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Review

Genetic variability of innate immunity impacts human susceptibility to fungal diseases

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ABSTRACT

Fungi are a major threat in immunocompromised patients. Despite presenting similar degrees of immunosuppression, not all individuals at-risk ultimately develop fungal diseases. The traditional view of immune suppression as a key risk factor for susceptibility to fungal infections needs to be accommodated within new conceptual advances on host immunity and its relationship to fungal disease. The critical role of the immune system emphasizes the contribution of host genetic polymorphisms to fungal disease susceptibility. This review highlights the present knowledge on innate immunity genetics that associates with susceptibility to fungal diseases.

1. Introduction

In the last decades, modern and sophisticated medical care has prolonged and improved the lives of many individuals suffering from severely debilitating conditions. However, such advances have also resulted in an increased risk for fungal diseases, particularly among patients with prolonged neutropenia, treated with corticosteroids, or submitted to bone-marrow or solid-organ transplantation.[1] Invasive fungal diseases are associated with significant morbidity and an estimated mortality exceeding 50% in most studies.[2] Mortality has been reported to be as high as 95% in bone-marrow transplant recipients with invasive aspergillosis (IA), particularly in the context of disseminated disease.[3] In addition to *Aspergillus* species, several fungi have emerged as important opportunistic pathogens, including species of *Candida*, *Fusarium*, *Scedosporium* and the zygomycetes. Despite the availability of drugs, the emergence of antifungal-resistant strains and the failure of certain patients to respond to standard treatments have emphasized the need for the development of novel therapeutic agents.[4–6] This involves the study of both the pathogens, in terms of life cycles, invasion processes and virulence mechanisms, and the host, whose own defense mechanisms determine the response to the infectious challenge and the ensuing pathology. In this regard, it is pertinent to acknowledge the variable degrees of virulence often presented by different isolates of the same fungal species (e.g., *Paracoccidioides brasiliensis*) that can contribute to the development of more or less severe fungal disease, independently of the genetic circumstances of the host.[7] On the other hand, regarding the host genetic background, significantly varying patterns of susceptibility are also known to be displayed by different inbred strains of mice to the same fungal pathogen.[8] Over the last few years, significant advances into the genetic basis of immune disorders leading to severe and recurrent infections have been made.[9] It is now clear that host genetic factors play a major role in determining differential susceptibility to infectious diseases of humans. In this review, we will discuss major findings and recent progress on the genetic variables of the innate immune system contributing to susceptibility to fungal diseases.

2. Human genetics: exploring susceptibility to infectious diseases

Differences in human susceptibility to infectious diseases have been widely reported, the best known example being malaria.[10] Although malaria illustrates a genetic variant of the host resulting

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in reduced susceptibility to disease, there is also evidence that genetic variants can lead to increased occurrence of infections. A study comparing the causes of death among adopted children with those of either their biological or adoptive parents concluded that the risk of death from infection was increased 5.8-fold when biological parents also prematurely died from the same cause.[11] This study, which effectively separated genetic and environmental confounders, confirmed the substantial genetic effect involved in susceptibility to infection.

Although humans are identical at most of the 3 billion base pairs in their genome, inter-individual variation is present in approximately 0.01% of the genome.[12] The most common genetic variation is the single nucleotide polymorphism (SNP), in which two alternative bases occur at appreciable frequency (>1%) within a population. Another type of genetic mutation is the variable number of tandem repeats (VNTR), consisting of sequence repeats ranging from a single to thousands of base pairs.[13] Many genetic variants are 'silent' with no effect on gene products. In general, functionally significant effects are most likely when polymorphisms are associated with amino acid substitutions in the gene product, when a deletion/insertion results in a frameshift in the coding region, or when the polymorphism directly affects gene transcription, RNA splicing, mRNA stability or mRNA translation. Only 1.5% of SNPs are thought to be located in the coding regions, with the functions of nearly all SNPs located outside gene coding or regulatory regions remaining unknown. These variations, however, are not randomly distributed within the genome, but rather depend on the particular genomic region, as well as on selective pressure.[14] Hence, it is reasonable to speculate that genes involved in important immune pathways represent a potential source of variability regarding susceptibility to infectious diseases. In fact, these attractive candidates have already prompted a number of key human studies on genetic variation contributing to susceptibility or outcome of fungal diseases, as discussed throughout this review.

