes of the *CCR6* tagSNP rs2301436 and SNP rs3093023 were significantly associated with the occurrence of cGVHD (Table 4). The other significant variable was previous aGVHD. For tagSNP rs2301436, the cumulative incidence of cGVHD was 10% for the GG genotype versus 38% and 33% for the AG and AA genotypes, respectively ($P = .004$) (Figure 1A and Table 4). In addition, the recipient genotype at tagSNP rs2301436 also was associated with cGVHD in univariate analysis, with an incidence of 39% for the heterozygous AG genotype versus 17% and 20% for the homozygous AA and GG genotypes, respectively ($P = .007$) (Figure 1B and Table 4). For *CCR6* SNP rs3093023, the cumulative incidence of cGVHD was 15% for the GG genotype versus 39% and 32% for the AG and AA genotypes, respectively ($P = .02$) (Figure 1C and Table 4). In addition, the recipient genotype at SNP rs3093023 was associated with cGVHD in univariate analysis, with an incidence of 41% for the heterozygous AG genotype versus 19% for both the homozygous AA and GG genotypes ($P = .02$) (Figure 1D and Table 4). Although we found an association between the heterozygous genotype AG in recipients and cGVHD in both SNPs, these associations did not seem very plausible, given that comparison of the A allele versus G allele frequencies revealed no risk allele. No significant associations between the *IL-23R* SNPs rs11209026 and rs11805303 with the incidence of cGVHD were found (data not shown).

In multivariate analysis, along with aGVHD, the donor genotype at rs2301436 and rs3093023 remained significantly associated with the incidence of cGVHD. Interestingly, the GG genotype versus the AA and AG genotypes in donors showed a protective effect for rs2301436 (odds ratio [OR]: 0.16; 95% confidence interval [CI]: 0.05-0.51; $P = .002$), as well as for rs3093023 (OR: 0.24; 95% CI: 0.09-0.65; $P = .005$) (Table 4). In contrast, the influence of female to male HSCT was not significant in multivariate analysis ($P = .37$). These data indicate that the GG donor genotype at tagSNP rs2301436 and SNP rs3093023 in the *CCR6* region is protective for cGVHD.

TRM, Relapse, and OS Were Not Significantly Influenced by the Polymorphisms Studied

The median follow-up was 68 months (range, 8-157 months) for surviving patients and 8 months

Table 4. Influence of Clinical Factors on Chronic GVHD, Relapse, and IFD at Day 100: Univariate and Multivariate Analyses

Outcome Parameter	Variable	Univariate		Multivariate	
		OR	*P* Value	OR	*P* Value
Chronic GVHD	Donor rs2301436 GG vs AA/AG	0.19 (0.06-0.58)	.004	0.16 (0.05-0.51)	.002
	Recipient rs2301436 AG vs AA/GG	2.86 (1.32-6.16)	.007	2.23 (0.97-5.42)	.06
	Donor rs3093023 GG vs AA/AG	0.31 (2.12-0.76)	.02	0.24 (0.09-0.65)	.005
	Recipient rs3093023 AG vs AA/GG	2.92 (1.37-6.49)	.02	3.02 (0.09-0.65)	.01
	Acute GVHD	4.50 (2.07-9.77)	.0001	4.69 (2.00-10.95)	.0004
	Sex combination	1.58 (0.70-3.52)	.27	1.54 (0.60-3.93)	.37
Relapse	Recipient rs2301436 AA vs AG/GG	2.21 (1.00-4.88)	.05	1.88 (0.74-4.76)	.18
	Chronic GVHD	0.38 (0.16-0.87)	.02	0.26 (0.10-0.68)	.006
	Prophylactic donor lymphocyte infusion	0.64 (0.30-1.35)	.24	0.34 (0.14-0.86)	.02
	Diagnosis (CML vs other)	4.97 (2.5-12.07)	.0004	4.86 (1.73-13.62)	.003
	Conditioning, Ida vs non-Ida	0.49 (0.24-1.04)	.06	0.49 (0.20-1.23)	.13
IFD at day 100	Donor rs2301436 GG vs AA/AG	2.60 (1.10-6.13)	.03	3.59 (1.41-11.25)	.008
	Donor rs3093023 GG vs AA/AG	2.18 (0.88-5.40)	.09	2.15 (0.89-5.36)	.01
	Acute GVHD	2.08 (0.86-5.01)	.10	2.41 (0.89-6.53)	.08
	Conditioning, Ida vs non-Ida	3.17 (0.90-11.24)	.07	3.32 (0.86-12.78)	.08
	Dectin-1 Y238X recipient vs wild-type	0.76 (0.16-3.70)	.73	0.46 (0.08-2.64)	.38

