Host Genetic Signatures of Susceptibility to Fungal Disease

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Abstract Our relative inability to predict the development of fungal disease and its clinical outcome raises fundamental questions about its actual pathogenesis. Several clinical risk factors are described to predispose to fungal disease, particularly in immunocompromised and severely ill patients. However, these alone do not entirely explain why, under comparable clinical conditions, only some patients develop infection. Recent clinical and epidemiological studies have reported an expanding number of monogenic defects and common polymorphisms associated

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with fungal disease. By directly implicating genetic variation in the functional regulation of immune mediators and interacting pathways, these studies have provided critical insights into the human immunobiology of fungal disease. Most of the common genetic defects reported were described or suggested to impair fungal recognition by the innate immune system. Here, we review common genetic variation in pattern recognition receptors and its impact on the immune response against the two major fungal pathogens *Candida albicans* and *Aspergillus fumigatus*. In addition, we discuss potential strategies and opportunities for the clinical translation of genetic information in the field of medical mycology. These approaches are expected to transfigure current clinical practice by unleashing an unprecedented ability to personalize prophylaxis, therapy and monitoring for fungal disease.

1 Introduction: Genetic Regulation of the Host-Fungus Interaction

An increasing number of fungal diseases has been documented over the past two decades. Although we are constantly exposed to fungi, only a few species can cause disease in healthy individuals. Opportunistic and otherwise commensal fungi can instead trigger life-threatening infections in individuals with acquired or treatment-induced immunodeficiencies, such as patients receiving hematopoietic stem-cell (HSCT) or solid organ (SOT) transplantation, or undergoing anticancer therapy (Pfaller and Diekema 2010). Many species of fungi are responsible for invasive infections, although more than 90% of all reported fungal-related deaths result from infection with species belonging to one of four genera: *Candida*, *Aspergillus*, *Cryptococcus* and *Pneumocystis*. These diseases are estimated to affect more than 2 million people annually and, in some cases, can have mortality rates that exceed 50% (Brown et al. 2012).

Despite several clinical risk factors typically associated with fungal disease have been disclosed, the actual mechanisms underlying infection in the human host remain largely undefined. Like many infectious diseases, fungal infections are characterized by significant interindividual variability in their development, progression and resolution. While a significant contribution to infection might be credited to virulence traits and the ability of fungi to adapt to the human host, recent evidence has highlighted a dominant role of heritable factors (Cunha et al. 2013; Smeekens et al. 2013b; Wojtowicz and Bochud 2014). Our current understanding of the genetic basis of fungal disease has stemmed from the study of individuals with rare monogenic defects and from cohort-based studies to identify common polymorphisms associated with disease (Lionakis and Levitz 2017). In addition, mouse studies illustrating disparities in susceptibility to experimental infection between inbred strains have also strengthened the concept of genetic control of susceptibility to fungal disease (Durrant et al. 2011; Radovanovic et al. 2011).

Clinical and basic research during the last decade has provided exciting new insights into the molecular and cellular players involved in the host-fungus interaction and antifungal host defense (Netea et al. 2015; van de Veerdonk et al. 2017). The improved understanding of the genetic mechanisms that regulate host immunity to fungi represents an opportunity for the development of new and more effective approaches to preventing and treating fungal diseases. In addition, future studies addressing the genetic architecture of both the host and the fungus and how it regulates the outcome of their interaction are expected to further support clinical translation and personalized medical interventions in fungal diseases (Oliveira-Coelho et al. 2015; Rello et al. 2018). In this review, we explore recent advances in the immunogenetics of fungal disease and how it modulates innate immunity to the two major fungal pathogens *Candida albicans* and *Aspergillus fumigatus*. Also discussed is how an improved understanding of the genetic regulation of the host-fungus interaction is expected to reform the clinical management of fungal disease by paving the way toward precision medical interventions based on individual host genetics.

2 Pattern Recognition Receptors in Fungal Immunity

Our understanding of innate immunity was revolutionized in the early 90s with the groundbreaking concept of selective recognition of conserved pathogen-associated molecular patterns (PAMPs) by germline-encoded pattern recognition receptors (PRRs) (Takeuchi and Akira 2010). Although there are remarkable differences in the way different classes of pathogens are perceived by the immune system, the first step in developing a proper innate immune response is widely acknowledged to require fungal sensing by PRRs (Fig. 1). Owing to its inherent dynamic composition and variable cellular localization of the different constitutive components during the interaction with the host, the cell wall is considered the most abundant source of fungal PAMPs, such as β-1,3-glucans and mannans (Gow and Hube 2012; Latge et al. 2017).

The main families of PRRs include Toll-like receptors (TLRs), C-type lectin receptors (CLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) and retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs) (Netea et al. 2012). Following the recognition of their cognate ligands, PRRs induce mechanisms responsible for pathogen clearance, including the secretion of cytokines and chemokines, phagocytosis and production of reactive oxygen species (ROS), and orchestrate complex immunoregulatory processes resulting in the activation of adaptive immunity (Patin et al. 2018). The proficiency of fungal recognition and interaction with the membrane-associated PRRs also relies to a large extent on the opsonization by different families of soluble pattern recognition molecules, including collectins, pentraxins, ficolins and components of the complement pathway (Bidula and Schelenz 2016). Of note, studies have highlighted that these molecules may be exploited as a possible alternative or adjuvants to