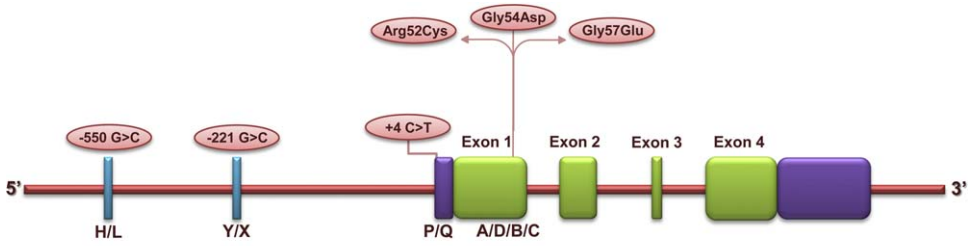
Human disease-association studies can identify multiple interacting disease genes and respective pathways, providing a comprehensive understanding of the etiology of disease. However, as information on human genetic variability increases, it is critical that study design and data analysis receives thorough attention to ensure that spurious associations are not reported and that genuine disease genes are identified. Frequently cited concerns in association studies regard case definition. In this regard, it is imperative that appropriate and uniform diagnostic criteria for the disease being studied are used, especially if subgroup analysis involving the stage or severity of the disease is performed. There are also several pitfalls in control recruitment. Ideally, cases and controls should be matched by ethnic origin, age, and gender. Moreover, it is also important to note that the power for association detection depends on several factors, including the frequency of risk allele/genotype, the relative risk conferred by disease-associated allele/genotype, the correlation between the genotyped marker and risk allele, sample size, disease prevalence and heterogeneity, and genetic heterogeneity of the sampled population.

3. Genetic variants of pattern recognition receptors and susceptibility to fungal diseases

Host defense mechanisms influence both fungal disease manifestation and severity. The immune system of vertebrates consists of two inter-related components, the innate and adaptive responses, jointly required for the resolution of most infections.[15] The innate immune response is based on a restricted number of evolutionarily conserved germline-encoded receptors, the pattern recognition receptors (PRRs), which recognize highly conserved microbial structures, enabling the host to rapidly recognize a broad range of pathogens.[16] Microbial recognition induces an inflammatory response through the production of cytokines and chemokines, activating and recruiting other cells to the site of infection, ultimately initiating the adaptive arm of the immune response. During infection in vivo, microbial recognition is likely to occur through multiple interactions at multiple sites involving many receptors.

3.1. Soluble PRRs

The collectin subfamily of lectins includes members such as mannose-binding lectin (MBL), pentraxin 3 (PTX3), and lung surfactant proteins (SPs). MBL is known to strengthen the innate immune response by interacting with pathogens and immune



Promoter alleles	MBL expressed	Structural alleles	Serum MBL resulting from extended haplotype
HYP	High	A	High
		D	Non-functional
LYQ	High	A	High
		C	Non-functional
LYP	Medium	A	Medium
		B	Non-functional
LXP	Low	A	Low

Figure 1. The structure of *MBL2* and the functional impact of SNPs in *MBL2*. The six loci of the most common mutations are shown above the gene, with the letters below them representing the alleles for wild-type and mutant variants. Three are located at codons 52, 54, and 57, encoding for variants D, B, and C, respectively. The wild-type allele that codes for the formation of high-order oligomers is assigned the letter A. Three additional polymorphisms at positions −550 (H/L), −221 (Y/X), and +4 (P/Q) are located in the 5′-flanking region of *MBL2*. Consequently, an individual expressing the sequence HYP will produce high levels of MBL. Whilst in theory, any combination sequence of H/L, Y/X, and P/Q should be possible, linkage disequilibrium leads to the formation of four main promoter sequences: HYP, LYQ, LYP, and LXP.

