MAJOR ARTICLE

IL1B and *DEFB1* Polymorphisms Increase Susceptibility to Invasive Mold Infection After Solid-Organ Transplantation

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Background. Single-nucleotide polymorphisms (SNPs) in immune genes have been associated with susceptibility to invasive mold infection (IMI) among hematopoietic stem cell but not solid-organ transplant (SOT) recipients.

Methods. Twenty-four SNPs from systematically selected genes were genotyped among 1101 SOT recipients (715 kidney transplant recipients, 190 liver transplant recipients, 102 lung transplant recipients, 79 heart transplant recipients, and 15 recipients of other transplants) from the Swiss Transplant Cohort Study. Association between SNPs and the end point were assessed by log-rank test and Cox regression models. Cytokine production upon *Aspergillus* stimulation was measured by enzyme-linked immunosorbent assay in peripheral blood mononuclear cells (PBMCs) from healthy volunteers and correlated with relevant genotypes.

Results. Mold colonization (n = 45) and proven/probable IMI (n = 26) were associated with polymorphisms in the genes encoding interleukin 1β *(IL1B; rs16944*; recessive mode, $P = .001$ for colonization and $P = .00005$ for IMI, by the log-rank test), interleukin 1 receptor antagonist (*IL1RN*; *rs419598*; $P = .01$ and $P = .02$, respectively), and β-defensin 1 (*DEFB1*; *rs1800972*; $P = .001$ and $P = .0002$, respectively). The associations with *IL1B* and *DEFB1* remained significant in a multivariate regression model ($P = .002$ for *IL1B rs16944*; $P = .01$ for *DEFB1 rs1800972*). The presence of 2 copies of the rare allele of *rs16944* or *rs419598* was associated with reduced *Aspergillus*-induced interleukin 1β and tumor necrosis factor α secretion by PBMCs.

Conclusions. Functional polymorphisms in *IL1B* and *DEFB1* influence susceptibility to mold infection in SOT recipients. This observation may contribute to individual risk stratification.

Keywords. innate immunity; single nucleotide polymorphism; genetic susceptibility; aspergillosis; invasive mold infections; solid organ transplant; interleukin-1 beta.

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Over 100 000 solid-organ transplantations (SOTs) are performed worldwide each year [1]. Despite recent improvements in the management of SOT recipients, infectious complications after transplantation remain a challenging issue [2]. In particular invasive aspergillosis can occur in up to 3% of SOT recipients and is associated with a mortality rates of 20%–76% [3, 4]. Risk factors for the development of invasive aspergillosis include type and level of immunosuppression, use of renal replacement therapy, older age, and cytomegalovirus

(CMV) disease [3, 5]. However, not all patients with these risk factors develop invasive mold infections (IMIs), while some patients without these risk factors do, making it difficult to predict the individual-level risk of developing IMI.

Over the last decade, a series of studies have identified common genetic polymorphisms that are associated with the development of invasive aspergillosis among hematopoietic stem cell transplant recipients and other patients with oncohematological conditions [6, 7]. The identification of specific genetic variants may improve individual risk stratification and allow the development of personalized management strategies, as well as the use of prophylaxis or specific surveillance in individuals at high risk to develop invasive aspergillosis [6]. To date, no studies examined the role of such genetic polymorphisms on the susceptibility to fungal infections among SOT recipients. To our knowledge, this study is the first to explore the role of host genetics in susceptibility to IMI in SOT recipients.

MATERIALS AND METHODS

Patients and Study Design

The Swiss Transplant Cohort Study (STCS) is a large, nationwide, well-documented prospective cohort including all SOT recipients followed at 6 Swiss university transplantation centers (Basel, Bern, Geneva, Lausanne, St. Gallen, and Zurich) since May 2008 [8]. Patient data were systematically collected on standardized case report forms at enrollment, 6 months after transplantation, and every 12 months after transplantation.

Infectious complications were systematically evaluated by an infectious diseases specialist on the basis of clinical, histological, radiological, and mycological evidence and reported on a separate case report form. Medical records for patients reported to have fungal colonization or IMI were revised by an independent investigator (T. L.) [9]. Proven or probable IMI was defined on the basis of standardized European Organisation for Research and Treatment of Cancer/Mycoses Study Group guidelines and definitions [10] and adapted International Society for Heart and Lung Transplantation guidelines and definitions unique for lung transplant recipients, such as anastomotic bronchial infections or tracheobronchitis [11]. Colonization was defined by microscopic or culture detection of a mold from a specimen from a nonsterile site, including sputum, bronchoalveolar lavage, bronchial brush, sinus aspirate, or urine samples, in the absence of clinical signs/symptoms for infection. Patients who received a diagnosis of mold colonization and/or IMI before transplantation and/or who previously received an organ transplant were excluded. CMV infection was classified as asymptomatic replication, viral syndrome, or probable and proven disease as previously reported [12].

Ethics Statement

All patients provided a written informed consent for participation in the STCS (including genetic analyses). The protocol was approved by the independent ethics committees of each Swiss participating center (University Hospital of Lausanne; University Hospitals of Geneva; University Hospital Zürich; Cantonal Hospital St. Gallen; Inselspital, Bern University Hospital; Clinica Luganese, Lugano; and University Hospital of Basel).

Fresh venous blood specimens were collected for functional studies from healthy volunteers who provided a written informed consent. The protocol was approved by the local ethics committee (Radboud University Nijmegen Medical Center, the Netherlands).

Peripheral Blood Mononuclear Cell (PBMC) Isolation and In Vitro–Stimulation Assays

PBMCs were isolated using Ficoll-Paque Plus density gradient centrifugation (GE Healthcare, Zeist, the Netherlands) as described previously [13]. Cells were subsequently stimulated with 1×10^7 live or heat-inactivated *Aspergillus fumigatus* conidia per milliliter for 24 hours or 7 days, respectively. Afterward, concentrations of the cytokines tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), interleukin 1 receptor antagonist (IL-1Ra), interleukin 17 (IL-17), interleukin 2 (IL-22; all from R&D Systems, Minneapolis Minnesota) and interferon γ (IFN-γ; Sanquin, Amsterdam, the Netherlands) were measured in cell supernatants by enzyme-linked immunosorbent assays according to the manufacturer's protocol.