CML indicates chronic myeloid leukemia; Ida, idarubicin.

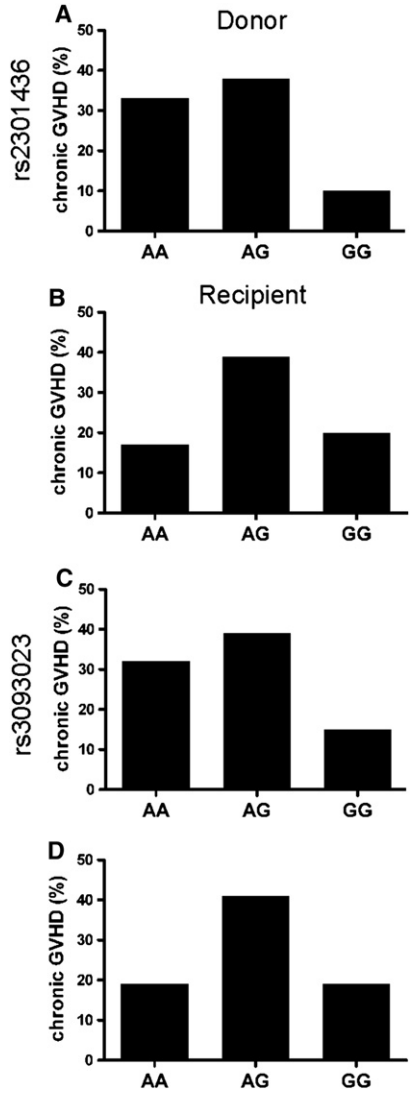

Figure 1. Influence of genotype of rs2301436 and rs3093023 on the incidence of chronic GVHD. (A) Recipients receiving a stem cell graft from a homozygous donor for rs2301436 (ie, genotyped as GG) showed a significantly lower incidence of chronic GVHD ($P = .004$, univariate analysis; $P = .002$, multivariate analysis). (B) Recipients genotyped as AG showed a higher incidence of chronic GVHD ($P = .007$, univariate analysis; $P = .06$, multivariate analysis). (C) Recipients receiving a stem cell graft from a homozygous donor for rs3093023 (ie, genotyped as GG) showed a significantly lower incidence of chronic GVHD ($P = .01$, univariate analysis; $P = .005$, multivariate analysis). (D) Recipients genotyped as AG showed a higher incidence of chronic GVHD ($P = .006$, univariate analysis; $P = .01$, multivariate analysis).

(range, 0-90 months) for the patients who died. Relapse, including molecular, genetic, and hematologic relapse, occurred in 54 of 154 evaluable patients (35%). Known risk factors for relapse were significant in our multivariate analysis, with a diagnosis of chronic myelogenous leukemia, absence of cGVHD, and lack of prophylactic donor lymphocyte infusion increasing the risk of relapse (Table 4). Recipient genotype at *CCR6* tagSNP rs2301436 was associated with the incidence of relapse (AA, 50%; AG, 27%; GG, 38%; $P = .05$ for AA vs AG/GG); however, this association was not significant on multivariate analysis (Table 4). No effects were observed for any of the other SNPs tested.

Relapse-free survival and OS at 5 years were 37.5% and 55%, respectively, and 1-year TRM was 21%. None of the investigated SNPs showed any association with these outcome parameters.