effector cells.[17] Since it was first reported, MBL deficiency has been consistently associated with increased susceptibility to infections, particularly when adaptive immunity is compromised (e.g., in early childhood or following chemotherapy[18,19]). Two MBL genes exist, named *MBL1* and *MBL2*. Both are found on chromosome 10, although work into *MBL1* has found it to be a pseudogene;[20] consequently, MBL in humans is only expressed by *MBL2*. Six functional polymorphisms are known in *MBL2*, and each may affect protein levels (Figure 1). Three are non-synonymous polymorphisms leading to amino acid changes, which dramatically reduce functional MBL levels by impairing assembly of MBL monomers into functional oligomers.[21] Three additional polymorphisms are located in the 5′-flanking region of *MBL2*, affecting transcriptional activity, reducing levels of circulating MBL.[22] Although MBL may still be produced at a level which reflects its expression, the fact that it is non-functional will manifest as a deficiency of MBL. Thus, through the combination of structural and promoter polymorphisms, MBL concentrations can vary considerably in the apparently healthy individual.

The variant D of *MBL2* was shown to associate with chronic cavitary pulmonary aspergillosis (CCPA), a subacute and slowly destructive form of aspergillosis.[23] Interestingly, unlike CCPA, no significant association was observed between the same variant and allergic bronchopulmonary aspergillosis (ABPA), a hypersensitivity disease affecting mainly patients with asthma or cystic fibrosis. However, ABPA patients showed a significant association with the G+1011A polymorphism in intron 1 of *MBL2*, resulting in eosinophilia and elevated plasma MBL levels and MBL activity, which is related with the pathogenesis of the disease.[24] In transplantation, the incidence of invasive fungal diseases after allogeneic hematopoietic stem cell transplantation (HSCT) was instead correlated with donor MBL-low genotypes.[25] The same authors reported an additional non-synonymous polymorphism in MBL-associated serine protease 2 (*MASP2*), known to lead to reduced MBL function, to be an independent predictive factor for invasive fungal disease after HSCT, although this result deserves further validation owing to the low number of recipients with the MASP2 variant.[25] Nevertheless, this study shows that independent factors from both donors and recipients play an important role in the outcome of bone-marrow transplantation.

Vulvovaginal candidiasis (VVC), together with its recurrent form (rVVC), is one of the most prevalent vaginal infections. The variant B of *MBL2* has been associated with either an increased risk[26–29] or no risk[30] of rVVC or VVC, despite the fact that a reduction in vaginal concentrations of MBL in women with rVVC has been consistently observed in the presence of such variant.[26,28,29] These observations suggest that MBL deficiency could be an important risk factor for rVVC. Additionally, low MBL plasma levels were also reported to facilitate abdominal Candida infections in patients with secondary peritonitis, independently of other risk factors.[31] Despite this evidence suggesting an important role of MBL in Candida infections, *MBL2* polymorphisms were not associated with chronic disseminated candidiasis (CDC) in immunocompromised patients, particularly those undergoing therapy for acute leukemia.[32] This suggests that additional innate immune gene polymorphisms may be involved in susceptibility to Candida infection or, as recently suggested, in Candida carriage.[33] In this regard, diabetic or healthy asymptomatic individuals who have detectable levels of *Candida spp* in the oral cavity have a significantly increased incidence of the wild-type allele at position −44 (C−44G) in the *DEFB1* gene encoding β-defensin-1 (hBD-1).[33] Since this polymorphism is located in the 5′-untranslated region of *DEFB1*, variations in the translation and/or transcription of hBD-1 may explain the expression variability among individuals. However, its relevance to Candida carriage in HIV-infected patients and the development of oropharyngeal candidiasis is currently unknown.