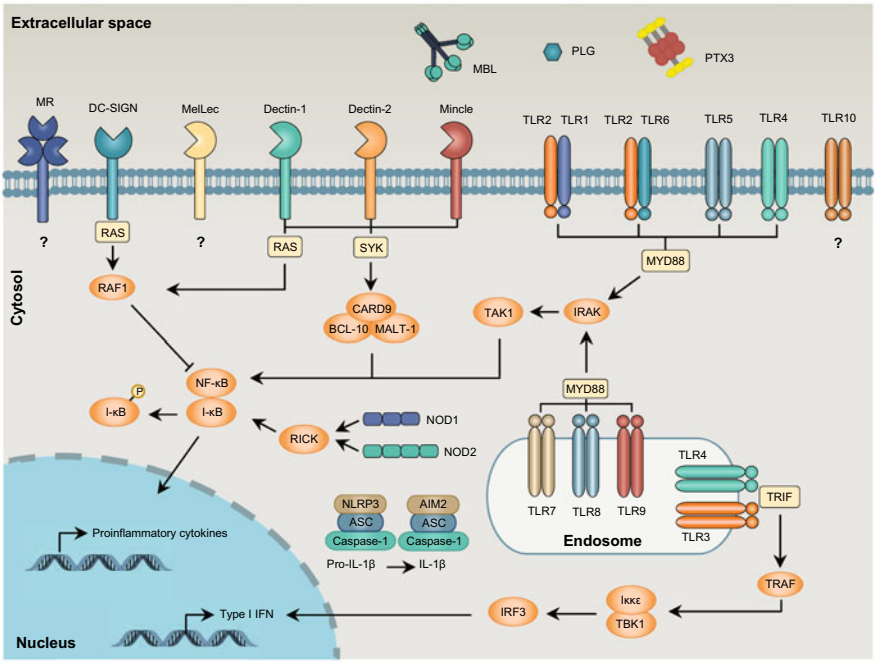

Fig. 1 Overview of PRRs and downstream signaling pathways. PRRs are expressed on the cell surface or are present in endosomes or the cytosol, where they detect PAMPs and activate downstream signaling pathways leading to the transcription of cytokines and chemokines, whereas soluble PRRs bind and opsonize fungi, facilitating their elimination. Upon stimulation, TLRs activate two disparate pathways that involve myeloid differentiation primary response protein 88 (MYD88) and/or TIR domain-containing adaptor protein inducing IFN-β (TRIF). Crosstalk between TLR signaling cascades underlies the activation of different cellular processes, including the transcription of proinflammatory cytokines and chemokines, and type I IFN. On the other hand, CLRs trigger intracellular signaling pathways typically linked to the activation of the SYK kinase and CARD9, resulting in a cascade of downstream signaling and cellular activation that regulate and fine-tune NF-κB activation and cytokine gene expression. The stimulation of NLRs also regulates NF-κB activation via receptor-interacting protein-like interacting caspase-like apoptosis regulatory protein kinase (RICK), whereas the NLRP3 and AIM2 inflammasomes process bioactive proinflammatory cytokines, such as IL-1β

currently available antifungal therapy to increase their efficacy (Lo Giudice et al. 2012; Marra et al. 2014). Besides pathogen-derived molecules, PRRs are also critically important in responding to products released from damaged host cells during infection, including nucleic acids, alarmins and metabolic products, collectively termed damage-associated molecular patterns (DAMPs) (Cunha et al. 2012). Regardless of the PRRs or soluble molecules implicated in fungal recognition or opsonization, the activation of antifungal immune responses requires a coordinated regulation of the function and cellular localization of individual or cooperating receptors.

The role of TLRs in innate immunity was initially proposed following the observation that *Drosophila* lacking the hematocyte receptor Toll were extremely susceptible to infection with fungi and Gram-negative bacteria (Lemaitre et al. 1996). This discovery prompted the identification of mammalian TLRs shortly thereafter (Rock et al. 1998). Similar to other PRRs, TLRs are primarily expressed in cells of the immune system, including monocytes, neutrophils, basophils, eosinophils and natural killer cells, but also in cells of epithelial, endothelial and stromal origin (Netea et al. 2012). TLRs are characterized by containing extracellular domains with leucine-rich repeats that interact directly with PAMPs and cytoplasmic domains highly homologous to the sequences in the interleukin (IL)-1 and IL-18 receptors. After binding to their specific ligands, TLRs activate signaling cascades that promote the translocation of transcription factors to the nucleus, where they induce transcription of genes involved in inflammatory responses and mechanisms of pathogen clearance (Takeuchi and Akira 2010).

The large family of CLRs includes members such as dectin-1, dectin-2, mannose receptor, dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN), macrophage inducible C-type lectin (Mincle), macrophage C-type lectin (MCL), and the recently identified MelLec (Brown et al. 2018). These receptors have carbohydrate recognition domains and bind microbial polysaccharides and, for this reason, they have been widely implicated in the activation and regulation of antifungal immune responses (Salazar and Brown 2018). Among CLRs, dectin-1 was the first to be identified and is currently the best described receptor able to orchestrate the activation of adaptive immune responses to fungi (Dambuza and Brown 2015). Dectin-1 recognizes β-glucans (Brown and Gordon 2001) and triggers intracellular signaling pathways that, synergistically and through cross-regulatory mechanisms, culminate in the activation of nuclear factor (NF)-κB and cytokine gene expression (Geijtenbeek and Gringhuis 2009). Although most of the CLR family members have been implicated in antifungal immunity in one way or another, the fungal ligands for most of them remain elusive. One exception was recently provided by the identification of MelLec as a specific receptor for 1,8-dihydroxynaphthalene (DHN)-melanin, a process required for eliciting protective immunity to *A. fumigatus* (Stappers et al. 2018). We also refer the reader to several excellent reviews on the biology of CLRs (Brown et al. 2018; Dambuza and Brown 2015; Salazar and Brown 2018).