Genotyping

A total of 24 single-nucleotide polymorphisms (SNPs) in 21 genes were selected from the literature by performing a PubMed search until June 2012, using the keywords "candidemia", "candidiasis", "aspergillosis", and "SNP" and by searching previous reviews on fungal immunogenetics [6, 7]. Blood samples were obtained from all SOT recipients at the time of transplantation. Genomic DNA was extracted from blood specimens obtained from patients or healthy volunteers and stored in ethylenediaminetetraacetic acid–containing tubes, using the Gentra Puregene Blood Kit (Qiagen). Genotyping was performed using a customized GoldenGate Genotyping Assay on Veracode platform (Illumina, San Diego, California), unless otherwise indicated. Results were analyzed on a BeadXpress Reader according to standard protocols and quality controls. Additional SNPs were genotyped using the Competitive Allele-Specific PCR system (LGC Genomics, Herts, United Kingdom). For functional studies, genotyping of the *IL1B rs16944* and *IL1RN rs419598* variants was performed on the ABI-Prism StepOne thermocycler (Applied Biosystems), using predesigned SNP assays.

Statistical Analysis

Statistical analyses were performed in Stata13 (StataCorp, College Station, Texas), unless otherwise indicated. The cumulative

incidence of mold colonization and IMI by genetic variants at 36 months after the first transplantation was assessed by the log-rank test, with censoring at the date of the last follow-up visit or death. Associated variants were selected on the basis of log-rank test results and were further tested by univariate and multivariate Cox models. To estimate the independent contribution of each polymorphism to the end points, demographic and clinical factors previously associated with mold colonization and/or IMI, as described elsewhere [5, 9], were entered into multivariate stepwise regression models ($P < .2$), together with relevant genetic polymorphisms. Haplotypes were inferred using PHASE 2.1 (University of Washington, Seattle). The power calculation for Cox proportional hazard regression analysis was done using an R implementation of the power and sample size calculation for survival analysis of epidemiological studies (powerSurvEpi R package 0.0.6) [14]. The effect of the presence of SNPs on the *Aspergillus*-induced cytokine levels was determined by the Mann–Whitney *U* test. The data are presented as mean values ± standard error of the mean and were analyzed using GraphPad Prism v5.0 (San Diego, California).

RESULTS

Cohort Study

The study included 1101 white patients who received a SOT (670 received a kidney transplant, 190 received a liver transplant, 102 received a lung transplant, 79 received a heart transplant, 15 received an islet/pancreas transplant, and 45 received combined organ transplants) between May 2008 and December 2011 (Table 1). Mold colonization and IMI were diagnosed in 45 patients (4.1%) and 26 patients (2.4%), respectively. Most IMIs (17 [75%]) occurred >3 months after transplantation. The most frequent causative organism of IMI was *Aspergillus* species (21 IMIs [81%]); only 5 IMIs were due to other fungi, including *Fusarium* species (2 IMIs), *Alternaria* species (1 IMI), Zygomycetes species (1 IMI), and mixed pathogens (Zygomycetes and *Fusarium* species [1 IMI]). Factors significantly associated with IMI were identified and described elsewhere [9] and included in the multivariate analysis.

Genetic Risk Factors for Mold Colonization and IMI in SOT Recipients

The minor allele frequency of the 24 SNPs are shown in Table 2. Three SNPs that deviated from Hardy–Weinberg equilibrium were excluded from the analyses. The power to detect mold colonization and IMI was calculated for each SNP (Table 4).

To assess the risk of fungal disease according to the different SNPs, we estimated their associations with colonization and infection during the first 36 months after transplantation (Table 2). Mold colonization and IMI were both associated with SNPs in 3 different genes, including the genes encoding IL-1β (*IL1B*; *rs16944*; TT vs CT or CC; $P = .001$ and $P = .00005$,

Table 1. Demographic Characteristic of Solid-Organ Transplant (SOT) Recipients

Variable	Value
Age, y, median (IQR)	
Recipient	54 (19)
Donor[a]	53 (22)
Sex	
Recipient	
Male	730 (66)
Female	371 (34)
Donor[b]	
Male	573 (52)
Female	523 (48)
White race	1101 (100)
Duration of cold ischemia, d, median (IQR)	5.6 (7.3)
Transplanted organ	
Kidney	670 (61)
Liver	190 (17)
Lung	102 (9)
Heart	79 (7)
Pancreas and islets	15 (1)
Mixed[c]	45 (4)
Donor type[d]	
Deceased	801 (73)
Living related/unrelated	299 (27)
Rejection type[e]	
Acute cellular rejection	342 (31)
Acute humoral rejection	35 (3)
CMV characteristic	
Infection	279 (25)
Disease	61 (6)
Serostatus[f]	
D+ R+	350 (33)
D− R+	256 (24)
D− R−	245 (24)
D+ R−	218 (20)
Induction therapy[g]	
BAS	641 (61)
Anti-thymocyte globulin ± BAS	188 (18)
None	225 (21)
Maintenance regiment[h]	
Calcineurin inhibitors	817 (92)
Corticosteroids	910 (83)
MMF	721 (81)
AZA	31 (3)
mTOR inhibitors	54 (6)
Anti-IMI prophylaxis (wks 1–4)	74 (7)

Data are no. (%) of 1101 SOT recipients, unless otherwise indicated.

Abbreviations: AZA, azathioprine; BAS, basiliximab; CMV, cytomegalovirus; IMI, invasive mold infection; IQR, interquartile range; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin.

[a] Data were missing for 104 patients.

[b] Data were missing for 5 patients.

[c] Including kidney and pancreas (n = 24), kidney and liver (n = 10), kidney and kidney (n = 5), kidney and islets (n = 3), two kidneys and pancreas (n = 2), kindey and lung (n = 1).

[d] Data were missing for 2 patients.

[e] Reported at any time of follow-up.

[f] Data were missing for 32 patients.

[g] Data were missing for 47 patients.

[h] Reported at month 12.