PTX3 is the type member of the pentraxin family, playing an important role in microbe recognition and complement activation, acting non-redundantly against selected pathogens.[34] For instance, PTX3 may bind to Aspergillus conidia to facilitate phagocytosis. Accordingly, whereas PTX3-null mice are highly susceptible to IA,[34] PTX3 administration was shown to have a protective effect against IA in a murine model of allogeneic HSCT.[35] However, a study failed to associate polymorphisms in the human *PTX3* gene and susceptibility to particular phenotypes of aspergillosis, although not all regions of *PTX3* were sequenced (Pasqualotto et al., unpublished data). Additionally, a high degree of variability in PTX3 serum levels was observed, without any obvious pattern of expression associated with disease phenotype. Since the *PTX3* gene exists in the human genome as an antisense pair with the *VEPH* gene, studying the latter could be an interesting approach.

Allele variants in *SP-A2*, one of the genes encoding for functional SP-A, have also been shown to influence susceptibility to both ABPA and CCPA.[23,36] Although the precise effect of these polymorphisms on SP-A function is yet unknown, increased levels of total IgE antibodies and peripheral eosinophilia, as well as decreased lung function, were suggested as the causes for ABPA susceptibility.[36] The occurrence of distinct genotype combinations of *SP-A2* and *MBL2* in patients with pulmonary aspergillosis suggests that variations in these genes may contribute to the pathogenesis of the diverse clinical entities caused by the fungus.

It is of interest that the genetic bases for human disease susceptibility may also be evaluated by the screening of inbred murine strains. Recently, this approach was performed using an immunocompromised mice model of IA.[37] Through a haplotype-based computational genetic analysis of the survival data, the polymorphic gene encoding plasminogen (PLG), a regulatory molecule that binds to Aspergillus, was identified as a suitable candidate for Aspergillus susceptibility. Accordingly, a non-synonymous polymorphism causing an amino acid change (Asp472Asn) in human PLG was reported to affect the risk of developing IA in HSCT recipients, particularly after day 40 post-transplant.[37] In addition, there was an apparent gene-dosage effect: homozygous mutant individuals had a 5.6-fold increased risk of developing IA while heterozygotes had a 3.0-fold increased risk, relative to wild-type individuals. Besides shedding light into the role of the fibrinolytic system in the pathogenesis of IA, this approach identified a novel, biologically plausible, positional candidate gene, validating its use in the identification of less obvious disease-related genes.

To date, the vast majority of the genetic studies involving soluble PRRs and susceptibility to fungal disease have focused on the role of MBL. Indeed, a well-established link between genetically determined deficiencies in MBL levels and susceptibility to both mould and yeast infections in either immunocompetent or immunosuppressed hosts has been proposed (reviewed in Table 1). Genetic variants affecting other soluble molecules of crucial relevance in host antifungal defense, including proteins from the lung surfactant and β-defensins, have also been shown to influence susceptibility to fungal diseases.[23,33,36] On the other hand, polymorphisms in *PTX3* were demonstrated to be unlikely determinant factors of susceptibility to aspergillosis (Pasqualotto et al., unpublished data). Despite these advances, further studies identifying additional genes involved in fungal susceptibility are required. The recent approach developed by Zaas et al. using an immunocompromised mice model of IA represents a promising methodology to be used in the discovery of candidate genes involved in fungal disease,[37] information which will eventually allow the uncovering of additional human genetic variations putatively implicated in fungal disease.

Table 1
Human genetic association studies evaluating polymorphisms in soluble pattern recognition receptors (PRRs) and susceptibility to fungal diseases