Besides the mainly membrane-bound TLRs and CLRs, cytoplasmic PRRs are activated by pathogens following cell invasion or release of PAMPs into the cytoplasm. Although the fungal ligands that are recognized by NLRs have not been identified, their activation during infection results in the assembly of multimeric protein complexes named inflammasomes that are primarily responsible for converting inactive pro-IL-1β and pro-IL-18 into bioactive cytokines (van de Veerdonk et al. 2015). The NLR family pyrin domain containing 3 (NLRP3) and the NLR family CARD domain containing 4 (NLRC4) inflammasomes were specifically credited with a prominent role in the immune response to *C. albicans* (Borghi et al. 2015; Cheng et al. 2011; Hise et al. 2009; Tomalka et al. 2011). The NLRP3 and AIM2 inflammasomes were instead shown to form a dual cytoplasmic surveillance

system that orchestrates protective immune responses against *A. fumigatus* (Karki et al. 2015). The contribution of NLRs to the immune response against fungi nonetheless extends beyond inflammasome formation. Fungal chitin has been demonstrated to dampen inflammatory responses to *C. albicans* by inducing the immunoregulatory cytokine IL-10 via the activation of NOD-containing receptor 2 (NOD2) and TLR9 (Wagener et al. 2014). More recently, NOD1 was found to play an inhibitory role in the host defense against *A. fumigatus* by suppressing dectin-1 expression and cytokine responses responsible for optimal fungal killing (Gresnigt et al. 2017). Taken together, these observations support the existence of highly dynamic regulatory mechanisms modulated by intracellular fungal recognition with important consequences to the overall antifungal immune response.

3 Genetic Defects in Pattern Recognition Receptors and Susceptibility to Fungal Disease

Genetic variation in the genes encoding PRRs can influence susceptibility to diseases caused by a wide range of fungal pathogens (Carvalho et al. 2010). As for most infectious diseases, genetic defects in the different families of PRRs have been widely linked with susceptibility to fungal disease (Netea et al. 2012). These associations highlight the complexity of the host genetic signatures influencing antifungal host defense. Here, we discuss the most relevant genetic variation in PRRs, its association with susceptibility to infection by *Candida* and *Aspergillus*, and their functional consequences to the activation of innate immune responses.

3.1 Toll-like Receptors

Following the early description of TLRs, genetic variation in these genes was proposed to underlie significant interindividual differences in susceptibility to infectious and inflammatory diseases (Netea et al. 2012). Although monogenic or primary immunodeficiencies affecting TLR signaling promote large effect sizes, this is typically a rare (or very rare) event on the general population that has not been implicated in the development of fungal disease thus far. However, TLRs are characterized by remarkable genetic diversity due to strong selective pressures during their evolution, particularly in the number of single nucleotide polymorphisms (SNPs) that lead to substitutions in amino acid residues (Quach et al. 2013). As such, before the advent of next-generation sequencing and genome-wide association studies, polymorphisms in TLRs were considered biologically plausible targets for involvement in susceptibility to infectious diseases, including fungal infections (Cunha et al. 2010b). Table 1 summarizes relevant genetic variants in TLRs and their association with susceptibility to infection by *Candida* and *Aspergillus*.

Table 1 Genetic variation in TLRs and susceptibility to infection by *Candida* and *Aspergillus*

Gene (s)	SNP(s)	Nucleotide change	Amino acid change	Disease(s)	References
TLR1	rs5743611	G > C	R80T	IPA	Kesh et al. (2005)
	rs4833095	C > T	N248S	IPA	Kesh et al. (2005)
	rs5743618	T > G	S602I	Candidemia	Plantinga et al. (2012)
TLR2	rs5743704	C > A	P631H	RVVC	Rosentul et al. (2014)
TLR3	rs3775296	G > T	–	IPA	Carvalho et al. (2012b)
TLR4	rs4986790	A > G	D299G	IPA and CPA	Bochud et al. (2008), Carvalho et al. (2008), de Boer et al. (2011), Koldehoff et al. (2013)
	rs4986791	C > T	T399I	IPA	Bochud et al. (2008), Koldehoff et al. (2013)
TLR5	rs5744168	C > T	R392X	IPA	Grube et al. (2013)
TLR6	rs5743810	C > T	S249P	IPA	Kesh et al. (2005)
TLR9	rs5743836	C > T	–	ABPA	Carvalho et al. (2008)

The first nucleotide (and corresponding amino acid) is the ancestral nucleotide and therefore is considered the wild-type allele. *SNP* single nucleotide polymorphism; *IPA* invasive pulmonary aspergillosis; *RVVC* recurrent vulvovaginal candidiasis; *CPA* chronic pulmonary aspergillosis; *ABPA* allergic bronchopulmonary aspergillosis

The TLR4 sequence variants rs4986790 (D299G) and rs4986791 (T399I) constitute an haplotype that has been reported to alter the leucine-rich repeat region of the receptor and decrease the efficiency of ligand recognition (Arbour et al. 2000). The presence of this haplotype in donors of allogeneic HSCT was associated with the development of invasive pulmonary aspergillosis (IPA) in the corresponding patients (Bochud et al. 2008). Although this association was validated in independent HSCT cohorts (de Boer et al. 2011; Koldehoff et al. 2013) and in immunocompetent individuals suffering from chronic aspergillosis (Carvalho et al. 2008), other studies have failed to replicate the findings (Carvalho et al. 2009; Fisher et al. 2017; Kesh et al. 2005). The TLR4 haplotype was reported to delay immune reconstitution after transplant (Koldehoff et al. 2013), and this could represent a key mechanism justifying its association with IPA in specific patient populations. The exact mechanism by which TLR4 variants may influence antifungal immune responses remains however unknown – particularly since no fungal ligands (or endogenous molecules released during infection) have been identified to date – and this may also support the lack of association with other fungal diseases, including candidemia (Plantinga et al. 2012).