Table 2. Association Between Genetic Polymorphisms and Mold Colonization or Invasive Mold Infection (IMI) in Solid-Organ Transplant (SOT) Recipients

Gene (nt, aa Change)[a]	Reference SNP ID	MAF	HWE	*P* value[b]	
				Mold Colonization (n = 45)	IMI (n = 26)
IL1B (–511 C/T)	*rs16944*	0.33	0.160	.001[c]	.00005[d]
DEFB1 (–44 C/G)	*rs1800972*	0.18	1.000	.001[c]	.0002[e]
IL1RN (2018T/C)	*rs419598*	0.25	0.320	.01	.02
TLR9 (–1237 C/T)	*rs5743836*	0.13	0.060	.2	.04
INFG (874 T/A)	*rs2069705*	0.33	0.086	.05	.06
PLG (D472N)	*rs4252125*	0.30	0.340	.4	.1
CD209 (–139 A/G)	*rs2287886*	0.36	0.610	.2	.2
IL10 (–1082 A/G)	*rs1800896*	0.44	0.220	.8	.2
TLR6 (S249P)	*rs5743810*	0.35	0.180	.1	.4
TNF (–308 G/A)	*rs1800629*	0.14	0.010	.2	.4
SFTPA2 (A91P)	*rs17886395*	0.14	0.900	.004	.5
CXCL10 (1642G/C)	*rs3921*	0.41	0.280	.6	.5
MBL (G54D)	*rs1800450*	0.15	0.099	.8	.4
IL10 (–819 C/T)	*rs1800871*	0.27	0.820	.8	.6
TLR1 (R80 T)	*rs5743611*	0.07	0.500	.6	.7
TLR4 (D299G)	*rs4986790*	0.05	0.540	.8	.8
IL1A (–889 C/T)	*rs1800587*	0.29	0.620	.6	.8
MASP2 (D105G)	*rs72550870*	0.02	0.150	.8	.8
CLEC7A (Y238X)	*rs16910526*	0.08	0.340	.7	.8
TLR3 (L412F)	*rs3775291*	0.29	1.000	.9	.9
IL23R (R381Q)	*rs11209026*	0.07	0.811	.7	.7

Data are for 1101 SOT recipients, unless otherwise indicated.

Abbreviations: aa, amino acid; *CD209*, gene encoding CD209; *CLEC7A*, gene encoding C-type lectin domain 7; *CXCL10*, gene encoding CXC-chemokine ligand 10; *DEFB1*, gene encoding β-defensin 1; HWE, Hardy-Weinberg equilibrium; ID, identification; *INFG*, gene encoding interferon γ; *IL1A*, gene encoding interleukin 1α; *IL1B*, gene encoding interleukin 1β; *IL1RN*, gene encoding interleukin 1 receptor antagonist; *IL10*, gene encoding interleukin 10; *IL23R*, gene encoding interleukin 23 receptor; MAF, minor allele frequency; *MASP2*, gene encoding mannan-binding lectin serine peptidase 2; *MBL*, gene encoding mannose binding lectin; nt, nucleotide; *PLG*, gene encoding plasminogen; *SFTPA2*, gene encoding surfactant protein A2; SNP, single-nucleotide polymorphism; *TLR1*, gene encoding Toll-like receptor 1; *TLR3*, gene encoding Toll-like receptor 3; *TLR4*, gene encoding Toll-like receptor 4; *TLR6*, gene encoding Toll-like receptor 6; *TLR9*, gene encoding Toll-like receptor 9; *TNF*, gene encoding tumor necrosis factor.

[a] Three SNPs that deviated from HWE (*TLR1 rs5743618*, *IL4 rs2243250*, and *CARD9 rs10870077*) were excluded from the analyses.

[b] By the log-rank test, recessive mode (patients homozygous for the rare alleles are compared to the other patients). Five patients who were colonized with mold before transplantation, of whom 2 also developed IMI before transplantation, were removed from the analyses.

[c] Significant after Bonferroni correction for multiple testing (21 tests; $P = .02$).

[d] Significant after Bonferroni correction for multiple testing (21 tests; $P = .001$).

[e] Significant after Bonferroni correction for multiple testing (21 tests; $P = .004$).

respectively), β-defensin 1 (*DEFB1*; *rs1800972*; CC vs GG or CG; $P = .001$ and $P = .0002$, respectively), and IL-1Ra (*IL1RN*; *rs419598*; CC vs CT or TT; $P = .01$ and $P = .02$, respectively; Figure 1). In addition, we observed a significant association between a SNP in the gene encoding surfactant-associated protein 2 (*SFTPA2*; *rs17886395*; GG vs CC or CG) and mold colonization ($P = .004$) but not infection ($P = .5$). However, the number of cases carrying this polymorphism was very small (Supplementary Figure 4).

To determine whether the SNPs were independent risk factors for mold colonization and IMI, we used multivariate Cox stepwise regression models, after adjustment for all relevant covariates (Table 3). The final model for colonization still included *IL1B rs16944* (hazard ratio [HR], 2.52; 95% confidence interval [CI], 1.18–5.36; $P = .02$), *DEFB1 rs1800972* (HR, 6.11; 95% CI, 2.28–16.4; $P = .0003$), and *IL1RN rs419598* (HR, 3.35; 95% CI, 1.31–8.58; $P = .01$). The final model for IMI still included *IL1B rs16944* (HR, 4.29; 95% CI, 1.71–10.8; $P = .002$) and *DEFB1 rs1800972* (HR, 4.73; 95% CI, 1.46–15.3; $P = .01$) but not *IL1RN rs419598*. Associations were stronger when the SNPs were combined together (for *IL1B rs16944* and *DEFB1 rs1800972*: HR, 4.94 [95% CI, 2.06–11.8; $P = .003$]; for *IL1B rs16944* and *IL1RN rs419598*: HR, 4.64 [95% CI, 1.92–11.2; $P = .0006$]; Supplementary Figure 1). To account for a possible confounding role of antifungal prophylaxis, the analyses were repeated after removal of patients who received antimold

