

PTX3 Polymorphisms and Invasive Mold Infections After Solid Organ Transplant

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Donor *PTX3* polymorphisms were shown to influence the risk of invasive aspergillosis among hematopoietic stem cell transplant recipients. Here, we show that *PTX3* polymorphisms are independent risk factors for invasive mold infections among 1101 solid organ transplant recipients, thereby strengthening their role in mold infection pathogenesis and patients' risk stratification.

Keywords. innate immunity; SNP; *PTX3*; genetic susceptibility; solid organ transplant.

Invasive mold infections (IMIs) represent an important cause of morbidity and mortality in transplant recipients [1, 2]. Although specific risk factors have been identified in both hematopoietic stem cell transplant (HSCT) and solid organ

transplant (SOT) recipients, such as patient age, comorbidities, conditioning regimen, cytomegalovirus (CMV) infection, renal failure, and level of immunosuppression [1, 3], it is still difficult to accurately predict which patients will develop this complication [4].

An increasing number of studies are highlighting a role for genetic polymorphisms in susceptibility to invasive fungal infections [4]. So far, due to numerous limitations, existing data have not supported the use of such polymorphisms for individual risk stratification in the clinical practice. A major limiting factor is the inability to replicate the association, especially when studies are performed in populations that differ in terms of baseline characteristics or immunosuppressive regimen [4].

Pentraxin 3 (*PTX3*) is a soluble pattern recognition receptor (PRR) produced by neutrophils, dendritic cells, macrophages, and epithelial cells that has been shown to exert important antifungal protection [5]. Polymorphisms in the *PTX3* gene in the donor have been recently associated with increased susceptibility to invasive aspergillosis (IA) among HSCT recipients [6]. Here, we show that polymorphisms in *PTX3* also increase susceptibility to IMIs among SOT recipients. This observation strengthens the role of *PTX3* polymorphisms in immune defenses against fungal pathogen and their potential use as a predictor for infection in clinical practice.

MATERIALS AND METHODS

Patients and Study Design

The Swiss Transplant Cohort Study (STCS) is a large national cohort of SOT recipients followed at 6 Swiss university centers [7, 8]. For the present study, SOT recipients enrolled prospectively from May 2008 to December 2011 who provided written informed consent for participation to genetic studies within the STCS were included. The protocol was approved by the ethics committees of all participating centers. Patients' data were collected at enrollment, at 6 months, and every 12 months after transplantation on standardized case report forms. Mold colonization and proven or probable IMI were diagnosed according to 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) definitions as previously described [8, 9]. Only patients undergoing their first organ transplant were included. Patients who had mold infection before undergoing transplantation (n = 5) were disqualified from the study.

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^aThe members of the Swiss Transplant Cohort Study Group are listed in the Appendix.

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Genotyping

Genomic DNA was extracted from patients' blood using the GenTra Puregene Blood Kit (Qiagen, Hombrechtikon, Switzerland). Three single-nucleotide polymorphisms (SNPs) in *PTX3*, including rs2305619 (+281A/G), rs1840680 (+1449A/G), and rs3816527 (+734A/C [D48A]), were selected on the basis of previous observations [6]. The rs2305619 and rs1840680 SNPs were genotyped as a part of a customized GoldenGate Genotyping Assay (BeadXpress, Veracode technology, Illumina). The rs3816527 SNP was genotyped using competitive allele-specific polymerase chain reaction (KASP, LGC Genomics, Hoddesdon, Herts, United Kingdom).

Statistical Analysis

Statistical analysis was performed using Stata version 13.1 software (StataCorp LP, College Station, Texas). The association between mold colonization and IMI by *PTX3* variants were assessed by 36-month cumulative incident curves (with censoring at loss to follow-up or death date) and by using the log-rank test [8]. Stepwise Cox regression model were used to estimate risk factors that were independently associated with the phenotypes. Based on previous studies [6], the associations were tested for the recessive mode of inheritance. Linkage disequilibrium and Hardy-Weinberg equilibrium were assessed using the *pwld* and *hwe* programs implemented in Stata. Because rs2305619 and rs1840680 were in almost perfect linkage disequilibrium ($R^2 = 0.99$), analyses are shown only for rs2305619. *PTX3* haplotypes were generated using PHASE version 2.1 (University of Washington, Seattle). Power calculation was performed using the *powerSurvEpi* package 0.0.6 in R (R Core Team, Vienna, Austria).

