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

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Abstract

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

Methods: 24 SNPs from systematically selected genes were genotyped among 1101 SOT recipients (715 kidneys, 190 liver, 102 lungs, 79 hearts, 15 other) from the Swiss Transplant Cohort Study. Association between SNPs and the endpoint were assessed by log-rank test and Cox regression models. Cytokine production upon *Aspergillus* stimulation was measured by ELISA in 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 interleukin-1 beta *(IL1B*, *rs16944*; log-rank test, recessive mode, colonization P=0.001 and IMI P=0.00005), interleukin-1 receptor antagonist (*IL1RN*, *rs419598*; P=0.01 and P=0.02) and β-defensin-1 (*DEFB1*, *rs1800972*; P=0.001 and P=0.0002, respectively). The associations with *IL1B* and *DEFB1* remained significant in a multivariate regression model (*IL1B rs16944* P=0.002; *DEFB1 rs1800972* P=0.01). Presence of two copies of the rare allele of *rs16944* or *rs419598* was associated with reduced *Aspergillus*-induced IL-1β and TNFα 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.

Introduction

Over 100'000 solid organ transplants (SOT) 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 ranging 20-76% [3, 4]. Risk factors for the development of invasive aspergillosis include the type and level of immunosuppression, use of renal replacement therapy, older age, and CMV disease [3, 5]. However, not all patients with these risk factors develop invasive mold infections (IMI), while some patients without these risk factors do, making it difficult to predict the risk to develop IMI at the individual level.

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 onco-hematological patients [6, 7]. The identification of specific genetic variants may improve individual risk stratification and allow the development of personalized management strategies, as well as to use 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. We explored for the first time the role of host genetics in susceptibility to IMI in a nationwide cohort of 1101 SOT recipients.

Materials and Methods

Patients and study design. The STCS is a large, nationwide, well documented prospective cohort including all SOT recipients followed at six Swiss University transplant centers (Basel, Bern, Geneva, Lausanne, St Gallen and Zurich) since May 2008 [8]. Patient data were systematically collected at enrollment, at six months and every 12 months after transplant on standardized case report forms.

Infectious complications were systematically evaluated by an infectious disease specialist based on clinical, histological, radiological and mycological evidence and reported on a separate case report form. Charts from patients reported to have fungal colonization or IMI were revised by an independent investigator (TL) [9]. Proven or probable IMI, was defined based on standardized EORTC/MSG guidelines definitions [10] and adapted ISHLT guidelines 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 of a non-sterile site, including sputum, bronchoalveolar lavage, bronchial brush, sinus aspirate samples or urine in the absence of clinical signs/symptoms for infection. Patients who were diagnosed with mold colonization and/or IMI before transplant, and/or had received previous organ transplantation, 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 to the STCS (including genetic analyses). The protocol was approved by the independent ethics Committees of each Swiss participating center (University Hospital of Lausanne (CHUV); University Hospitals

of Geneva (HUG); University Hospital Zürich (USZ); Cantonal Hospital St. Gallen (KSSG); Inselspital, Bern University Hospital; Clinica Luganese, Lugano; University Hospital of Basel). For functional work fresh venous blood was collected 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 cells (PBMCs) isolation and *in vitro* stimulation assays. PBMCs were isolated using Ficoll®-Paque Plus (GE healthcare, Zeist, The Netherlands) density gradient centrifugation method as described previously [13]. Cells were subsequently stimulated with $1x10^7$/ml live or heat-inactivated *Aspergillus fumigatus* conidia for 24 hours or 7 days, respectively. Afterwards concentrations of the cytokines TNFα, IL-1β, IL-1Ra, IL-17, IL-22 (R&D systems, Minneapolis MN, USA) and IFNγ (Sanquin, Amsterdam, the Netherlands) were measured in cell supernatants by ELISA according to the manufacturer's protocol.

Genotyping. A total of 24 SNPs in 21 genes were selected from the literature (Table 2) by performing a PubMed search until June 2012 using the keywords: candidemia, candidiasis, aspergillosis, SNP and/or previous reviews on fungal immunogenetics [6, 7]. Blood samples were obtained from all SOT recipients at the time of transplantation. Genomic DNAs was extracted from patients or healthy volunteer EDTA blood using the Gentra Puregene Blood Kit (Qiagen). Genotyping was performed using a customized GoldenGate Genotyping Assay on Veracode® platform (Illumina®, San Diego, CA, USA), unless otherwise indicated. Results were analyzed on a BeadXpress® Reader according to standard protocols and quality controls. Additional SNPs were genotyped using Competitive Allele-Specific PCR (KASP™) system (LGC Genomics, Herts, UK). For functional studies genotyping of the *IL1B rs16944* and *IL1RN rs419598* variants was performed using pre-designed SNP assays on the ABI-Prism StepOne thermocycler (Applied Biosystems®).