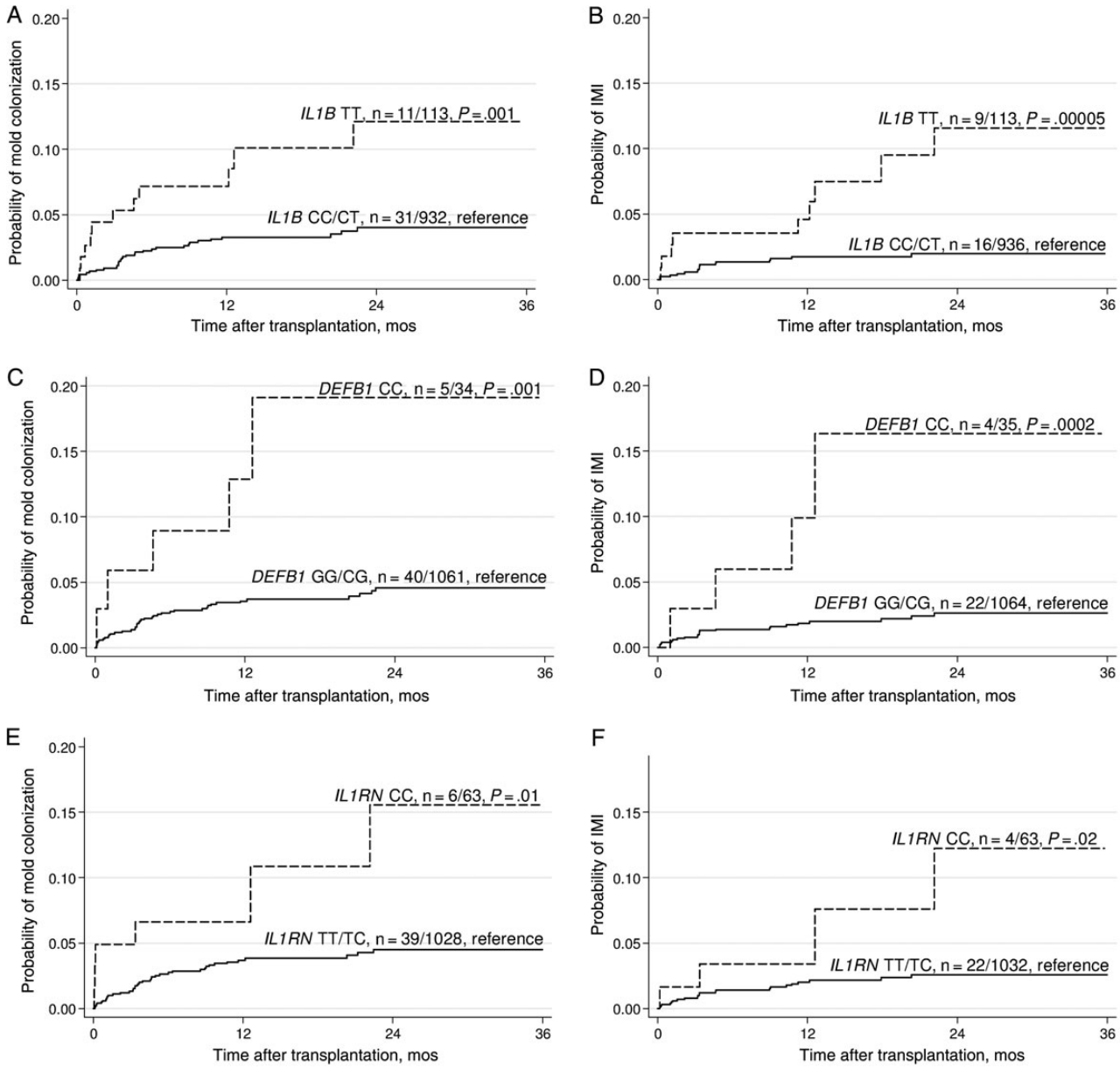

Figure 1. Cumulative incidence of mold colonization (n = 45) and invasive mold infection (IMI; n = 26), according to polymorphisms in genes encoding interleukin 1β (*IL1B*; *rs16944*; *A* and *B*, respectively), β-defensin 1 (*DEFB1*; *rs1800972*; *C* and *D*, respectively), and interleukin 1 receptor antagonist (*IL1RN*; *rs419598*; *E* and *F*, respectively), in solid-organ transplant recipients. Patients with mold colonization or IMI before engraftment were excluded from the analyses. *P* values were calculated by the log-rank test, recessive mode (patients homozygous for the rate alleles are compared to the other patients). For the *IL1B rs16944* and *DEFB1 rs1800972* single-nucleotide polymorphisms, *P* values remained significant after correction for multiple testing (21 tests; *IL1B rs16944*, colonization *P* = .02, IMI *P* = .001; and *DEFB1 rs1800972*, colonization *P* = .02, IMI *P* = .004).

prophylaxis. The associations between the SNPs in *IL1B*, *DEFB1*, and *IL1RN* were still significant on repeat analysis (not shown).

IL1B rs16944 and *IL1RN rs419598* Risk Haplotype for Mold Colonization and IMI

IL1B and *IL1RN* are located within an approximately 400 kb region on chromosome 2q13-21. We therefore analyzed whether haplotypic combinations of *IL1B rs16944* and *IL1RN rs419598* SNPs further influenced mold colonization and IMI (Figure 2). Carriage of the *rs16944-rs419598* C-T haplotype was associated with a decreased risk of both mold colonization and IMI (C-T haplotype vs all others: HR, 0.34 [95% CI, .18–.63; *P* = .0007] and 0.21 [95% CI, .10–.45; *P* = .00008], respectively). Conversely, carriage of the T-C haplotype was associated with an increased risk for both phenotypes (T-C haplotype vs all others:

Table 3. Independent Factors Associated With Mold Colonization and Invasive Mold Infection (IMI) in Solid-Organ Transplant Recipients

	Mold Colonization (n = 42)		IMI (n = 25)	
Variable	HR (95% CI)	*P* value[a]	HR (95% CI)	*P* value[a]
IL1B rs16944 (TT vs CC/CT)[b]	2.52 (1.18–5.36)	.02	4.29 (1.71–10.8)	.002[c]
DEFB1 rs1800972 (CC vs GG/GC)[b]	6.11 (2.28–16.4)	.0003[d]	4.73 (1.46–15.3)	.01
IL1RN rs419598 (CC vs TT/TC)[b]	3.35 (1.31–8.58)	.01	2.50 (.75–8.29)	.1
Lung or heart transplantation	11.5 (5.83–22.6)	<.0001	3.12 (1.21–8.03)	.02
MMF	0.32 (.16–.63)	.001	0.14 (.06–.33)	<.0001
Tacrolimus	0.52 (.27–1.03)	.06	0.45 (.19–1.09)	.1
Corticosteroids	. . .	. . .	3.03 (.67–13.7)	.1
Acute/chronic rejection	. . .	. . .	2.35 (.94–5.83)	.07
CMV infection/disease	1.83 (.89–3.72)	.1	2.68 (1.11–6.50)	.03
Recipient age (per year)	1.04 (1.01–1.06)	.008	1.06 (1.02–1.10)	.004

The total number of patients in the multivariate analysis (n = 1047) was slightly lower than in the univariate analysis (n = 1101), owing to missing covariates. Five patients who were colonized with mold before transplantation, of whom 2 also developed IMI before transplantation, were removed from the analyses.

Abbreviations: CI, confidence interval; CMV, cytomegalovirus; *DEFB1*, gene encoding β-defensin 1; HR, hazard ratio; *IL1B*, gene encoding interleukin 1β; *IL1RN*, gene encoding interleukin 1 receptor antagonist; MMF, mycophenolate mofetil.

[a] By Cox proportional multivariate analysis, with stepwise regression variable selection. The variables included in the initial model were recipient age and sex, CMV infection or disease, mycophenolate mofetil, tacrolimus and corticosteroid treatment, acute/chronic rejection, and type of transplanted organ.