Donor GG Genotype at rs2301436 Influences the Occurrence of IFD

The incidences of candidemia and invasive mold infections at 100 days were 10% and 7.5%, respectively (Table 1). The GG genotype at rs2301436 in donors was associated with a higher incidence of IFD (32%) as opposed to AA (12%) and AG (16%) ($P = .03$, GG vs AA/AG) (Table 4 and Figure 2). In multivariate analysis, donor rs2301436 genotype was the only significant risk factor, although the presence of aGVHD and an idarubicin-containing conditioning regimen both showed a trend toward more IFD (Table 4). Donor rs3093023 GG genotype was accompanied by an increased incidence of IFD, but this was not statistically significant (24% vs 15% for AA and 12% for AG; $P = .22$). In a previous study, we determined the gene status of the dectin-1 polymorphism Y238X, which is known to influence *Candida* colonization and possibly invasive aspergillosis in HSCT recipients [29-31]; however, we found no association between the dectin-1 genotype and IFD at day 100. These data suggest that the GG genotype at *CCR6* tagSNP rs2301436 influences posttransplantation immunity against fungal infections.

We also evaluated the association with Gram-positive bacteremia for all 4 tested SNPs and found none. Data on cytomegalovirus and Epstein-Barr virus reactivation were incomplete and insufficient to perform proper statistical analysis, although Epstein-Barr virus reactivation apparently was not influenced by any of the SNPs.

DISCUSSION

The role of polymorphisms in non-HLA genes in the outcome of allogeneic HSCT is a subject that has

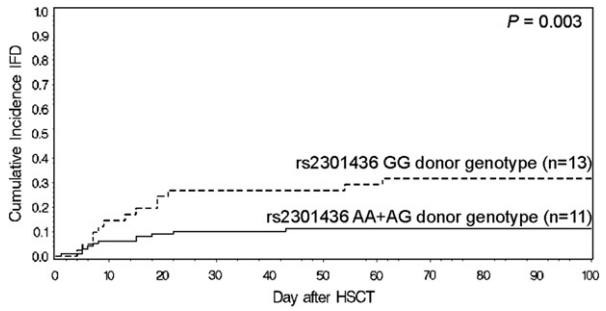

Figure 2. Cumulative incidence of IFD at day 100. Donor GG genotype at CCR6 tagSNP rs2301436 was associated with a higher incidence of IFD (dashed line) compared with AA and AG genotypes (black line) ($P = .003$, log-rank test).

received much attention in the last few years. The particular focus here is on SNPs in genes involved in the immune system. However, with few exceptions, a general problem with genetic association studies is that documented associations are very context-dependent and thus often difficult to confirm and reproduce. This also seems to apply to the rs11209026 *IL-23R* polymorphism; a previous large study failed to confirm an impact on GVHD in the particular setting of unrelated donor HSCT [32]. In the present study, we were not able to reproduce the repeatedly described protection against aGVHD of the donor GA genotype at SNP rs11209026 [17-19]. This GA genotype, resulting in Arg381Gln, is believed to perturb the IL-23 receptor function, reducing alloreactive T cell responses that mediate aGVHD. We believe that we could not confirm this genetic association mainly because of the practice of ex vivo T cell depletion in all of our transplants, as opposed to Elmaagacli et al. [17], who did not perform any T cell depletion. The allele frequency was comparable in the 2 studies (A allele frequency $\approx$ 6%), but the incidence of GVHD was lower in our study, which is directly related to the use of T cell–depleted stem cell grafts. T cell depletion most likely results in delayed T cell recovery, which might influence the pathogenesis of aGVHD. Altering the kinetics and reducing the impact of T cell function might alter the influence genetic variations, such as those in the IL-23/Th17, pathway as well. Context dependency is a recurring theme in studies on genetic associations, and discrepancies are often found, emphasizing the need for caution when generalizing results from previous association studies.