RESULTS

The study included 1101 white patients who received an SOT from kidney (n = 670), liver (n = 190), lung (n = 102), heart (n = 79), islet/pancreas (n = 15), or combined organs (n = 45). Among those, 45 were diagnosed with mold colonization (21 lung, 11 kidney, 7 heart, 4 liver, and 2 mixed organ recipients; [Supplementary Table 1](#)), and 26 developed IMIs (11 kidney, 5 lung, 5 heart, 3 liver, and 2 mixed organ recipients). IMIs were caused by *Aspergillus* species (n = 21 [81%]) or other fungi (*Fusarium* [n = 2], *Alternaria* [n = 1], *Zygomycetes* [n = 1], and mixed pathogens [*Zygomycetes* and *Fusarium*, n = 1]). The *PTX3* rs2305619 and rs3816527 SNPs had minor allele frequencies (MAFs) of 0.48 and 0.42, respectively, and both were at Hardy-Weinberg equilibrium ([Supplementary Tables 2 and 3](#)).

To assess the risk of mold colonization and IMI according to *PTX3* polymorphisms, we first analyzed the 36-month cumulative incidence of colonization and infection after transplantation in patients carrying the different genotypes/diplotypes. These incidences were significantly higher among patients

carrying the rs3816527 AA genotype compared with those carrying the CC or CA genotype (colonization, 0.0621 vs 0.0320, log-rank test $P = .03$; IMI, 0.0394 vs 0.0166, $P = .03$; [Figure 1A and 1B](#)). Similar but less significant associations were observed when comparing patients carrying the rs2305619 GG genotype with those carrying the AA or AG genotype (colonization $P = .09$ and IMI $P = .08$; [Supplementary Figure 1A and 1B](#)) or when comparing patients carrying the h2/h2 diplotype (combining the minor alleles of both rs2305619 and rs3816527) with the other diplotypes (colonization $P = .08$ and IMI $P = .07$; [Supplementary Figure 2A and 2B](#)).

To determine whether the polymorphisms were independent risk factors for the fungal phenotypes, we used multivariate stepwise Cox regression models adjusted for all relevant covariates. The associations between rs3816527 and fungal colonization or infection were even more significant after adjustment for age and sex, CMV infection or disease, CMV serostatus, immunosuppressive drugs, acute/chronic rejection, and/or type of transplanted organ (colonization hazard ratio [HR], 2.57 [95% confidence interval {CI}, 1.42–4.65], $P = .002$ and IMI HR, 3.18 [95% CI, 1.45–6.98], $P = .004$; [Supplementary Table 4](#)). Significant associations were also observed for rs2305619 (colonization HR, 1.97 [95% CI, 1.06–3.58], $P = .03$ and IMI HR, 2.29 [95% CI, 1.04–5.03], $P = .04$; [Supplementary Table 5](#)) and the h2/h2 diplotype (colonization HR, 2.06 [95% CI, 1.12–3.79], $P = .02$ and IMI HR, 2.43 [95% CI, 1.11–5.34], $P = .03$; [Supplementary Table 6](#)).

Because the occurrence of colonization and IMIs was significantly higher among thoracic transplant recipients, we performed a supplementary analysis that was limited to this group of patients. The associations between *PTX3* polymorphisms and fungal colonization and infection were even stronger, especially for rs3816527 (log-rank test: colonization $P = .002$ and IMI $P = .006$; [Figure 1C and 1D](#); multivariate model: colonization HR, 3.64 [95% CI, 1.67–7.92], $P = .001$; IMI HR, 7.33 [95% CI, 1.86–28.9], $P = .004$; [Supplementary Table 4](#)). Significant associations were also observed for rs2305619 (colonization HR, 2.74 [95% CI, 1.26–5.96], $P = .01$ and IMI HR, 5.30 [95% CI, 1.41–19.9], $P = .01$; [Supplementary Figure 1C and 1D](#) and [Supplementary Table 5](#)) and for the h2/h2 diplotype (colonization HR, 3.06 [95% CI, 1.39–6.75], $P = .006$ and IMI HR, 5.68 [95% CI, 1.56–20.7], $P = .009$; [Supplementary Figure 2C and 2D](#), [Supplementary Table 6](#)).