[b] Genetic associations are for recessive mode (patients homozygous for the rare alleles are compared to the other patients).

[c] Significant after Bonferroni correction for multiple testing (21 tests; *P* = .04).

[d] Significant after Bonferroni correction for multiple testing (21 tests; *P* = .006).

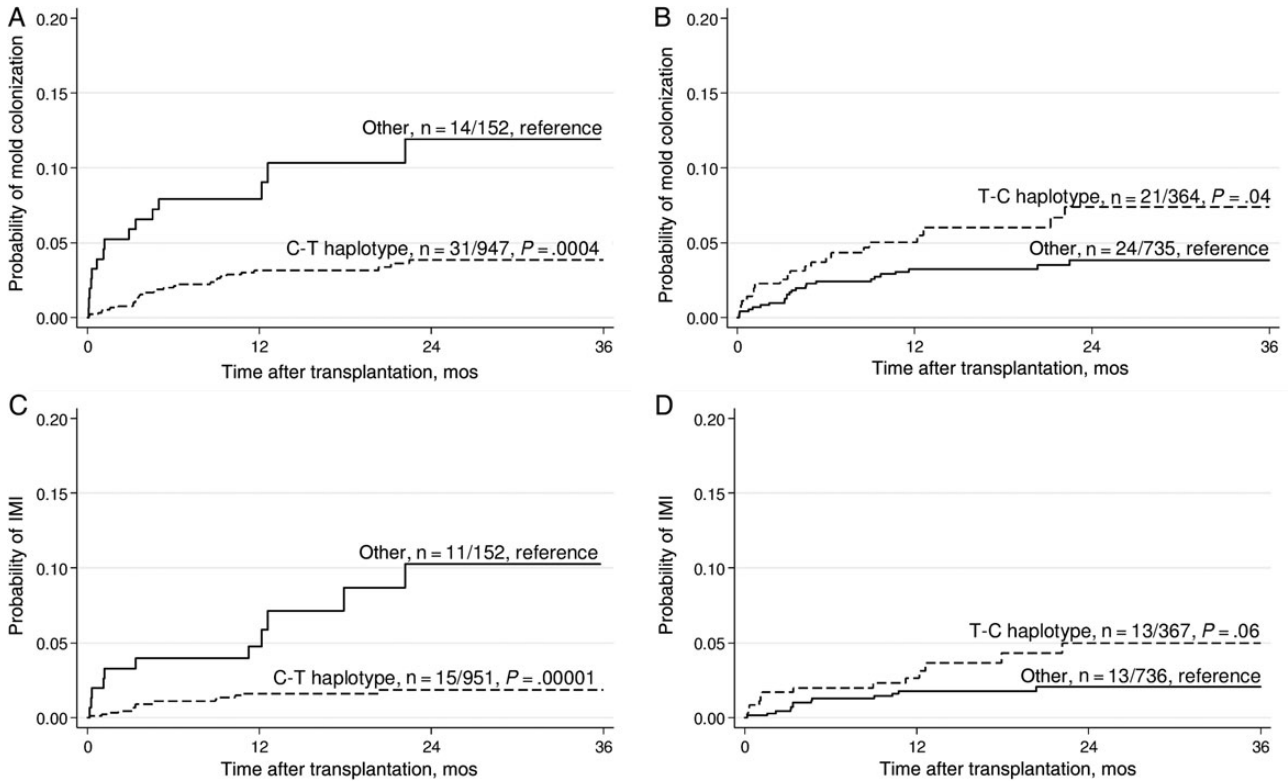

Figure 2. Cumulative incidence of mold colonization (*A* and *B*) and invasive mold infection (IMI; *C* and *D*) according to *rs16944-rs419598* C-T (*A* and *C*) or T-C (*B* and *D*) haplotypes of polymorphisms in the genes encoding interleukin 1β (IL-1β) and interleukin 1 receptor antagonist (IL1-Ra) among solid-organ transplant recipients. *P* values were calculated by the log-rank test by using the dominant mode of inheritance (patients carrying 1 or 2 copies of each haplotype are compared to the other patients). Patients with mold colonization or IMI before engraftment were excluded from the analyses.

HR, 1.83 [95% CI, 1.02–3.29; $P = .04$] and 2.09 [95% CI, .97–4.50; $P = .06$], respectively).

Effect of *IL1B rs16944* and *IL1RN rs419598* SNPs on *Aspergillus*-Induced Cytokine Release

To determine whether the SNPs associated with IMI and colonization had measurable biological effects, we analyzed the production of different cytokines that are involved in antifungal host defense, including IL-1β, IL-1Ra, TNF-α, IL-17, IL-22, and IFN-γ in PBMCs from 73 healthy volunteers, after stimulation with live or heat-inactivated *A. fumigatus* conidia (Figure 3). PBMCs from volunteers carrying the *IL1B rs16944* TT genotype produced lower amounts of IL-1β ($P = .01$), TNF-α ($P = .03$), and IL-22 ($P = .03$) after stimulation with *A. fumigatus,* compared with PBMCs from volunteers carrying the TC and CC genotypes. However, the production of IL-1Ra, IL-17, and IFN-γ was not significantly influenced by *IL1B rs16944*. PBMCs from volunteers carrying the *IL1RN rs419598* CC genotype produced lower amounts of IL-1β ($P = .03$) and TNF-α ($P = .04$) after stimulation with *A. fumigatus*, compared with PBMCs from volunteers carrying the TC and TT genotypes. However, the production of IL-1Ra itself, as well as IL-22, IL-17 and IFN-γ, was not influenced by *IL1RN rs419598*.

DISCUSSION

While a number of investigators have reported associations between genetic polymorphisms and susceptibility to invasive aspergillosis among patients with onco-hematological conditions [6, 7], the role of such polymorphisms has not been studied among SOT recipients. We are the first to report an association between polymorphisms in *IL1B*, its antagonist *IL1RN*, and *DEFB1* on the susceptibility to IMI in this population.