Studying a second gene prominent in the IL-23/Th17 pathway, the chemokine receptor CCR6, we found significant associations between donor genotype at rs2301436 and rs3093023 and the occurrence of cGVHD. For rs2301436, the incidence of IFD was increased significantly, whereas for rs3093023, only a trend toward a higher incidence was seen. The GG genotype of rs2301436 was protective for cGVHD, but conferred a high risk for developing IFD. SNP rs2301436 is considered a tagSNP for the *CCR6* gene, but the SNP itself lies within the *FGFR10P* gene [22]. Functional consequences of the SNP itself, and of the CCR6 phenotype corresponding with the tagSNP, are not yet completely known. However, the A allele of rs2301436 recently has been associated with a modestly increased risk for both Crohn's disease and rheumatoid arthritis, with a protective effect attributed to the G allele [23]. Kochi et al. [22] showed that two different polymorphisms, both located within the *CCR6* gene and in linkage disequilibrium with tagSNP rs2301436, regulate the expression of CCR6. Therefore, we tested the impact of one of these polymorphisms, SNP rs3093023, on the clinical outcome after allogeneic HSCT and also observed a protective effect of the G allele. Although no direct functional consequences for these specific *CCR6*-associated polymorphisms are known, functional studies of other polymorphisms in strong linkage disequilibrium with rs3093023 have shown that a triallelic dinucleotide polymorphism correlates with CCR6 expression levels as well as with the presence of IL-17 in patients with rheumatoid arthritis [22]. In the absence of data on any functional consequences from the genotype of tagSNP rs2301436 or SNP rs3093023 regarding CCR6, we propose 2 general hypotheses that are not mutually exclusive. One hypothesis is that the GG genotype at *CCR6* SNPs rs2301436 and rs3093023 might represent the normal CCR6 variant, as opposed to what might be the dysfunctional A allele. Kochi et al. [22] reported that in activated cells, expression levels of CCR6 increased with the number T alleles. Normal CCR6 levels are associated with normal functioning APCs and thus contribute to intact mucosal immune homeostasis [33]. Because distorted immune homeostasis predisposes to GVHD [34], the GG genotype could decrease the risk of excessive inflammation. In addition, normal CCR6-mediated Th17 homing might result in impaired Th1 responses, with decreased alloreactivity and tissue damage protecting subjects from GVHD [8], and decreased Th1-mediated antifungal defenses, resulting in more infections [35,36]. This hypothesis relies strongly on the assumption that Th1 cells are more important to the onset of cGVHD than Th17 cells.

An alternative hypothesis could be that the GG genotype at the *CCR6* SNPs might correspond to low levels of the CCR6 protein, impairing homing of APCs and Th17 cells to GVHD target tissues and infection sites, as well as to T cell priming sites [37]. Reduced APC function results in reduced alloreactive T cell responses and GVHD, along with an increased risk of fungal infections due to less effective phagocyte recruitment [38]. Alternatively, reduced Th17 homing might reduce GVHD, given that a role for Th17 in humans has been suggested [39]. In addition, impaired Th17 responses might perturb host antifungal defenses, increasing the risk for IFD [40]. This hypothesis strongly relies on the assumption of an important, but as-yet unconfirmed, role for Th17 in human SCT and cGVHD and IFD.

The present study is the first to show an association with the donor genotype of the *CCR6* tagSNP rs2301436 and the occurrence of cGVHD and IFD, as well as an association with the donor genotype of the *CCR6* SNP rs3093023, and we believe that our findings provide new directions for further research. We conclude that patients undergoing allogeneic HSCT might benefit from typing for these particular SNPs when searching for a suitable stem cell donor, although further research on *CCR6* gene variation is needed.