Reference	Association study	Gene	SNPs/haplotypes	Relevant findings
Vaid et al.[23]	CCPA	*MBL2*	Variant D (codon 52)	The variant allele ($p \leq 0.02$) and genotype ($p \leq 0.05$) were associated with CCPA ($n = 15$), but not ABPA
Donders et al.[27]	rVVC	*MBL2*	Variant B (codon 54)	Women suffering from rVVC ($n = 109$) were more likely to carry the variant B of *MBL2* than controls (20 vs. 6.6%, $p = 0.01$)
Liu et al.[29]	VVC	*MBL2*	Variant B (codon 54)	Cervicovaginal MBL concentrations and gene variant frequency were both significantly higher in women with VVC ($n = 51$) than controls ($p < 0.01$)
Kaur et al.[24]	ABPA	*MBL2*	G+1011	A significant association of high MBL levels with ABPA ($n = 11$) in comparison to the controls ($p < 0.01$) was observed
Granell et al.[25]	IA	*MBL2*	O/O or LXA/O[a]	Donor MBL-low genotype (38% vs. 12%, $p = 0.01$) was associated with a higher probability of IA in HSCT ($n = 106$ donor–patient sibling pairs)
van Till et al.[31]	AYI	*MBL2*	A/O or O/O	A higher proportion of variant patients had an AYI during early peritonitis than wild-type patients ($n = 88$) (39% vs.16%, $p = 0.012$)
Saxena et al.[36]	ABPA	*SP-A2*	G+1649C and A+1660G	*SP-A2* polymorphisms contribute to genetic predisposition and severity of clinical markers of ABPA ($n = 10$)
Vaid et al.[23]	CCPA	*SP-A2*	G+1649C	The C/C genotype was significantly associated with CCPA ($n = 12$) ($p \leq 0.05$)
Zaas et al.[37]	IA	*PLG*	Asp472Asn	Risk of IA at day >40 after HSCT was 5.6-fold higher in Asn/Asn vs. Asp/Asp individuals ($n = 83$ IA cases)
Granell et al.[25]	IA	*MASP2*	Asp105Gly	Recipient *MASP2* variant (67% vs. 14%, $p = 0.01$) was associated with a higher probability of IA in HSCT ($n = 106$ donor-patient sibling pairs)
Jurevic et al.[33]	Candida carriage	*DEFB1*	C−44G	A strong association with Candida carriage was observed in both diabetic ($n = 43$) and non-diabetic individuals

ABPA, allergic bronchopulmonary aspergillosis; AYI, abdominal yeast infection; CCPA, chronic cavitary pulmonary aspergillosis; DEFB1, β-defensin-1; HSCT, hematopoietic stem cell transplantation; IA, invasive aspergillosis; MASP2, MBL-associated serine protease 2; MBL, mannose-binding lectin; PLG, plasminogen; rVVC, recurrent vulvovaginal candidiasis; SNP, single nucleotide polymorphism; SP-A2, surfactant protein A2; VVC, vulvovaginal candidiasis.
[a] O/O and LXA/O are MBL-low haplotypes. LX represents an MBL promoter haplotype. Variants D, B and C are collectively named O, while A indicates the wild-type.

3.2. Membrane-bound PRRs

The Drosophila protein Toll, originally identified as a transmembrane receptor required for the establishment of dorsoventral polarity in developing fly embryos,[38] was shown to be required to mount effective antifungal responses.[39] The observation that Toll-deficient Drosophila were highly susceptible to fungal infection led to the assumption that mammalian TLRs also participated in antifungal immunity. In fungal infections, the different impact of TLRs on the innate and adaptive immunity is consistent with the ability of each individual TLR to activate specialized antifungal effector functions on innate immune cells, such as the respiratory burst, degranulation, and production of chemokines and cytokines.[40–42] TLR2, TLR4, and TLR9 signaling has been shown to contribute to host responses against fungi both in mice (reviewed in ref. 43) and humans.[44]

Two important co-segregated polymorphisms – Asp299Gly and Thr399Ile – resulting in lipopolysaccharide (LPS) hyporesponsiveness, are present in the *TLR4* gene.[45] These polymorphisms were described to be present at a substantially higher proportion among individuals hyporesponsive to inhaled LPS. Epithelial cells derived from these individuals were found to be unable to mediate LPS signaling in vitro.[45] This report was followed by several studies confirming an association between these polymorphisms and the incidence of septic shock due to Gram-negative bacteria.[46] Regarding fungal disease, the Asp299Gly polymorphism was recently shown to be associated with susceptibility to CCPA[44] and IA in allogeneic HSCT recipients of unrelated donors.[47] The fact