DISCUSSION

A number of studies have reported associations between polymorphisms in host immune genes and susceptibility to fungal infections in immunocompromised patients [4]. Many were limited by several factors, including a lack of replication and/or the absence of functional evidence supporting the association [4]. Polymorphisms in *PTX3* in the donor have been recently

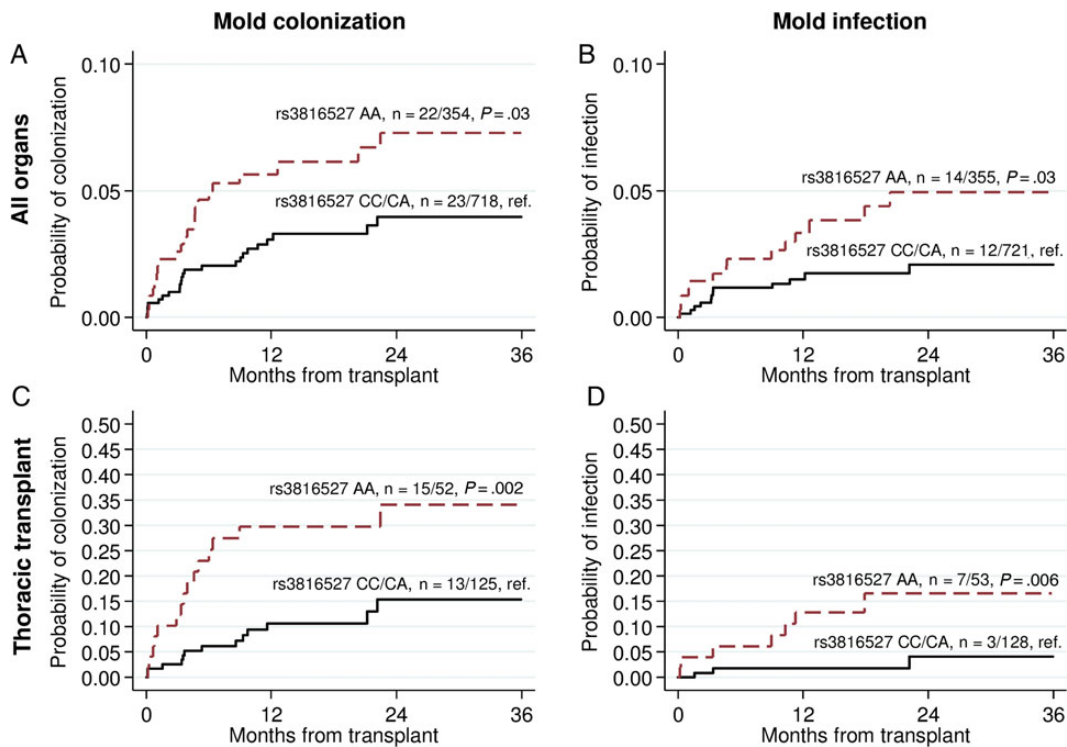


Figure 1. Cumulative incidence of mold colonization and invasive mold infection according to *PTX3* rs3816527 single-nucleotide polymorphism in all (A and B) and thoracic (C and D) solid organ transplant recipients. Patients who were colonized or infected with mold before transplantation were excluded from the analyses. *P* values were calculated by log-rank test, recessive mode (patients homozygous for the rare alleles are compared to the others).

associated with an increased risk for the development of IA among HSCT recipients [6]. We report for the first time an association between such polymorphisms and susceptibility to mold colonization and IMIs among SOT recipients. Thus, the validation in a different patient population suggests that *PTX3* polymorphisms may represent a valuable marker of increased risk for fungal infection.

The 2 *PTX3* polymorphisms have a relatively high frequency (MAF, approximately 0.4) [6] compared with polymorphisms previously associated with IA, such as rs4986790/1 in Toll-like receptor 4 (TLR4; MAF, approximately 0.05) [10] and rs16910526 in Dectin-1 (MAF, 0.08) [11]. Rare SNPs require very large cohorts for replication, whereas frequent ones can be replicated in smaller datasets. The association between *PTX3* polymorphisms was initially replicated in 2 independent cohorts of HSCT recipients from different centers [6]. In the present study, we provide further validation in a population whose clinical condition and type of immunosuppressive regimen is different. Thus, these polymorphisms may be more universal than other population-specific risk factors.