The *IL1B* gene encodes the cytokine IL-1β, which is essential in host defense against *Aspergillus* infection [15]. IL-1β is a potent proinflammatory cytokine that recruits neutrophils, which are crucial for clearing *Aspergillus* [16], to the lungs during infection. Resting *Aspergillus* conidia in the respiratory epithelium are detected by alveolar macrophages and/or dendritic cells (DCs). These cells express a wide variety of pattern-recognition receptors (PRRs) [17] that detect molecular patterns from the fungal cell wall (eg, o-linked mannan, galactomannan, and β-[1–3]-glucan) [7]. Whereas macrophages produce TNF-α and IL-1β upon recognizing *Aspergillus*, resulting in the recruitment of neutrophils and monocytes, activated DCs will migrate to lymph nodes to induce protective T-helper cell activity. IL-1β induces T-helper type 17 (Th17) responses that are characterized by the production of IL-17, leading to increased recruitment of neutrophils. Additionally, the activated T-helper cells induce IL-22 responses that will stimulate production of defensins by epithelial cells [7, 13]. Thus, IL-1β is a key player in the induction of protective innate and adaptive anti-*Aspergillus* host defense.

Because of its potent inflammatory capacity, the IL-1β response must be tightly controlled. This is underlined by the observation that patients with a mutation in IL-1Ra have severe inflammation of the skin and bones because of uncontrolled neutrophil influx and increased Th17 responses [18]. Moreover IL-1Ra knockout mice were shown to be fully protected from developing invasive aspergillosis [19]. Importantly, galactosaminogalactan, an antiinflammatory cell wall component of *A. fumigatus*, was able to induce IL-1Ra in vivo and consequently suppress the IL-1β pathway, leading to increased susceptibility to invasive aspergillosis [19]. IL-1β binds to the IL-1 receptor, and this results in the recruitment of a second receptor (IL-1RacP) [20] that activates signaling transduction pathways, thereby exerting potent inflammatory activities. IL-1Ra also binds to the IL-1 receptor but prevents recruitment of the second receptor and thus does not activate signal transduction pathways [20]. Therefore, the bioactivity of IL-1β is controlled by IL-1Ra [21].

The polymorphisms associated with mold colonization and IMI in this study are located within the IL-1 cluster, located in chromosome 2, encompassing both *IL1B* and *IL1RN*. We found that the minor alleles of *rs16944* and *rs1143627* within *IL1B* were associated with an increased risk of mold colonization and IMI in SOT recipients. Consistent with our observation, the minor allele of *rs16944* tended to be associated with an increased risk of invasive pulmonary aspergillosis in a case-control study of 110 neutropenic patients with hematological malignancies [22]. The minor alleles of these SNPs were also associated with susceptibility to different bacterial and viral infections. Two studies showed an association between *rs16944* and/or *rs1143627* and mortality due to meningococcal disease [23]. Studies among Chinese patients also showed an association between both SNPs and susceptibility to sepsis after major trauma [24] and 2009 pandemic influenza A(H1N1) virus [25].

It has been previously shown that *rs16944*, located at position −511, which corresponds to a putative AP-2 binding site, and *rs1143627*, located at position −31 within the TATA box, are functional polymorphisms that could be responsible for alteration of promoter activity and thus be able to modulate expression and secretion of IL-1β in vitro [26]. In our study, PBMCs from individuals carrying 2 copies of a rare allele of *IL1B rs16944* had diminished IL-1β release upon *Aspergillus* stimulation. In line with our data, human monocytes from individuals carrying the minor allele of *rs1143627* had moderated transcriptional activity of *IL1B* promoter in response to lipopolysaccharide (LPS) [26]. Moreover, carriers of minor alleles of *rs16944* or *rs1143627* had significantly lower IL-1β levels in LPS-induced PBMCs [27]. However, other studies have reported that the minor allele of *rs16944* was correlated with increased transcriptional activity of the promoter in vitro upon LPS stimulation and high IL-1β secretion [28, 29]. There is no clear