Statistical analysis. Statistical analyses were performed in Stata13® (StataCorp LP, College Station, Texas, USA), 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 or death. Associated variants were selected based on the log-rank test and were further tested by uni- and multivariate Cox models. In order to estimate the independent contribution of each polymorphism to the endpoints, demographic and clinical factors previously associated with mold colonization and/or IMI (as described elsewhere [9], [5]), were entered into multivariate stepwise regression models ($P<0.2$) together with relevant genetic polymorphisms. Haplotypes were inferred using PHASE 2.1 (University of Washington, Seattle, WA, USA). Power calculation for Cox proportional hazard regression 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 presence of SNPs on the *Aspergillus*-induced cytokine levels was determined by the Mann-Whitney U test. The data are presented as mean ± standard error of the mean (SEM) and were analysed using Graphpad Prism v5.0 (San Diego, CA, USA).

Results

Cohort Study

The study included 1101 Caucasian patients who received solid organ transplantation (670 kidneys, 190 liver, 102 lungs, 79 hearts, 15 islets/pancreas and 45 combined organs) between May 2008 and December 2011 (**Table 1**). Mold colonization and IMI were diagnosed in 45 (4.1%) and 26 (2.4%) patients, respectively. Most IMI occurred >3 months after transplantation (N=17, 75%). The most frequent causative organism of IMI was *Aspergillus spp.* (N=21, 81%);

only five IMIs were due to other fungi, including *Fusarium spp.* (N=2), *Altenaria spp.* (N=1), *Zygomycetes spp.* (N=1) and mixed pathogens (*Zygomycetes* and *Fusarium spp.,* N=1). 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 patients

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 the cumulative incidence of colonization and infection during the first 36 months after transplantation (**Table 2**). Mold colonization and IMI were both associated with SNPs in three different genes, including *IL1B* (*rs16944* TT versus CT or CC; log-rank test P=0.001 and P=0.00005), β-defensin 1 (*DEFB1, rs1800972* CC versus GG or CG, P=0.001 and P=0.0002) and the interleukin-1 receptor antagonist (*IL1RN, rs419598* CC versus CT or TT, P=0.01 and P=0.02, respectively, **Figure 1**). In addition, we observed a significant association between a SNP in surfactant-associated protein 2 and mold colonization (*SFTPA2*, rs17886395 GG versus CC or CG, log-rank test P=0.004) but not infection (P=0.5). However, this association was due to a small number of individuals (**Figure S4**).

To determine whether the SNPs were independent risk factors for the 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* (HR=2.52, CI 1.18-5.36, P=0.02), *DEFB1 rs1800972* (HR=6.11, CI 2.28-16.4, P=0.0003) and *IL1RN rs419598*

(HR=3.35, CI 1.31-8.58, P=0.01). The final model for IMI still included *IL1B rs16944* (HR=4.29, CI 1.71-10.8, P=0.002), *DEFB1 rs1800972* (HR=4.73, CI 1.46-15.3, P=0.01), but not *IL1RN rs419598*. Associations were stronger when the SNPs were combined together (for *IL1B rs16944* and *DEFB1 rs1800972*, HR=4.94; CI 2.06-11.8; P=0.003; for *IL1B rs16944* and *IL1RN rs419598* HR=4.64, CI 1.92-11.2, P=0.0006; **Figure S1**). In order to account for a possible confounding role of antifungal prophylaxis, the analyses were repeated after removal of patients who received an anti-mold prophylaxis. The associations between the SNPs in *IL1B, DEFB1* and *IL1RN* were still significant (not shown).

IL1B rs16944 **and** ***IL1RN rs419598*** **risk haplotype for mold colonization and IMI.**

IL1B and IL1RN genes are located within a ~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 other, HR=0.34, CI 0.18-0.63, P=0.0007 and HR=0.21, CI 0.10-0.45, P=0.00008, respectively). Reversely, carriage of the T-C haplotype was associated with an increased risk for both phenotypes (T-C haplotype vs. all other, HR=1.83, CI 1.02-3.29, P=0.04 and HR=2.09, CI 0.97-4.50, P=0.06, respectively).

Effect of ***IL1B rs16944*** **and** ***IL1RN rs419598*** **polymorphisms on** ***Aspergillus*****-induced cytokine release.**

In order 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-1 receptor antagonist (IL-1Ra), TNFα, IL-17, IL-22

and IFNγ in PBMCs from 73 healthy volunteers, after stimulation with live or heat-inactivated *A. fumigatus* conidia, respectively (**Figure 3**). PBMCs from volunteers carrying the *IL1B rs16944* TT genotype produced lower amounts of IL-1β (P=0.01), TNFα (P=0.03) and IL-22 (P=0.03) after stimulation with *A. fumigatus,* compared to PBMCs from volunteers carrying the TC and CC genotypes. However, the production of IL-1Ra, IL-17 and IFNγ was not significantly influenced by *IL1B rs16944.* PBMCs from volunteers carrying the *IL1RN rs419598* CC genotype produced lower amounts of IL-1β (P=0.03) and TNFα (P=0.04) after stimulation with *A. fumigatus*, compared to PBMCs from volunteers carrying the TC and TT genotypes. However, the production of IL-1Ra itself as well as IL-22, IL-17 and IFNγ was not influenced by *IL1RN rs419598*.