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REFERENCES

1. Baddley JW, Andes DR, Marr KA, et al. Factors associated with mortality in transplant patients with invasive aspergillosis. *Clin Infect Dis*. 2010;50:1559-1567.
2. Ferrara JL, Levine JE, Reddy P, et al. Graft-versus-host disease. *Lancet*. 2009;373:1550-1561.
3. Lee SJ, Vogelsang G, Flowers ME. Chronic graft-versus-host disease. *Biol Blood Marrow Transplant*. 2003;9:215-233.
4. Toubai T, Sun Y, Reddy P. GVHD pathophysiology: is acute different from chronic? *Best Pract Res Clin Haematol*. 2008;21: 101-117.
5. Miossec P, Korn T, Kuchroo VK. Interleukin-17 and type 17 helper T cells. *N Engl J Med*. 2009;361:888-898.
6. Annunziato F, Romagnani S. Do studies in humans better depict Th17 cells? *Blood*. 2009;114:2213-2219.
7. Costa-Rodriguez EV, Rivino L, Geginat J, et al. Surface phenotype and antigenic specificity of human interleukin-17–producing T helper memory cells. *Nat Immunol*. 2007;8:639-646.
8. Wang C, Kang SG, Lee J, et al. The roles of CCR6 in migration of Th17 cells and regulation of effector T-cell balance in the gut. *Mucosal Immunol*. 2009;2:173-183.
9. Khader SA, Gaffen SL, Kolls JK. Th17 cells at the crossroads of innate and adaptive immunity against infectious diseases at the mucosa. *Mucosal Immunol*. 2009;2:403-411.
10. Van de Veerdonk FL, Gresnigt MS, Kullberg BJ, et al. Th17 responses and host defense against microorganisms: an overview. *BMB Rep*. 2009;42:776-787.
11. Steinman L. Mixed results with modulation of TH-17 cells in human autoimmune diseases. *Nat Immunol*. 2010;11:41-44.
12. Litjens NH, Van de Wetering J, Van Besouw NM, et al. The human alloreactive $CD4^+$ T-cell repertoire is biased to a Th17 response, and the frequency is inversely related to the number of HLA class II mismatches. *Blood*. 2009;114:3947-3955.
13. Benghiat FS, Charbonnier LM, Vokaer B, et al. Interleukin-17–producing T helper cells in alloimmunity. *Transplant Rev (Orlando)*. 2009;23:11-18.
14. Zou W. Regulatory T cells, tumour immunity and immunotherapy. *Nat Rev Immunol*. 2006;6:295-307.
15. Carlson MJ, West ML, Coghill JM, et al. In vitro–differentiated TH17 cells mediate lethal acute graft-versus-host disease with severe cutaneous and pulmonary pathologic manifestations. *Blood*. 2009;113:1365-1374.
16. Chen X, Vodanovic-Jankovic S, Johnson B, et al. Absence of regulatory T-cell control of TH1 and TH17 cells is responsible for the autoimmune-mediated pathology in chronic graft-versus-host disease. *Blood*. 2007;110:3804-3813.
17. Elmaagacli AH, Koldehoff M, Landt O, et al. Relation of an interleukin-23 receptor gene polymorphism to graft-versus-host disease after hematopoietic cell transplantation. *Bone Marrow Transplant*. 2008;41:821-826.
18. Gruhn B, Intek J, Pfaffendorf N, et al. Polymorphism of interleukin-23 receptor gene but not of NOD2/CARD15 is associated with graft-versus-host disease after hematopoietic stem cell transplantation in children. *Biol Blood Marrow Transplant*. 2009;15:1571-1577.
19. Wermke M, Maiwald S, Schmelz R, et al. Genetic variations of IL23R(1143A>G) and BPI(A645G) but not NOD2 are associated with acute GvHD after allogeneic transplantation. *Biol Blood Marrow Transplant*. 2010;16:1718-1727.
20. Mullally A, Ritz J. Beyond HLA: the significance of genomic variation for allogeneic hematopoietic stem cell transplantation. *Blood*. 2007;109:1355-1362.
21. Van der Velden WJ, Blijlevens NM, Maas FM, et al. NOD2 polymorphisms predict severe acute graft-versus-host and treatment-related mortality in T-cell–depleted haematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2009;44:243-248.