There is strong evidence for the involvement of *PTX3* in the immune responses against *Aspergillus* species [5]. *PTX3* can directly bind *Aspergillus* conidia by recognizing galactomannan,

thereby acting as an opsonizing factor for complement activation and subsequent phagocytosis [12]. *PTX3* can also interact with PRRs such as Dectin-1 or TLR4 to increase fungal pattern recognition and thus promote adaptive immune responses [12, 13]. In vivo, *PTX3* knockout mice have been shown to be highly susceptible to IA due to defective recognition of *A. fumigatus* by macrophages and their phagocytic activities as well as imbalanced adaptive responses to this fungus [5].

In addition, there is evidence that polymorphisms in *PTX3* are associated with reduced immunity against fungal pathogens. The missense +734A rs3816527 allele was suggested to influence *PTX3* messenger RNA stability, thereby affecting its secondary structure and leading to its lower expression. *PTX3* variants were also associated with reduced *PTX3* production in neutrophils with defective phagocytic activities and reduced *Aspergillus* clearance [6]. Of note, neutrophils originate from the donor stem cells in HSCT and from the recipient in SOT. Consistently, polymorphisms associated with IA in the previous study of HSCT patients were issued from the donor [6], whereas those associated with IMIs in the present study of SOT recipients are from the recipient. As most patients who develop infection are previously colonized, it is difficult to determine whether the polymorphisms influence colonization alone, or colonization and infection.

Our findings indicate that specific genetic polymorphisms in *PTX3* are responsible for susceptibility to IMIs in SOT recipients. This study reinforces the validity of *PTX3* polymorphisms as an important risk factor for mold infection risk stratification in immunocompromised patients.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (<http://cid.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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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Morgan J, Wannemuehler KA, Marr KA, et al. Incidence of invasive aspergillosis following hematopoietic stem cell and solid organ transplantation: interim results of a prospective multicenter surveillance program. *Med Mycol* **2005**; 43(suppl 1):S49-58.
2. Pappas PG, Alexander BD, Andes DR, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). *Clin Infect Dis* **2010**; 50:1101-11.
3. Neofytos D, Treadway S, Ostrand D, et al. Epidemiology, outcomes, and mortality predictors of invasive mold infections among transplant recipients: a 10-year, single-center experience. *Transpl Infect Dis* **2013**; 15:233-42.
4. Wojtowicz A, Bochud PY. Host genetics of invasive *Aspergillus* and *Candida* infections. *Semin Immunopathol* **2015**; 37:173-86.
5. Garlanda C, Hirsch E, Bozza S, et al. Non-redundant role of the long pentraxin *PTX3* in anti-fungal innate immune response. *Nature* **2002**; 420:182-6.
6. Cunha C, Aversa F, Lacerda JF, et al. Genetic *PTX3* deficiency and aspergillosis in stem-cell transplantation. *N Engl J Med* **2014**; 370:421-32.
7. Koller MT, van Delden C, Muller NJ, et al. Design and methodology of the Swiss Transplant Cohort Study (STCS): a comprehensive prospective nationwide long-term follow-up cohort. *Eur J Epidemiol* **2013**; 28:347-55.

8. Wojtowicz A, Gresnigt MS, Lecompte T, et al. *IL1B* and *DEFB1* polymorphisms increase susceptibility to invasive mold infection after solid organ transplantation. *J Infect Dis* **2015**; 211:1646-57.
9. 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**; 46:1813-21.
10. Bochud PY, Chien JW, Marr KA, et al. Toll-like receptor 4 polymorphisms and aspergillosis in stem-cell transplantation. *N Engl J Med* **2008**; 359:1766-77.
11. Cunha C, Di Ianni 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**; 116:5394-402.
12. Salvatori G, Campo S. Current understanding of *PTX3* protective activity on *Aspergillus fumigatus* infection. *Med Mycol* **2012**; 50:225-33.
13. Bozza S, Campo S, Arseni B, et al. *PTX3* binds MD-2 and promotes TRIF-dependent immune protection in aspergillosis. *J Immunol* **2014**; 193:2340-8.

APPENDIX

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