Discussion

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

The *IL1B* gene encodes for the cytokine IL-1β that is essential in host defense against *Aspergillus* infection [15]. IL-1β is a potent pro-inflammatory cytokine that recruits neutrophils to the lungs during infection, which are crucial for clearing *Aspergillus* [16]. 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 (e.g. o-linked mannan, galactomannan and β-(1-3)-glucan) [7]. Whereas macrophages produce TNFα 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_H17 responses that are characterized by the production of IL-17, leading to an 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, IL-1β responses need to 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 due to uncontrolled neutrophil influx and increased T_H17 responses [18]. Moreover IL-1Ra knockout mice were shown to be fully protected from developing invasive aspergillosis [19]. Importantly galactosaminogalactan, an anti-inflammatory 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. 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 H1N1 pandemic influenza A virus [25].

It has been previously shown that *rs16944* located at position -511 corresponding to a putative AP-2 binding site and *rs1143627* located at position -31 within TATA box, are functional polymorphisms that could be responsible for alteration of promoter activity and thus being able to modulate expression and secretion of IL-1β *in vitro* [26]. In our study, PBMCs from individuals carrying two copies of rare allele of *IL1B rs16944* had diminished IL-1β release upon *Aspergillus* stimulation. In line with our data, human monocytes from individuals carrying 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 explanation for these discrepant observations, but they may be due, at least in part, to the use of different cells or tissues and/or stimuli, stimulating concentrations or durations, or 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 reduced TNFα production in response to *Aspergillus.* TNFα 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* VNTR, 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 (HPV) 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 TNFα. This is consistent with a previous study, in which the *VNTR*2* was associated with decreased IL-1β in the gastric mucosa as well as in PBMCs stimulated with LPS [28, 33]. Altogether, these data suggest that *IL1RN* participates to the regulation of *Aspergillus*-mediated cytokines production, and that *IL1RN* polymorphism may reduce the ability to clear the pathogen.

DEFB1 encodes human β-defensin 1, a member of the defensins family. Defensins are encoded by 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 a chemotactic activity on immune cell 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 *C. albicans* [37]. To our knowledge, the antimicrobial properties of this β-defensin against other fungal pathogens have not been investigated so far.

The minor allele of *rs1800972* SNP in *DEFB1* was associated with an increased risk of mold colonization and IMI in SOT. In line with our findings, the same allele was previously associated with higher oral *Candida* carriage among diabetic and non-diabetic patients [38]. The *rs1800972* SNP is located at position -44 of 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 cytokine such as IL-1β and TNFα, 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 collagen containing soluble CLRs (collectins). The 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 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 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 SNPs allele frequency and sample size as detailed in Table 4.

By using a list of 24 polymorphisms from 21 genes previously associated with susceptibility to fungal infections, we showed that SNPs from three genes (*IL1B*, *IL1RN* and *DEFB1*) were associated with mold colonization or IMI in Caucasian SOT recipients. Mold colonization may have been underestimated, as bronchoscopy is not routinely performed, especially in non thoracic 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* (encoding Dectin-1), that were previously reported to be associated with invasive aspergillosis by several investigators [6, 7]. This may be due to several reasons, 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 a limited statistical power, especially for infrequent SNPs, as detailed in Table 4. Larger studies and studies in patient from non-Caucasian ethnicities will be needed to replicate such associations.

We report an association between polymorphisms in three 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 the recipient age, cytomegalovirus co-infection [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. Altogether, 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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Footnote

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Conflict of Interests

The authors have declared that no competing interests exist.

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Meetings where information has been previously presented

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Figure legend

Figure 1

Cumulative incidence of mold colonization (N=45) and invasive mold infection (N=26) according to *IL1B rs16944* (panel A, and B, respectively), *β-defensin 1 rs1800972* (*DEFB1,* panel C and D, respectively) and interleukin-1 receptor antagonist *rs419598* (*IL1RN*, panel E, and F, respectively) polymorphisms in solid organ transplant recipients. Patients who were colonized or infected with mold before engraftment were excluded from the analyses. P values were calculated by log-rank test, recessive mode (patients homozygous for the rate alleles are compared to the other). For *IL1B rs16944* and *DEFB1 rs1800972* SNPs P values remained significant after correction for multiple testing (21 tests; colonization for both polymorphisms P=0.02; infection P=0.001, and P=0.004, respectively).