Genetic variation in TLRs other than TLR4 has also been proposed as an important risk factor for fungal disease. Earlier studies have reported associations of the polymorphisms rs5743611 (R80T) and rs4833095 (N248S) in TLR1 and rs5743810 (S249P) in TLR6 with IPA (Kesh et al. 2005), but only TLR6 has been shown to be required for the human immune response to *A. fumigatus* (Rubino et al. 2012). TLR1 also appears to be an important repository of genetic variability

increasing susceptibility to candidemia (Plantinga et al. 2012). Although the precise mechanism(s) whereby TLR1 influences the risk of candidemia remains elusive, the variant rs5743618 (I602S) has been shown to impair the trafficking of TLR1 to the cell surface and to result in decreased NF-κB activation and proinflammatory cytokine responses to TLR1 agonists (Johnson et al. 2007; Wurfel et al. 2008). In addition, and despite it was not associated with systemic *Candida* infection (Plantinga et al. 2012), the rs5743704 (P631H) SNP in TLR2 was implicated in the development of idiopathic recurrent vulvovaginal candidiasis (RVVC) (Rosentul et al. 2014). Finally, the variant rs5743836 in the promoter of TLR9 was associated with the development of allergic bronchopulmonary aspergillosis (ABPA) (Carvalho et al. 2008), although this study still requires confirmation.

Another interesting example regards TLR5, the receptor for flagellin expressed by flagellated bacteria (Hayashi et al. 2001), and in which a SNP leading to an early stop codon (R392X) has been shown to disrupt flagellin recognition (Hawn et al. 2003). In HSCT recipients, the presence of this variant was associated with the development of IPA (Grube et al. 2013), thereby suggesting a likely important antifungal function of TLR5 in the non-hematopoietic compartment. However, functional data is not available, and additional studies are warranted to identify the so far unsuspected mechanisms (and the ligand) by which TLR5 might influence susceptibility to IPA. In any case, the fact that R392X presents with a relatively common frequency without imposing a primary immunodeficiency phenotype suggests a non-essential role for TLR5 in host defense (Wlasiuk et al. 2009).

Despite classically acknowledged as a prototypical receptor for double stranded RNA (Zhang et al. 2013), TLR3 has been implicated in fungal recognition and activation of adaptive immune responses. In particular, the regulatory variant rs3775296 in TLR3 was demonstrated to increase the risk of IPA after HSCT (Carvalho et al. 2012b). Cross-presenting dendritic cells harboring this variant displayed an impaired expression of TLR3 and sensing of fungal RNA, which ultimately resulted in the defective priming of memory CD8(+) T-cell responses to *A. fumigatus*. This study was recently supported by evidence demonstrating a similar association with severe asthma with fungal sensitization (Overton et al. 2017). Although there is no evidence implicating this regulatory variant in susceptibility to infections by *Candida*, the non-synonymous SNP rs3775291 (L412F) in TLR3 was however detected more frequently in patients suffering from chronic mucocutaneous candidiasis (CMC) (Nahum et al. 2011).

This study represents a critical example of how genetic defects in TLRs (and other PRRs) may influence adaptive immune responses, in addition to fungal sensing and innate immunity. Although this has yet to be addressed, there is an unequivocal need to consider the genetic profile of the patient during diagnostic approaches based on the measurement of fungal-specific adaptive immune responses (Koehler et al. 2018; Potenza et al. 2013). The same applies to immunotherapeutic strategies focused on the direct or indirect manipulation of cytokines, since there are many examples of genetic variants that influence cytokine production and function (Cunha et al. 2017; Johnson et al. 2012). In conclusion, the success of novel diagnostic and immunotherapeutic approaches for fungal diseases

will only be possible if guided by personalization based on the interindividual variability in immune function, particularly for genetic variants with well-established functional consequences.

3.2 C-Type Lectin Receptors

Given the well-established role of CLRs in the coordination of antifungal immune responses, their genetic variation has been extensively implicated in susceptibility to fungal disease (Table 2). The importance of dectin-1 in the recognition of β-glucan and activation of antifungal immunity has been demonstrated in mouse studies but also in patients with recurrent fungal infections carrying the early stop codon polymorphism rs16910526 (Y238X) (Ferwerda et al. 2009; Taylor et al. 2007). This variant results in a truncated form of dectin-1 lacking several amino acids within the carbohydrate recognition domain, which underlies its decreased expression at the surface of myeloid cells and a defective production of cytokines, particularly IL-17, after stimulation with β-glucan or *C. albicans* (Ferwerda et al. 2009). Following the initial identification of Y238X, and given its specific impact on the activation of Th17-mediated immunity, it has since been vastly implicated in mucosal and gastrointestinal fungal colonization (De Luca et al. 2013; Plantinga et al. 2009; Usluogullari et al. 2014). This functional axis is further demonstrated by primary immunodeficiencies due to rare mutations in STAT3, IL-17RA and IL-17F that impair Th17 immunity and are associated with CMC and aspergillosis (Lionakis and Levitz 2018). Because the cellular localization of the different dectin-1 isoforms regulates the signaling quality of antifungal immunity (Carvalho et al. 2012c; Fischer et al. 2017), it is also tempting to speculate that this may be one additional mechanism through which the Y238X variant may contribute to infection. Of note, another non-synonymous variant in dectin-1 (rs16910527; I223S) was instead associated with lower levels of interferon (IFN)-γ and the risk of oropharyngeal candidiasis in HIV patients (Plantinga et al. 2010). This suggests that different pathogenetic mechanisms are likely in place depending on the specific structural consequences of genetic variants to dectin-1 function.