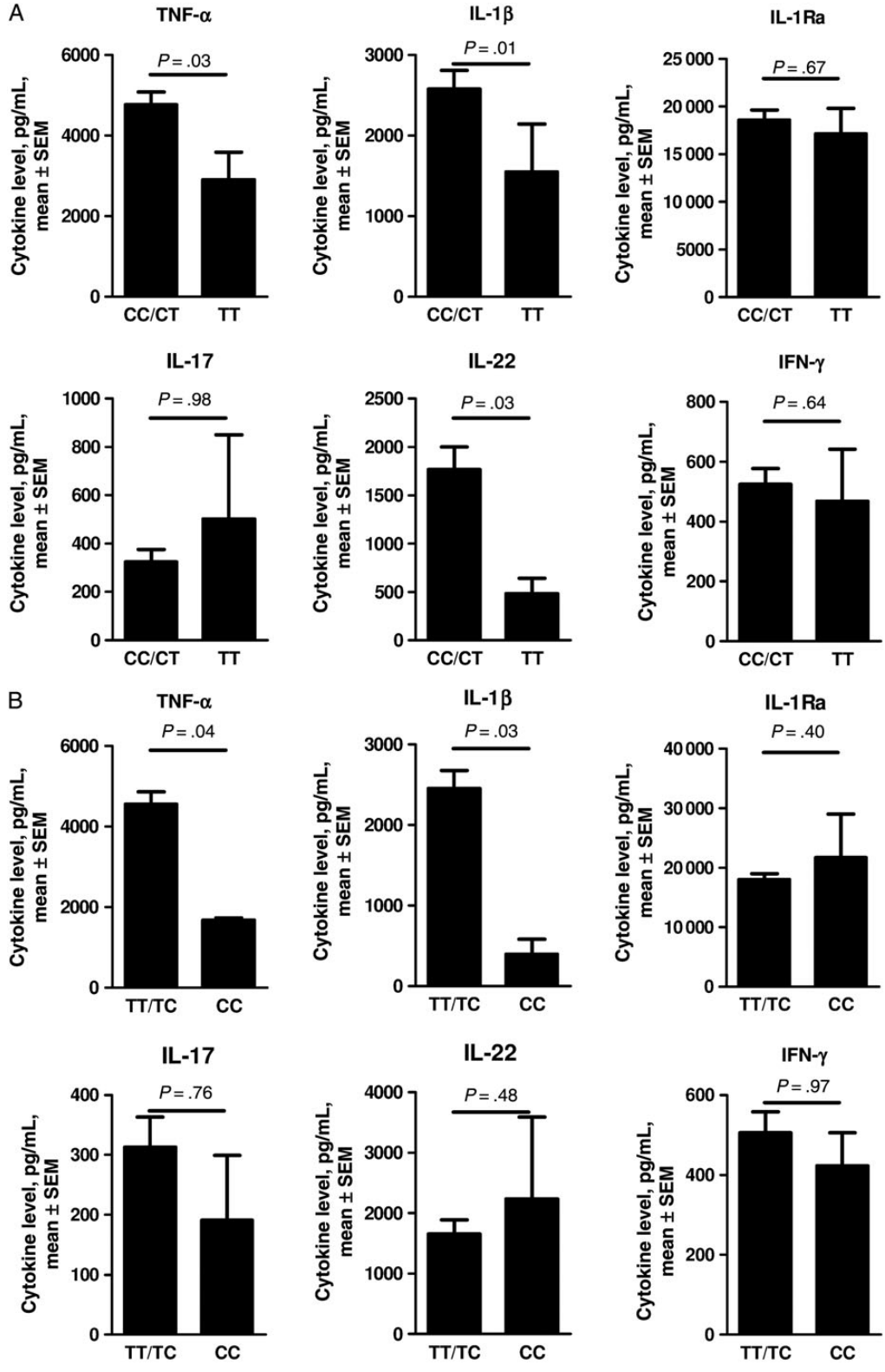

Figure 3. Levels of *Aspergillus*-induced tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), interleukin 1 receptor antagonist (IL-1Ra), interleukin 17 (IL-17), interleukin 22 (IL-22), and interferon γ (IFN-γ) release by human peripheral blood mononuclear cells (PBMCs), depending on the presence or absence of single-nucleotide polymorphisms (SNPs) in 2 copies of the genes encoding IL-1β (*IL1B; rs16944*; *A*) or IL-1Ra (*IL1RN*; *rs419598; B*). PBMCs from 73 healthy volunteers with different *IL1B rs16944* or *IL1RN rs419598* genotypes were stimulated either with live *Aspergillus fumigatus* conidia for 24 hours (for TNF-α, IL-1β, and IL-1Ra) or heat-inactivated *A. fumigatus* conidia for 7 days (for IL-17, IL-22, and IFN-γ). The cytokine levels were measured in cell culture supernatants. Differences in the effects of SNPs on cytokine levels were calculated by the Mann–Whitney *U* test. The data are presented as mean ± standard error of the mean (SEM).

explanation for these discrepant observations, but they may be due, at least in part, to the use of different cells or tissues; the use of different stimuli, stimulating concentrations, or durations; and/or the failure to account for other, so far unknown, regulating factors. We found that the *IL1B rs16944* polymorphism was not only associated with less IL-1β production, but also with reduced TNF-α production in response to *Aspergillus*. TNF-α is another essential cytokine in antifungal host defense, and anti-TNF treatment has previously been associated with invasive aspergillosis [24, 30].

The *IL1RN rs419598* SNP, which is in strong linkage disequilibrium with the *IL1RN* variable number tandem repeat (VNTR) sequence, has also been associated with mold colonization and IMI [31]. Consistent with this finding, the minor allele of *rs419598* was previously associated with severity of meningococcal disease and genital human papillomavirus clearance [23, 32]. To our knowledge, the functional role of *rs419598* has not been explored so far. In the present study, the minor allele of *rs419598* did not influence IL-1Ra production in PBMCs from volunteers stimulated with *A. fumigatus*. However, *rs419598* carriers had significantly lower levels of IL-1β and TNF-α. This is consistent with a previous study, in which *VNTR*2* was associated with decreased IL-1β production in the gastric mucosa, as well as in PBMCs stimulated with LPS [28, 33]. Together, these data suggest that *IL1RN* participates in the regulation of *Aspergillus*-mediated cytokine production and that *IL1RN* polymorphisms may reduce the ability to clear the pathogen.

Table 4. Power to Detect an Association Between Genetic Polymorphisms and Mold Colonization or Invasive Mold Infection (IMI) in Solid-Organ Transplant Recipients

			Colonization (Frequency 4.1%)					IMI (Frequency 2.4%)				
			Power to Detect Colonization			Observed Value		Power to Detect IMI			Observed Value	
Gene (nt, aa Change)[a]	Reference SNP ID	MAF	HR, 3	HR, 4	HR, 5	HR	*P* value[b]	HR, 3	HR, 4	HR, 5	HR	*P* value[b]
IL10 (–1082 A/G)	*rs1800896*	0.44	0.62	0.90	0.98	0.89	.8	0.29	0.57	0.77	0.34	.1
CXCL10 (1642G/C)	*rs3921*	0.41	0.61	0.89	0.98	0.79	.6	0.28	0.55	0.76	0.67	.5
CD209 (–139 A/G)	*rs2287886*	0.36	0.58	0.87	0.97	0.50	.2	0.26	0.52	0.72	0.28	.2
TLR6 (S249P)	*rs5743810*	0.35	0.57	0.86	0.97	1.78	.1	0.25	0.51	0.71	1.47	.4
IL1B (–511 C/T)[c]	*rs16944*	0.33	0.55	0.85	0.96	2.98	.002	0.24	0.49	0.70	4.67	.0002
INFG (874 T/A)	*rs2069705*	0.33	0.55	0.85	0.96	0.17	.08	0.24	0.49	0.70	0.00	1.0
PLG (D472N)	*rs4252125*	0.30	0.51	0.82	0.95	1.45	.4	0.22	0.46	0.66	2.24	.1
IL1A (–889 C/T)	*rs1800587*	0.29	0.50	0.81	0.94	1.24	.6	0.21	0.45	0.65	0.81	.8
TLR3 (L412F)	*rs3775291*	0.29	0.50	0.81	0.94	1.10	.8	0.21	0.45	0.65	0.94	.9
IL10 (–819 C/T)	*rs1800871*	0.27	0.47	0.78	0.93	1.02	1.0	0.20	0.42	0.62	0.56	.6
IL1RN (2018T/C)	*rs419598*	0.25	0.44	0.75	0.91	2.84	.02	0.18	0.39	0.58	3.36	.03
DEFB1 (–44 C/G)	*rs1800972*	0.18	0.30	0.59	0.79	4.08	.003	0.12	0.27	0.42	5.91	.001
MBL (G54D)	*rs1800450*	0.15	0.24	0.49	0.70	0.80	.8	0.09	0.21	0.34	0.00	1.0
SFTPA2 (A91P)	*rs17886395*	0.14	0.22	0.45	0.66	4.79	.009[d]	0.08	0.19	0.31	0.00	1.0
TNF (–308 G/A)	*rs1800629*	0.14	0.22	0.45	0.66	0.00	1.0	0.08	0.19	0.31	0.00	1.0
TLR9 (–1237 C/T)	*rs5743836*	0.13	0.19	0.41	0.61	2.27	.3	0.07	0.17	0.28	4.13	.054
CLEC7A (Y238X)	*rs16910526*	0.08	0.09	0.21	0.34	0.00	1.0	0.03	0.08	0.13	0.00	1.0
TLR1 (R80T)	*rs5743611*	0.07	0.07	0.17	0.28	0.00	1.0	0.03	0.06	0.11	0.00	1.0
IL23R (R381Q)	*rs11209026*	0.07	0.07	0.17	0.28	0.00	1.0	0.03	0.06	0.11	0.00	1.0
TLR4 (D299G)	*rs4986790*	0.05	0.04	0.09	0.16	0.00	1.0	0.02	0.04	0.06	0.00	1.0
MASP2 (D105G)	*rs72550870*	0.02	0.01	0.02	0.03	0.00	1.0	0.005	0.009	0.01	0.00	1.0