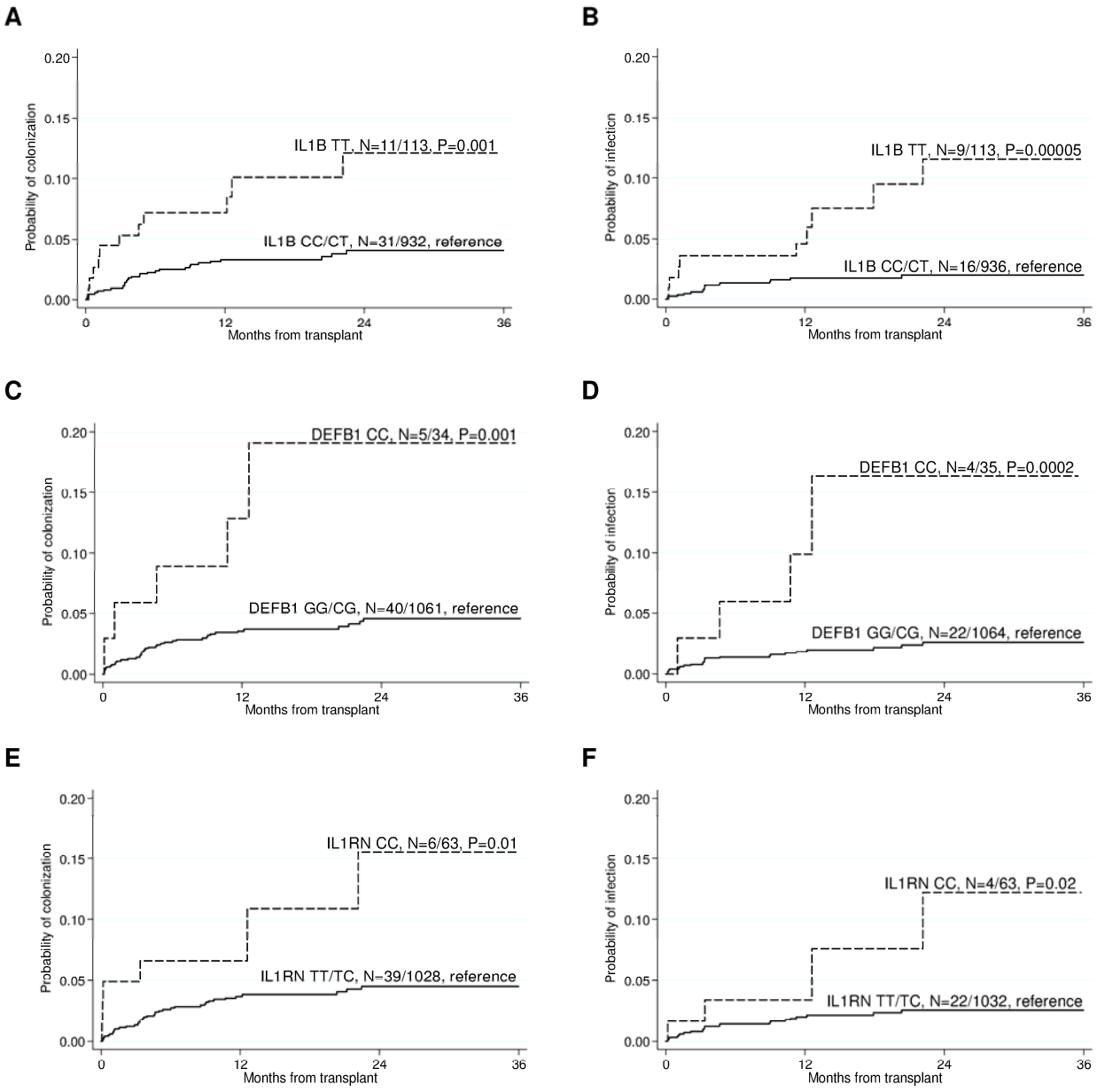
Figure 2

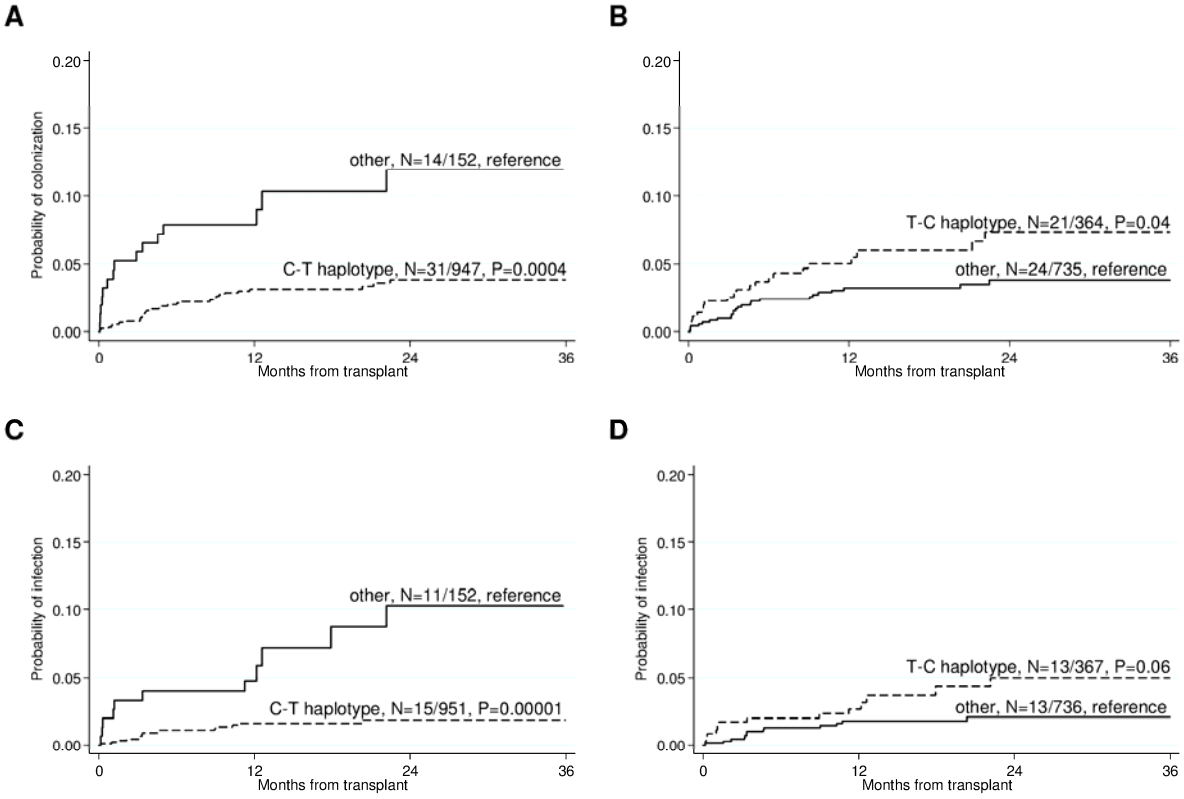
Cumulative incidence of mold colonization (Panel A and B) and invasive mold infection (Panel C and D) according to *rs16944-rs419598* C-T (Panel A and C) or T-C (Panel B and D) haplotype of *IL1B* and *IL1RN polymorphisms* in solid organ transplant recipients. P values were calculated by the log-rank test by using the dominant mode of inheritance (patients carrying one or two copies of each haplotype are compared to the other). Patients who were colonized or infected with mold before engraftment were excluded from the analyses.

Figure 3

Levels of *Aspergillus*-induced TNFα, IL-1β, IL-1Ra, IL-17, IL-22, IFNγ release by human peripheral blood mononuclear cells depending on presence or absence of two copies of *IL1B rs16944* (A) or *IL1RN rs419598* (B) SNPs. Peripheral blood mononuclear cells (PBMCs)

from 73 healthy volunteers with different *IL1B rs16944* or *IL1RN rs419598* genotypes were stimulated either with live *A. fumigatus* conidia for 24 hours (for TNFα, IL-1β, IL-1Ra) or heat inactivated *A. fumigatus* conidia for 7 days (for IL-17, IL-22, IFNγ). The cytokine levels were measured in cell culture supernatants and given in pg/ml. The effect of SNPs on cytokine levels were calculated by Mann-Whitney U test. The data are presented as mean ± standard error of the mean (SEM).





