PTX3 Polymorphisms and Invasive Mold Infections after Solid Organ Transplantation

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Donor polymorphisms in \textit{PTX3} were previously associated with susceptibility to invasive aspergillosis in hematopoietic stem cell transplant recipients. Here, we show that \textit{PTX3} polymorphisms also increase the risk of mold colonization and infection when detected among solid organ transplant recipients.

Abstract

Donor \textit{PTX3} polymorphisms were shown to influence the risk of invasive aspergillosis among hematopoietic stem cell transplant recipients. Here, we show that \textit{PTX3} polymorphisms are independent risk factors for invasive mold infection among 1101 solid organ transplant recipients, thereby strengthening their role in mold infection pathogenesis and patient’s risk stratification.
Introduction

Invasive mold infections (IMI) represent an important cause of morbidity and mortality in transplant recipients [1, 2]. While specific risk factors have been identified in both hematopoietic stem-cell (HSCT) and solid organ transplant (SOT) recipients, such as patient age, co-morbidities, conditioning regiments, cytomegalovirus (CMV) infection, renal failure, reoperation 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 was shown to exert important antifungal protection [5]. Polymorphisms in 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 IMI among SOT recipients. This observation strengthens the role of these polymorphisms in immune defenses against fungal pathogen and its potential use as a predictor for infection in the clinical practice.
Materials and Methods

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

Genotyping. Genomic DNA was extracted from patient’s 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 based on 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 PCR (KASP™) system (LGC Genomics, UK).

Statistical analysis. Statistical analysis was carried out by using Stata 13.1® software (StataCorp LP, College Station, Texas, USA). The association between mold colonization and IMI by PTX3 variants were assessed by 36-months cumulative incident curves (with censoring at lost to follow-up or death date) and by using the log-rank test [8]. Furthermore, 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. The linkage disequilibrium (LD) and Hardy-Weinberg equilibrium (HWE) tests were assessed by using the pwld and hwe softwares implemented in Stata. Since rs2305619 and rs1840680 were in almost perfect LD ($R^2=0.99$), analyses are shown only for rs2305619. 

PTX3 haplotypes were generated using PHASE version 2.1 (University of Washington, Seattle, WA, USA). Power calculation was performed by using powerSurvEpi package 0.0.6 in R (R Core Team, Vienna, Austria).

**Results**

The study included 1101 Caucasian patients who received a SOT form kidney (N=670), liver (N=190), lung (N=102), heart (N=79), islet/pancreas (N=15), or combined organ transplants (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 S1) and 26 developed IMI (11 kidney, 5 lung, 5 heart, 3 liver and 2 mixed organ recipients). IMI was mainly caused by *Aspergillus* species (N=21 [81%]) or due to 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 (MAF) of 0.48 and 0.42, respectively, and both were at HWE (supplementary Tables S2 and S3).

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 to those carrying the CC or CA genotypes (colonization 0.0621 versus 0.0320, log rank test $P=0.03$; IMI 0.0394 versus 0.0166,
P=0.03; supplementary Figure 1 A and B). Similar though less significant associations were observed when comparing patients carrying the rs2305619 GG genotype to those carrying the AA or AG genotypes (colonization P=0.09 and IMI P=0.08; supplementary Figure S1 A and B) or patients carrying the h2/h2 diplotype (combining the minor alleles of both rs2305619 and rs3816527) to the other diplotypes (colonization P=0.08 and IMI P=0.07; supplementary Figure S2 A and B).

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 sero-status, immunosuppressive drugs, acute/chronic rejection and/or type of transplanted organ (colonization HR=2.57, 95%CI 1.42-4.65, P=0.002 and IMI HR=3.18, 95%CI 1.45-6.98, P=0.004; supplementary Table S4). Significant associations were also observed for rs2305619 (colonization HR=1.97, 95%CI 1.06-3.58, P=0.03 and IMI HR=2.29, 95%CI 1.04-5.03, P=0.04; supplementary Table S5) and the h2/h2 diplotype (colonization HR=2.06, 95%CI 1.12-3.79, P=0.02 and IMI HR=2.43, 95%CI 1.11-5.34, P=0.03; supplementary Table S6).

Since the occurrence of colonization and IMI 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=0.002 and IMI P=0.006; Figure 1 C and D; multivariate model, colonization HR=3.64, 95%CI 1.67-7.92, P=0.001, IMI HR=7.33, 95%CI 1.86-28.9, P=0.004; supplementary Table S4). Significant associations were also observed for rs2305619 (colonization HR=2.74, 95%CI 1.26-5.96, P=0.01 and IMI HR=5.30, 95%CI 1.41-19.9, P=0.01; supplementary Figure S1 C and D, Table S5) and for the h2/h2
diplototype (colonization HR=3.06, 95% CI 1.39-6.75, \( P = 0.006 \) and IMI HR=5.68, 95% CI 1.56-20.7, \( P = 0.009 \); Figure S2 C and D, supplementary Table S6).

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 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 IMI 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 two PTX3 polymorphisms have a relatively high frequency (MAF \(~0.4\) ) [6] compared to polymorphisms previously associated with IA, such as rs4986790/1 in Toll-like receptor 4 (TLR4, MAF \(~0.05\) ) [10] and rs16910526 in Dectin-1 (MAF 0.08) [11]. Rare SNPs require very large cohorts for replication, while frequent ones can be replicated in smaller datasets. The association between PTX3 polymorphisms was initially replicated in two 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 spp [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 patterns 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 mRNA stability, thereby affecting its secondary structure and leading to its lower expression. PTX3 variants were also associated with a 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], while those associated with IMI in the present study of SOT are from the recipient. Since 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 IMI in SOT recipients. This study reinforces the validity of PTX3 polymorphisms as an important risk factor for mold infection risk stratification in immunocompromised patients.
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Conflict of Interest

The authors have no conflicting financial interests.

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References


Figure legend

Figure 1. Cumulative incidence of mold colonization and invasive mold infection according to *PTX3* rs3816527 SNP in all (panel A and B) and thoracic (panel 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 other).