22. Kochi Y, Okada Y, Suzuki A, et al. A regulatory variant in CCR6 is associated with rheumatoid arthritis susceptibility. *Nat Genet*. 2010;42:515-519.
23. Barrett JC, Hansoul S, Nicolae DL, et al. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat Genet*. 2008;40:955-962.
24. Schaap N, Schattenberg A, Bar B, et al. Outcome of transplantation for standard-risk leukaemia with grafts depleted of lymphocytes after conditioning with an intensified regimen. *Br J Haematol*. 1997;98:750-759.
25. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant*. 1995;15:825-828.
26. De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis*. 2008;46:1813-1821.
27. MacGregor RR, Beaty HN. Evaluation of positive blood cultures: guidelines for early differentiation of contaminated from valid positive cultures. *Arch Intern Med*. 1972;130:84-87.
28. Safrany E, Pazar B, Csongei V, et al. Variants of the IL23R gene are associated with ankylosing spondylitis but not with Sjőgren syndrome in Hungarian population samples. *Scand J Immunol*. 2009;70:68-74.
29. Chai LY, de Boer MG, Van der Velden WJ, et al. The Y238X stop codon polymorphism in the human β-glucan receptor dectin-1 and susceptibility to invasive aspergillosis. *J Infect Dis*. 2011;203:736-743.
30. Cunha C, Di Lanni M, Bozza S, et al. Dectin-1 Y238X polymorphism associates with susceptibility to invasive aspergillosis in hematopoietic transplantation through impairment of both recipient- and donor-dependent mechanisms of antifungal immunity. *Blood*. 2010;116:5394-5402.
31. Plantinga TS, Van der Velden WJ, Ferwerda B, et al. Early stop polymorphism in human DECTIN-1 is associated with increased *Candida* colonization in hematopoietic stem cell transplant recipients. *Clin Infect Dis*. 2009;49:724-732.
32. Nguyen Y, Al-Lehibi A, Gorbe E, et al. Insufficient evidence for association of NOD2/CARD15 or other inflammatory bowel disease–associated markers on GVHD incidence or other adverse outcomes in T-replete, unrelated donor transplantation. *Blood*. 2010;115:3625-3631.
33. Rimoldi M, Chieppa M, Salucci V, et al. Intestinal immune homeostasis is regulated by the crosstalk between epithelial cells and dendritic cells. *Nat Immunol*. 2005;6:507-514.
34. Penack O, Smith OM, Cunningham-Bussel A, et al. NOD2 regulates hematopoietic cell function during graft-versus-host disease. *J Exp Med*. 2009;206:2101-2110.
35. Chai LY, Van der Velden WJ, Marijnissen RJ, et al. Anti-aspergillus human host defense relies on type 1 T helper (Th1), rather than type 17 T helper (Th17), cellular immunity. *Immunology*. 2010;130:46-54.
36. Zelante T, De Luca A, Bonifazi P, et al. IL-23 and the Th17 pathway promote inflammation and impair antifungal immune resistance. *Eur J Immunol*. 2007;37:2695-2706.
37. Salazar-Gonzalez RM, Niess JH, Zammit DJ, et al. CCR6-mediated dendritic cell activation of pathogen-specific T cells in Peyer's patches. *Immunity*. 2006;24:623-632.
38. Phadke AP, Akangire G, Park SJ, et al. The role of CC chemokine receptor 6 in host defense in a model of invasive pulmonary aspergillosis. *Am J Respir Crit Care Med*. 2007;175:1165-1172.
39. Dander E, Balduzzi A, Zappa G, et al. Interleukin-17–producing T-helper cells as new potential player mediating graft-versus-host disease in patients undergoing allogeneic stem-cell transplantation. *Transplantation*. 2009;88:1261-1272.
40. Werner JL, Metz AE, Horn D, et al. Requisite role for the dectin-1 β-glucan receptor in pulmonary defense against *Aspergillus fumigatus*. *J Immunol*. 2009;182:4938-4946.