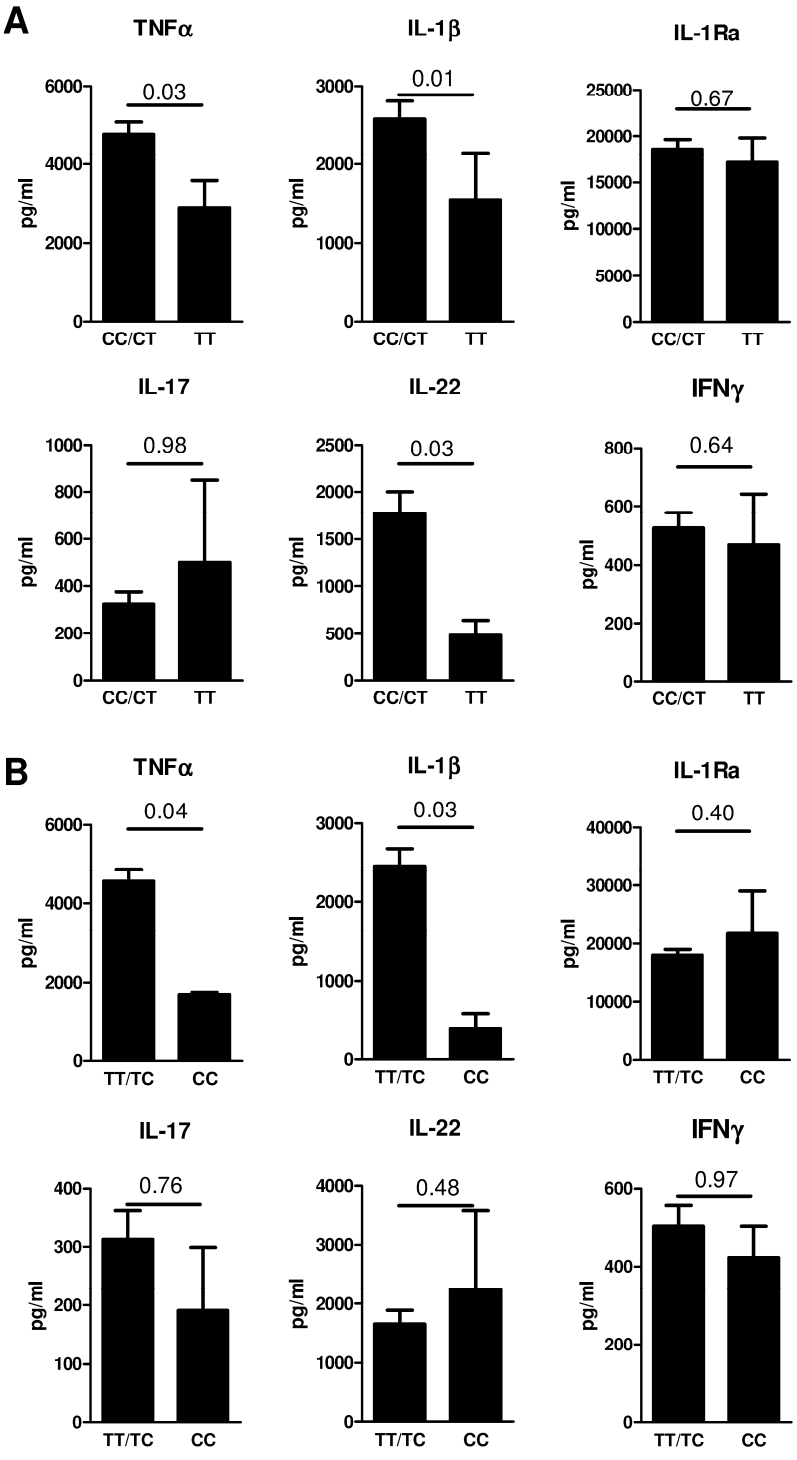


Table 1. Demographic characteristic of the solid organ transplant recipients (N=1101)

Variable	N	(%)
Recipient age (median years; iqr)	54	(19)
Donor age (median years; iqr) [1]	53	(22)
Recipient sex M/F	730 / 371	(66 / 34)
Donor sex M/F [2]	573 / 523	(52 / 48)
Caucasian ethnicity	1101	(100)
Duration of cold ischemia (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 [3]	45	(4)
Donor type [4]		
Deceased	801	(73)
Living related/unrelated	299	(27)
Rejection type [5]		
Acute cellular rejection	342	(31)
Acute humoral rejection	35	(3)
CMV infection/disease	279/61	
CMV serostatus (N=1186) [6]		
D+ R+	350	(33)
D- R+	256	(24)
D- R-	245	(24)
D+ R-	218	(20)
Induction therapy [7]		
Basiliximab (BAS)	641	(61)
Anti-thymocyte globulin +/- BAS	188	(18)
None	225	(21)
Maintenance regiment [8]		
Calcineurin inhibitors	817	(92)
Corticosteroids	910	(83)
MMF	721	(81)
AZA	31	(3)
mTOR inhibitors	54	(6)
Anti IMI prophylaxis (weeks 1-4)	74	(7)

1 Data were missing in 104 patients.
2 Data were missing in 5 patients.
3 Including kidney-pancreas N=24, kidney-liver N=10, kidney-kidney N=5, kidney-islets N=3, kidney-kidney-pancreas N=2, kidney-lung N=1 transplants.
4 Data were missing in 2 patients.
5 Reported at any time of follow-up.
6 Data were missing in 32 patients.
7 Data were missing in 47 patients.
8 Reported at month 12.

Abbreviations: AZA: azathiopirine; IMI: invasive mold infection; MMF: mycophenolate mofetil

Table 2. Association of genetic polymorphism with mold colonization or invasive mold infection in solid organ transplant recipients (N=1101).