Although the clinical phenotype of patients carrying the dectin-1 stop codon is relatively mild and less severe than that of patients with classic CMC (Puel et al. 2010), the Y238X variant was found to strongly predispose HSCT recipients to the development of IPA (Chai et al. 2011; Cunha et al. 2010a). Additional variants in dectin-1, but also dectin-2 and DC-SIGN (CD209), were likewise correlated with the development of IPA in hematological patients (Fischer et al. 2016; Sainz et al. 2012). Importantly, the genetic deficiency of dectin-1 in both the hematopoietic and non-hematopoietic compartments was disclosed to synergize towards risk of infection (Cunha et al. 2010a), a finding that was validated in the largest HSCT patient cohort at-risk of IPA collected to date (Fisher et al. 2017) and that highlights the key role of dectin-1 in antifungal immunity across multiple cell types. Among the many biological processes that are regulated by dectin-1 in response to fungi,

Table 2 Genetic variation in CLRs and susceptibility to infection by *Candida* and *Aspergillus*

Gene(s)	SNP(s)	Nucleotide change	Amino acid change	Disease(s)	References
CARD9	rs4077515	G > A	S12N	ABPA	Xu et al. (2018)
CD209	rs4804800	G > A	–	IPA	Sainz et al. (2012)
	rs11465384	C > T	–	IPA	Sainz et al. (2012)
	rs7248637	A > G	–	IPA	Sainz et al. (2012)
	rs7252229	C > G	–	IPA	Sainz et al. (2012)
CLEC1A	rs2306894	C > G	G26A	IPA	Stappers et al. (2018)
CLEC7A	rs16910526	T > G	Y238X	*Candida* colonization, RVVC and IPA	Chai et al. (2011), Cunha et al. (2010a), De Luca et al. (2013), Fisher et al. (2017), Plantinga et al. (2009), Usluogullari et al. (2014)
	rs16910527	A > C	I223S	Oropharyngeal candidiasis	Plantinga et al. (2010)
	rs7309123	G > C	–	IPA	Fischer et al. (2016), Sainz et al. (2012)
	rs3901533	G > T	–	IPA	Sainz et al. (2012)

The first nucleotide (and corresponding amino acid) is the ancestral nucleotide and therefore is considered the wild-type allele. *SNP* single nucleotide polymorphism; *ABPA* allergic bronchopulmonary aspergillosis; *IPA* invasive pulmonary aspergillosis; *RVVC* recurrent vulvovaginal candidiasis

the production of ROS through the NADPH oxidase system and the activation of downstream clearance mechanisms is critically important, as reflected by the extreme susceptibility of patients with chronic granulomatous disease (CGD) to aspergillosis (Grimm et al. 2013; Kyrmizi et al. 2013). The role of dectin-1 in immunity to infection may however extend beyond the immediate activation of antifungal effector mechanisms. For example, recognition of β-glucan has been demonstrated to confer innate immune memory to infection – a process referred to as trained immunity (Netea and van der Meer 2017) – by regulating multiple processes of cellular metabolism (Arts et al. 2016; Bekkering et al. 2018; Cheng et al. 2014). In the future, it will be critical to assess the extent to which the Y238X variant predisposes to fungal disease by impairing the induction of "natural" trained immunity as the result of our constant exposure to fungi.

Mutations in caspase recruitment domain-containing protein 9 (CARD9), the adaptor molecule that transduces signals from dectin-1 and other CLRs, have been identified in patients suffering from mucocutaneous fungal infections (Glocker et al. 2009). Importantly, neutrophils from CARD9-deficient patients were found to display impaired phagolysosomal killing of unopsonized *C. albicans*, a phenotype that was independent of dectin-1 and NADPH oxidase activity, thereby explaining the variable clinical presentation of fungal infection in patients suffering from

dectin-1 and CARD9 deficiency and CGD (Gazendam et al. 2014). Human CARD9 deficiency was also found to predispose to extrapulmonary aspergillosis with sparing of the lungs through a mechanism involving the defective accumulation of neutrophils in infected tissue (Rieber et al. 2016). Of note, the common polymorphism rs4077515 (S12N) in CARD9 was recently implicated in the risk of ABPA (Xu et al. 2018). Mechanistically, work performed in knock-in mice expressing the mutated form of human CARD9 revealed the remarkable contribution of S12N to the activation of NF-κB subunit RelB, which in turn promoted the production of IL-5 in alveolar macrophages and the recruitment of eosinophils to drive Th2 cell-mediated allergic responses. Given the key role of CARD9 in orchestrating the signals collected from the different CLRs, it will be interesting to assess whether this or other common genetic variants may impact susceptibility to other forms of aspergillosis and eventually other fungal infections.