Abbreviations: aa, amino acid; *CD209*, gene encoding CD209; *CLEC7A*, gene encoding C-type lectin domain 7; *CXCL10*, gene encoding CXC-chemokine ligand 10; *DEFB1*, gene encoding β-defensin 1; HR, hazard ratio; HWE, Hardy-Weinberg equilibrium; ID, identification; *INFG*, gene encoding interferon γ; *IL1A*, gene encoding interleukin 1α; *IL1B*, gene encoding interleukin 1β; *IL1RN*, gene encoding interleukin 1 receptor antagonist; *IL10*, gene encoding interleukin 10; *IL23R*, gene encoding interleukin 23 receptor; MAF, minor allele frequency; *MASP2*, gene encoding mannan-binding lectin serine peptidase 2; *MBL*, gene encoding mannose binding lectin; nt, nucleotide; *PLG*, gene encoding plasminogen; *SFTPA2*, gene encoding surfactant protein A2; SNP, single-nucleotide polymorphism; *TLR1*, gene encoding Toll-like receptor 1; *TLR3*, gene encoding Toll-like receptor 3; *TLR4*, gene encoding Toll-like receptor 4; *TLR6*, gene encoding Toll-like receptor 6; *TLR9*, gene encoding Toll-like receptor 9; *TNF*, gene encoding tumor necrosis factor.

[a] Three SNPs that deviated from HWE (*TLR1 rs5743618*, *IL4 rs2243250*, and *CARD9 rs10870077*) were excluded from the analyses.

[b] By Cox regression, recessive mode (patients homozygous for the rare alleles are compared to other patients).

[c] In almost complete linkage disequilibrium ($R^2 = 0.99$) with the *IL1B* –31 T/C *rs1143627*.

[d] $P = .01$ for the additive mode (HR, 1.91; 95% confidence interval, 1.15–3.17).

DEFB1 encodes human β-defensin 1, a member of the defensin family. Defensins are encoded by a polymorphic gene cluster located within a 450-kb region on chromosome 8p23 [34]. They exert antimicrobial activity against a broad spectrum of pathogens; they also exert chemotactic activity on immune cells and are able to induce cytokine production [35, 36]. β-defensin 1 is constitutively expressed in lung epithelial cells and was shown to exert antimicrobial properties against *Candida albicans* [37]. To our knowledge, the antimicrobial properties of this β-defensin against other fungal pathogens have not been investigated.

The minor allele of the *rs1800972* SNP in *DEFB1* was associated with an increased risk of mold colonization and IMI in SOT recipients. In line with our findings, the same allele was previously associated with higher-level oral *Candida* carriage among diabetic and nondiabetic patients [38]. The *rs1800972* SNP is located at position −44 from the 5′ untranslated region of *DEFB1* and was predicted to alter a putative transcription factor binding site to this region of the gene [39, 40]. The minor allele of *rs1800972* has been previously associated with lower β-defensin 1 expression in the skin with increased nasal carriage of *Staphylococcus aureus* [41]. This allele may also decrease the expression of β-defensin 1 in the respiratory epithelium, with reduced ability to clear opportunistic pathogens such as mold under certain immunosuppressive conditions. Interestingly, the cytokine IL-22 is a potent inducer of defensins produced by epithelial cells [42, 43]. The *IL1B rs16944* SNP was associated with a significant reduction in *Aspergillus*-induced IL-22 production in human PBMCs. Therefore, it can be proposed that this SNP may increase susceptibility, not only because of the low production of essential innate immune system cytokines such as IL-1β and TNF-α, but also because of a reduced induction of IL-22 that might result in defective induction of defensins.

SFTPA2 encodes pulmonary surfactant-associated protein 2 (SP-A2), a member of the collagen-containing soluble C-type lectin receptors (collectins). SP-A2 was shown to play an important role in host response to *Aspergillus* by its ability to bind *A. fumigatus* conidia and thus enhance phagocytosis by neutrophils and macrophages [44]. Polymorphisms in the collagen region of *SFTPA2* (G1649C and/or A1660G) have been associated with chronic cavitary pulmonary aspergillosis [45] and allergic bronchopulmonary aspergillosis in asthmatic patients [46]. Here, we detected association between a G1649C polymorphism and mold colonization but not invasive mold infection in SOT recipients. The lack of association with infection may be due to insufficient statistical power due to the relatively small SNP allele frequency and sample size, as detailed in Table 4.

By using 24 polymorphisms from 21 genes previously associated with susceptibility to fungal infections, we showed that SNPs from 3 genes (*IL1B*, *IL1RN*, and *DEFB1*) were associated with mold colonization or IMI in white SOT recipients. Mold colonization may have been underestimated, as bronchoscopy is not routinely performed, especially in nonthoracic SOT recipients. Yet, the polymorphisms associated with colonization were also associated with IMI, suggesting that these polymorphisms influence both phenotypes, with a continuum from colonization to invasive infection. We were not able to detect an association with polymorphisms in other genes, such as *TLR4* or *CLEC7A* (which encodes dectin 1), that were previously reported to be associated with invasive aspergillosis by several investigators [6, 7]. This may be due to several factors, such as the inclusion of different study populations, fungal pathogens, and/or less invasive forms of infections. Importantly, the number of patients with mold colonization and/or IMI was small, leading to limited statistical power, especially for infrequent SNPs, as detailed in Table 4. Larger studies and studies in patients who are not white will be needed to replicate such associations.

We report an association between polymorphisms in 3 genes and mold colonization and IMI in a nationwide cohort of SOT recipients. Theses associations were found to be independent from previously known risk factors, such as recipient age and CMV coinfection [3]. The genes were formerly described as important components of immune defenses against fungal pathogens, and the associated polymorphisms were all shown to be functionally relevant. Together, these findings may contribute to a better understanding of the pathogenesis of IMI in SOT recipients and help in individual risk stratification in the future.

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Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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