				Mold colonization (N=45) [2]	IMI (N=26) [2]
Gene (nt|aa change) [1]	**Rs number**	**MAF**	**HWE**	**log-rank test P value** [3]	**log-rank test P value** [3]
IL1B (-511 C/T)	rs16944	0.33	0.160	0.001 [4]	0.00005 [6]
DEFB1 (-44 C/G)	rs1800972	0.18	1.000	0.001 [4]	0.0002 [5]
IL1RN (2018T/C)	rs419598	0.25	0.320	0.01	0.02
TLR9 (-1237 C/T)	rs5743836	0.13	0.060	0.2	0.04
INFG (874 T/A)	rs2069705	0.33	0.086	0.05	0.06
PLG (D472N)	rs4252125	0.30	0.340	0.4	0.1
CD209 (-139 A/G)	rs2287886	0.36	0.610	0.2	0.2
IL10 (-1082 A/G)	rs1800896	0.44	0.220	0.8	0.2
TLR6 (S249P)	rs5743810	0.35	0.180	0.1	0.4
TNF (-308 G/A)	rs1800629	0.14	0.010	0.2	0.4
SFTPA2 (A91P)	rs17886395	0.14	0.900	0.004	0.5
CXCL10 (1642G/C)	rs3921	0.41	0.280	0.6	0.5
MBL (G54D)	rs1800450	0.15	0.099	0.8	0.4
IL10 (-819 C/T)	rs1800871	0.27	0.820	0.8	0.6
TLR1 (R80T)	rs5743611	0.07	0.500	0.6	0.7
TLR4 (D299G)	rs4986790	0.05	0.540	0.8	0.8
IL1A (-889 C/T)	rs1800587	0.29	0.620	0.6	0.8
MASP2 (D105G)	rs72550870	0.02	0.150	0.8	0.8
CLEC7A (Y238X)	rs16910526	0.08	0.340	0.7	0.8
TLR3 (L412F)	rs3775291	0.29	1.000	0.9	0.9
IL23R (R381Q)	rs11209026	0.07	0.811	0.7	0.7

[1] Three SNPs that deviated from Hardy-Weinberg equilibrium (*TLR1 rs5743618*, *IL4 rs2243250* and *CARD9 rs10870077*) were excluded from the analyses.
[2] Five patients who were colonized with mold before transplant, among whom two also developed IMI before transplant, were removed from the analyses.
[3] P value was assessed by log-rank test, recessive mode (patients homozygous for the rare alleles are compared to the other).
[4] Significant after Bonferroni correction for multiple testing (21 tests; P=0.02).
[5] Significant after Bonferroni correction for multiple testing (21 tests; P=0.004).
[6] Significant after Bonferroni correction for multiple testing (21 tests; P=0.001).

Abbreviations: CARD9: caspase recruitment domain-9; CI: confidence interval; CLEC7A: C-type lectin domain 7; CXCL10: CXC-chemokine ligand-10; CD209: CD209 molecule; DEFB1: β-defensin 1; HR: hazard ratio; IL: interleukin; IL1RN: interleukin-1 receptor antagonist; ; IL23R: interleukin 23 receptor; IMI: invasive mold infection; MBL: mannose banding lectin; MASP2: mannan-binding lectin serine peptidase 2; MAF: minor allele frequency; PLG: plasminogen; SFTPA2: surfactant protein A2; TLR: toll-like receptor; TNF: tumor necrosis factor.

Table 3. Independent factors associated with mold colonization and invasive mold infections in solid organ transplant recipients.

	Mold colonization (N=42) [1]		IMI (N=25) [1]	
Variable	**HR (95%CI)**	**Cox P value [2]**	**HR (95%CI)**	**Cox P value [2]**
IL1B rs16944 (TT vs CC/CT) [3]	2.52 (1.18-5.36)	0.02	4.29 (1.71-10.8)	0.002 [5]
DEFB1 rs1800972 (CC vs GG/GC) [3]	6.11 (2.28-16.4)	0.0003 [4]	4.73 (1.46-15.3)	0.01
IL1RN rs419598 (CC vs TT/TC) [3]	3.35 (1.31-8.58)	0.01	2.50 (0.75-8.29)	0.1
Lung or heart transplantation	11.5 (5.83-22.6)	<0.0001	3.12 (1.21-8.03)	0.02
MMF	0.32 (0.16-0.63)	0.001	0.14 (0.06-0.33)	<0.0001
Tacrolimus	0.52 (0.27-1.03)	0.06	0.45 (0.19-1.09)	0.1
Corticosteroids	-	-	3.03 (0.67-13.7)	0.1
Acute/chronic rejection	-	-	2.35 (0.94-5.83)	0.07
CMV infection/disease	1.83 (0.89-3.72)	0.1	2.68 (1.11-6.50)	0.03
Recipient age (per year)	1.04 (1.01-1.06)	0.008	1.06 (1.02-1.10)	0.004

[1] The total number of patient in the multivariate analysis (N=1047) was slightly lower than in univariate analysis (N=1101) due to missing covariates. Five patients who were colonized with mold before transplant, among whom two also developed IMI before transplant, were removed from the analyses.

[2] Multivariate analysis assessed by 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.