MelLec (encoded by the *CLEC1A* gene) was recently characterized as the functional receptor for DHN-melanin from *A. fumigatus* (Stappers et al. 2018). This discovery was accompanied by the identification of a non-synonymous variant rs2306894 (G26A) in the cytoplasmic tail of MelLec, suggesting an influence on intracellular signal transduction rather than on the recognition of DHN-melanin. Accordingly, the presence of G26A in HSCT donors was found to strongly increase the risk of IPA in the corresponding recipient as the result of a broad defect in cytokine production by myeloid cells (Stappers et al. 2018). Although there is still much to be learned about the mechanisms through which MelLec orchestrates the immune response against *A. fumigatus* (Casadevall 2018), these findings demonstrate the key role of myeloid-expressed MelLec and its genetic variation to the human host defense against *A. fumigatus*.

3.3 NOD-like and RIG-I-like Receptors

Apart from the involvement of NLRs in inflammasome formation, the function of canonical receptors such as NOD1 and NOD2 in the host defense against fungi remained until recently poorly studied. Several polymorphisms in NOD1 and NOD2 have been typically associated with the development of many infectious and inflammatory diseases, namely Crohn's disease (Caruso et al. 2014), but not fungal infections. A recent genetic screening in HSCT patients and corresponding donors revealed however that the donor rs2066842 (P268S) variant was associated with protection from IPA (Gresnigt et al. 2018). Mechanistically, mononuclear cells harboring this variant displayed enhanced phagocytosis and killing capacity whereas NOD2 activation instead reduced the antifungal potential of these cells. Further supporting the suppressive effect of NOD2 activation on antifungal effector functions, NOD2 deficiency conferred resistance to experimental aspergillosis in a mouse model of infection. Altogether, the P268S variants in NOD2 represents one of the few available examples associated with genetically-determined protection

Table 3 Genetic variation in NLRs and RLRs and susceptibility to infection by *Candida* and *Aspergillus*

Gene (s)	SNP(s)	Nucleotide change	Amino acid change	Disease(s)	References
NLRP3	rs74163773	12,9,7,6 VNTR	–	RVVC	Jaeger et al. (2016), Lev-Sagie et al. (2009)
NOD2	rs2066842	C > T	P268S	IPA	Gresnigt et al. (2018)
IFIH1	rs3747517	G > A	H843R	Candidemia	Jaeger et al. (2015)
	rs1990760	C > T	A946T	Candidemia	Jaeger et al. (2015)

The first nucleotide (and corresponding amino acid) is the ancestral nucleotide and therefore is considered the wild-type allele. *SNP* single nucleotide polymorphism; *VNTR* variable number of tandem repeats; *RVVC* recurrent vulvovaginal candidiasis; *IPA* invasive pulmonary aspergillosis

from fungal infection and highlights the interesting possibility to block NOD2 signaling as a therapeutic intervention in IPA.

There is an intricate relationship between dectin-1 signaling and inflammasome formation in response to both *C. albicans* and *A. fumigatus* (Cheng et al. 2011; Said-Sadier et al. 2010). The variable number of tandem repeats (VNTR) rs74163773 in intron 4 of NLRP3 was reported to be more frequently detected in women suffering from RVVC (Lev-Sagie et al. 2009), a finding that was recently validated in a larger multicenter cohort (Jaeger et al. 2016). Importantly, both studies demonstrated that the levels of IL-1β were influenced by the number of intronic repeats carried by the patients, suggesting a regulatory role of this VNTR on NLRP3 inflammasome activity.

An important role in host defense against *C. albicans* has also been ascribed to genetic variants in other cytosolic receptors, namely RLRs. In particular, the missense variants rs1990760 (A946T) and rs3747517 (H843R) in MDA5 (*IFIH1*) were shown to be associated with systemic *Candida* infection as the result of an altered cytokine response (Jaeger et al. 2015). Like TLR3, this receptor for viral RNA also showed unsuspected effects in antifungal immunity, thereby suggesting intracellular receptors for fungal nucleic acids as novel targets amenable to therapeutic manipulation. Table 3 illustrates relevant genetic variants in NLRs and RLRs associated with fungal disease.

3.4 Soluble Pattern Recognition Receptors

In addition to the membrane-bound PRRs discussed in detail above, there are several soluble molecules that are endowed with the ability to interact with and bind to microbial polysaccharides without transducing intracellular signals and that function as opsonins to facilitate phagocytosis (Bidula and Schelenz 2016). Table 4 summarizes relevant genetic variants in soluble PRRs and their association with susceptibility to fungal disease. The mannose-binding lectin (MBL), a CLR that

Table 4 Genetic variation in soluble PRRs and susceptibility to infection by *Candida* and *Aspergillus*

Gene (s)	SNP(s)	Nucleotide change	Amino acid change	Disease(s)	References
MBL2	rs5030737	C > T	R52C	CPA	Crosdale et al. (2001), Vaid et al. (2007)
	rs1800450	G > A	G54D	RVVC	Babula et al. (2003), Donders et al. (2008), Giraldo et al. (2007), Nedovic et al. (2014), Wojitani et al. (2012)
PLG	rs4252125	A > G	D472N	IPA	Zaas et al. (2008)
PTX3	rs2305619	A > G	–	IPA	Cunha et al. (2014, 2015), Fisher et al. (2017)
	rs3816527	A > C	A48D	IPA and mold infection and colonization	Cunha et al. (2014, 2015), Wojtowicz et al. (2015)
	rs1840680	G > A	–	IPA and CPA	Cunha et al. (2014), He et al. (2018)

The first nucleotide (and corresponding amino acid) is the ancestral nucleotide and therefore is considered the wild-type allele. *SNP* single nucleotide polymorphism; *CPA* chronic pulmonary aspergillosis; *RVVC* recurrent vulvovaginal candidiasis; *IPA* invasive pulmonary aspergillosis

binds carbohydrate patterns from microorganisms and activates the lectin pathway of the complement system, stands out as one of the most well-known examples (Foo et al. 2015). Many studies have established genetic variation to be a major regulator of the levels and function of MBL in as much as 8% of individuals in the general population (Sprong and van Deuren 2008). Interestingly, these individuals do not display any obvious clinical phenotypes, suggesting that MBL deficiency may be, to a large extent, compensated by the redundancy of the humoral innate immune system, namely by the vast set of molecules that possess similar opsonic properties. Although not presenting as an outright immunodeficiency, the genetically-determined defect in the function of MBL is acknowledged as an important risk factor for infection, particularly in immunocompromised hosts. There are several known combinations of non-synonymous and promoter variants in the gene encoding MBL, either affecting the expression levels, its functional activity or both (Carvalho et al. 2010). Several studies have proposed a role for the rs1800450 (G54D) variant in MBL in the development of RVVC (Babula et al. 2003; Donders et al. 2008; Giraldo et al. 2007; Wojitani et al. 2012), and this was recently confirmed in a meta-analysis (Nedovic et al. 2014). In addition, the levels of circulating MBL were found to vary significantly during the course of invasive candidiasis (Damiens et al. 2012), although the extent to which genetic variation regulated this phenotype was not assessed. The same holds true for IPA, in which low circulating concentrations of MBL were detected in infected patients (Lambourne et al. 2009). Although there is no evidence for a contribution of genetic

variants in MBL to invasive disease, the development of chronic pulmonary aspergillosis was nonetheless linked with the presence of variable MBL alleles at codon 52 (Crosdale et al. 2001; Vaid et al. 2007). Because most of the studies that have addressed genetic variation in MBL and risk of fungal disease in the past were flawed by the limited number of patients analyzed, these associations need to be revisited in larger and well-characterized cohorts.

Another soluble PRR that has received a great deal of attention in the field of fungal diseases in the past is the long pentraxin-3 (PTX3) (Foo et al. 2015). This molecule has been shown to bind microbial moieties from a wide range of microorganisms, including bacteria, viruses, and fungi, particularly *A. fumigatus* (Garlanda et al. 2002). Although classic immunodeficiencies have not been linked to PTX3 deficiency, common polymorphisms have been disclosed as important risk factors across different infectious diseases, namely *Pseudomonas aeruginosa* colonization in cystic fibrosis patients (Chiarini et al. 2010) and urinary tract infections (Jaillon et al. 2014). Remarkably, and according to its nonredundant role in immunity to *A. fumigatus* in mouse models of infection (Garlanda et al. 2002), genetic variation in PTX3 was identified as a major risk factor for IPA after HSCT (Cunha et al. 2014). These findings were validated in a large, independent study (Fisher et al. 2017) and extended across different clinical settings, including solid organ transplant recipients (Cunha et al. 2015; Wojtowicz et al. 2015) and patients with chronic obstructive pulmonary disease (Cunha and Carvalho 2018; He et al. 2018). Collectively, these studies highlight genetic variation in PTX3 as robust host-derived markers for IPA and lay the foundations for well-designed clinical trials assessing their validity in the clinical setting.

Earlier studies have suggested binding of galactomannan to PTX3 (Garlanda et al. 2002). However, definitive evidence about the actual fungal ligand recognized by PTX3 is still lacking. This is in line with reports showing binding of PTX3 to the cell wall of *C. albicans* (Tierney et al. 2012), although no studies have been performed to date exploring the contribution of genetic variation in PTX3 to the risk of infections caused by *Candida*. Whatever the ligand(s) involved, PTX3 deficiency was found to hamper the normal alveolar expression of the protein and, at a cellular level, it impaired the antifungal effector mechanisms of neutrophils, namely phagocytosis and killing (Cunha et al. 2014). The specific impact of PTX3 deficiency on neutrophil function was corroborated by the loss of the genetic association in patients that developed IPA during severe neutropenia. However, additional mechanisms of antifungal host defense may also be influenced by PTX3 deficiency. The recent demonstration that PTX3 is a critical molecule bridging neutrophil function and B-cell function, namely class switching, plasmablast expansion and antibody production, represents one such example (Chorny et al. 2016). In addition, binding of PTX3 to myeloid differentiation protein 2, an adapter of the TLR4 signaling complex, is critically required for immune protection in experimental aspergillosis (Bozza et al. 2014). This raises the interesting possibility that the combined genetic deficiency of PTX3 and TLR4 might underlie a higher risk of IPA than the single defects alone, a hypothesis that requires confirmation.

The measurement of PTX3 in the bronchoalveolar fluids of mechanically ventilated patients was recently proposed to be endowed with the ability to identify microbiologically confirmed pneumonia (Mauri et al. 2014). The alveolar concentrations of PTX3 are known be determined at the genetic level (Cunha et al. 2014), and, as such, one could expect a further improvement to the diagnostic performance of PTX3 by knowing in advance the genotypic profile of the patient. In addition, PTX3 deficiency was also shown to influence the levels of alveolar cytokines in hematological patients suffering from IPA and to impact their ability in discriminating infection (Gonçalves et al. 2017). More important, reinstating the normal levels of PTX3 in vitro with the recombinant protein was sufficient to restore the efficacy of the antifungal effector functions of neutrophils (Cunha et al. 2014). Although data in the clinical setting is so far lacking, these observations support the potential applicability of PTX3 in novel prophylactic or therapeutic approaches for IPA in patients at-risk (Carvalho et al. 2012a). As stated above, the combined use of antifungal therapy and PTX3 treatment has been found to improve the efficacy of the drug alone in animal models of IPA (Lo Giudice et al. 2012; Marra et al. 2014).