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

[4] Significant after Bonferroni correction for multiple testing (N=21, P=0.006).

[5] Significant after Bonferroni correction for multiple testing (N=21, P=0.04).

Abbreviations: CI: confidence interval; CMV: cytomegalovirus; HR: hazard ratio; DEFB1: β-defensin 1; IL: interleukin; IL1RN: interleukin-1 receptor antagonist; IMI: invasive mold infection; MMF: mycophenolate mofetil

Table 4. Power to detect association of genetic polymorphism with mold colonization or invasive mold infection in solid organ transplant recipients.

				Colonization (Frequency 4.1%)					IMI (Frequency 2.4%)				
				Power to detect colonization			Observed values		Power to detect IMI			Observed values	
Gene (nt|aa change) [1]	rs number	MAF	HWE	HR=3	HR=4	HR=5	HR	Cox P [2]	HR=3	HR=4	HR=5	HR	Cox P [2]
IL10 (-1082 A/G)	rs1800896	0.44	0.220	0.62	0.90	0.98	0.89	0.8	0.29	0.57	0.77	0.34	0.1
CXCL10 (1642G/C)	rs3921	0.41	0.280	0.61	0.89	0.98	0.79	0.6	0.28	0.55	0.76	0.67	0.5
CD209 (-139 A/G)	rs2287886	0.36	0.610	0.58	0.87	0.97	0.50	0.2	0.26	0.52	0.72	0.28	0.2
TLR6 (S249P)	rs5743810	0.35	0.180	0.57	0.86	0.97	1.78	0.1	0.25	0.51	0.71	1.47	0.4
IL1B (-511 C/T) [4]	rs16944	0.33	0.160	0.55	0.85	0.96	2.98	0.002	0.24	0.49	0.70	4.67	0.0002
INFG (874 T/A)	rs2069705	0.33	0.086	0.55	0.85	0.96	0.17	0.08	0.24	0.49	0.70	0.00	1.0
PLG (D472N)	rs4252125	0.30	0.340	0.51	0.82	0.95	1.45	0.4	0.22	0.46	0.66	2.24	0.1
IL1A (-889 C/T)	rs1800587	0.29	0.620	0.50	0.81	0.94	1.24	0.6	0.21	0.45	0.65	0.81	0.8
TLR3 (L412F)	rs3775291	0.29	1.000	0.50	0.81	0.94	1.10	0.8	0.21	0·45	0.65	0.94	0.9
IL10 (-819 C/T)	rs1800871	0.27	0.820	0.47	0.78	0.93	1.02	1.0	0.20	0.42	0.62	0.56	0.6
IL1RN (2018T/C)	rs419598	0.25	0.320	0.44	0.75	0.91	2.84	0.02	0.18	0.39	0.58	3.36	0.03
DEFB1 (-44 C/G)	rs1800972	0.18	1.000	0.30	0.59	0.79	4.08	0.003	0.12	0.27	0.42	5.91	0.001
MBL (G54D)	rs1800450	0.15	0.099	0.24	0.49	0.70	0.80	0.8	0.09	0.21	0.34	0.00	1.0
SFTPA2 (A91P)	rs17886395	0.14	0.900	0.22	0.45	0.66	4.79	0.009 [3]	0.08	0.19	0.31	0.00	1.0
TNF (-308 G/A)	rs1800629	0.14	0.010	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	0.060	0.19	0.41	0.61	2.27	0.3	0.07	0.17	0.28	4.13	0.054
CLEC7A (Y238X)	rs16910526	0.08	0.340	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	0.500	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	0.811	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	0.540	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	0.150	0.01	0.02	0.03	0.00	1.0	0.005	0.009	0.01	0.00	1.0

[1] Three SNPs that deviated from Hardy-Weinberg equilibrium (*TLR1 rs5743618*, *IL4 rs2243250* and *CARD9 rs10870077*) were excluded from the analyses.
[2] P value was assessed by Cox regression, recessive mode (patients homozygous for the rare alleles are compared to the other).
[3] P value for the additive mode was 0.01, HR=1.91, CI 1.15-3.17.
[4] In almost complete linkage disequilibrium (LD=0.99) with the IL1B -31T/C *rs1143627*.

Abbreviations: CARD9: caspase recruitment domain-9; CI: confidence interval; CLEC7A: C-type lectin domain 7; CXCL10: CXC-chemokine ligand-10; CD209: CD209 molecule; DEFB1: β-defensin 1; HR: hazard ratio; IL: interleukin; IL1RN: interleukin-1 receptor antagonist; ; IL23R: interleukin 23 receptor; IMI: invasive mold infection; MBL: mannose banding lectin; MASP2: mannan-binding lectin serine peptidase 2; MAF: minor allele frequency; PLG: plasminogen; SFTPA2: surfactant protein A2; TLR: toll-like receptor; TNF: tumor necrosis factor.