Another example of a soluble PRR characterized by relevant genetic diversity regards plasminogen. By mining genetic data obtained from an unbiased screen of survival data in different strains of mice subjected to experimental aspergillosis, the non-synonymous variant rs4252125 (D472N) was correlated with the risk of IPA in patients undergoing HSCT (Zaas et al. 2008). These findings support the importance of additional pre-clinical studies testing different models of infection and evaluating additional immune-related readouts to assist in the discovery of human genetic variation with an important contribution to the risk of infection.

4 Clinical Translation of Host Genetics in Medical Mycology

Many studies over the past decade have implicated genetic variation in PRRs in the risk of developing fungal disease, particularly under predisposing clinical conditions such as those in the hematology setting (Cunha et al. 2011b; van der Velden et al. 2011). However, a great deal of work is still required to identify the actual causative alleles and their functional consequences, as well as to precisely pinpoint the biological mechanisms through which these influence the risk of infection. We have currently a large amount of genetic links to susceptibility to fungal disease at our disposal; however, clinical data supporting the translation of these critical insights into improved patient outcomes has been practically inexistent (Cunha et al. 2011a). This can be largely attributed to the relatively small effect size of the identified variants (since many carriers will not develop infection), which may negatively influence the discriminatory ability of the genetic profile to inform clinical decision-making. Although this limitation will hardly be countered soon

and the use of genetic information to predict the risk of fungal disease is unlikely to alter clinical practice in the near future, the predictive performance of the genetic information may benefit from advanced genetic screening strategies and mathematical models. The integration of host and pathogen genetic data into stratification models that also consider the clinical characteristics of the patients are expected to improve our current ability to predict risk and progression of disease, including the response to treatment and its duration, and adverse events. A first, modest step toward this goal was recently taken in a study that demonstrated that the concerted analysis of selected genetic and clinical factors into a predictive model could be used to guide preemptive therapy in hematological patients (White et al. 2018).

Although genetic data in the clinical setting has historically been investigated with the goal to develop risk stratification and diagnostic strategies, recent studies illustrating how genetic variation regulates processes of host immunity raises the exciting possibility that it may also be exploited to guide immunotherapy. Most of the clinical trials performed to date have failed to account for the significant impact that genetic variation may pose on subgroups of individuals, and this may partly explain the disappointing outcomes of trials involving anti-inflammatory agents for the treatment of sepsis (Cohen et al. 2015). There is therefore an urgent need to identify and characterize relevant genetic variation in the stratification of patients enrolled in clinical trials of immunomodulatory agents (Rello et al. 2018).

The application of next-generation sequencing and systems biology approaches provide a powerful tool to identify essential genes and pathways in the host-fungus interaction at a level of complexity that was not possible beforehand (Dix et al. 2016). Although in the field of fungal diseases, genetic association studies at the genome-wide level are scarce, a few examples exist which have enabled the identification of novel players controlling susceptibility to infection (Kumar et al. 2014; Smeekens et al. 2013a). For example, the integration of transcriptional data and functional genomics revealed an unanticipated role of type I IFN in the host defense against *Candida* (Smeekens et al. 2013a). Polymorphisms in type I IFN genes were found to modulate cytokine production and to drive a skew from a Th17 to a Th1 type of response, thereby predisposing to candidemia in critically ill patients. More recently, other functional genomics approaches combining whole genome information and immunological screenings have also provided invaluable evidence about the genetic regulation of cytokine production in humans in response to different stimuli, including fungi (Li et al. 2016a, b). By correlating genome-wide SNP genotypes with cytokine abundance in response to fungal stimulation, several cytokine quantitative trait loci (QTL), i.e., genetic variants that control cytokine production, were identified (Li et al. 2016a). Among them, a cytokine QTL at the NAA35/GOLM1 locus markedly influenced the levels of IL-6 produced and was associated with susceptibility to candidemia. Altogether, these studies support the need to consider the genetic contribution to immune phenotypic variation (e.g., cytokine production and immune cell function) to generate accurate maps of the human genomic architecture regulating susceptibility to fungal disease.

5 Conclusions

Our current understanding of the immunobiology of human fungal diseases has derived largely from studies addressing polygenic susceptibility. These studies have not only revealed the individual contribution of genetic variants but have also highlighted the complex interactions between adding effects of variants with small or modest effect sizes to the immune response. The current state-of-the-art is however not adequate for the needs of clinicians since it does not allow the accurate prediction of which individuals might develop fungal disease or that may benefit the most from intensive diagnostic surveillance or alternative prophylaxis and treatment strategies. Clinical translation of patient genetics will benefit from the concerted action of clinicians and researchers into the establishment of larger and well-characterized patient cohorts, as well as comprehensive functional testing to identify the biological mechanisms of association with infection. By unraveling molecules and pathways whose expression or function may be regulated at the genetic level may allow the possibility to target them with personalized immunotherapeutics aimed at restoring lacking or defective immune components (Cunha and Carvalho 2012). In conclusion, an improved understanding of how specific genetic signatures regulate susceptibility to fungal disease may revolutionize the field of medical mycology, opening new horizons and laying the foundations for personalized medical interventions based on individual genomics in patients who are at risk.

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