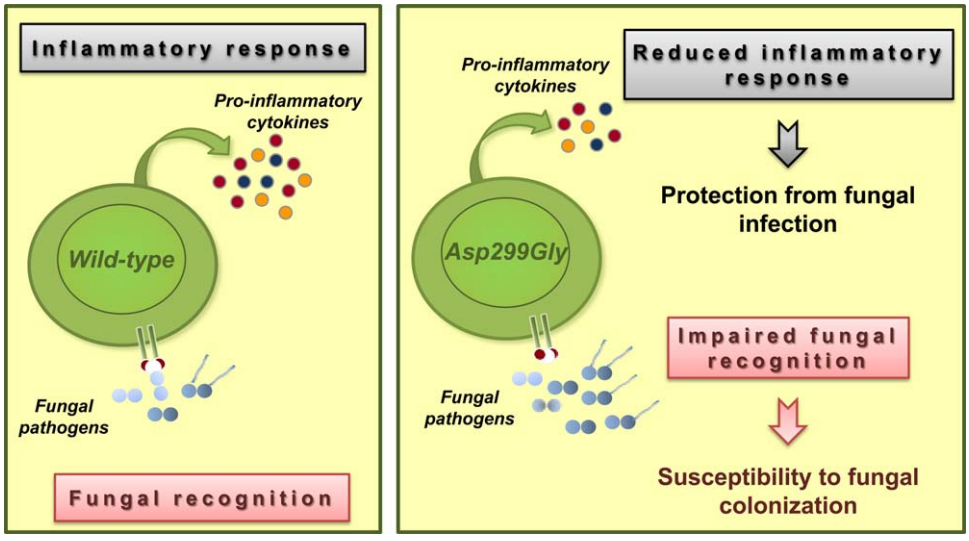


Figure 2. The Asp299Gly polymorphism of the *TLR4* gene may represent a 'double-edged sword' in allogeneic stem cell transplantation. The abnormal TLR4 extracellular domain may be hampering its function by impairing microbial recognition, eventually leading to fungal escape from immune surveillance and predisposing to fungal colonization. However, failure to recognize the fungus may be compensated by the lack of an exuberant inflammatory response to it, which may ultimately be harmful to the host, thus resulting in a protective effect from fungal infection.

that a previous study failed to link the presence of this polymorphism in HSCT recipients to susceptibility to IA[48] indicates that the contribution of this SNP may depend on the type of transplant. Additionally, in allogeneic HSCT recipients, we have recently observed an association between the presence of Asp299Gly and fungal colonization but not fungal pneumonia.[49] Therefore, fungal colonization may not predict susceptibility to infection in the presence of Asp299Gly. The positive correlation of this SNP with fungal colonization could be explained by the fact that the presence of an abnormal TLR4 extracellular domain may hamper its function by disrupting microbial recognition, eventually leading to fungal escape from immunosurveillance (Figure 2). However, *TLR4* polymorphisms have also been shown to have a protective effect in diseases associated with hyperinflammatory states.[50] Therefore, the failure to recognize the fungus may be compensated by the lack of an exuberant inflammatory response to it which may ultimately be harmful to the host. In this regard, we have recently found that a hyperinflammatory state, more than the fungus itself, may contribute to susceptibility to aspergillosis and other fungal infections.[4] Thus, by limiting the inflammatory response to the fungus, the Asp299Gly polymorphism could contribute to resistance to infection, despite evidence of fungal growth. It is interesting in this regard that the Asp299Gly polymorphism was recently shown to have a unique distribution with high prevalence in Africa and low prevalence in Europe, with the authors arguing that the benefit from reduced inflammation during malaria in Africa might have been counter-selected due to lack of inflammation in response to bacterial infections.[51]

The same *TLR4* polymorphisms were shown to contribute to a higher risk of Candida bloodstream infection, supposedly through an increased interleukin (IL)-10 production.[52] Patients with chronic mucocutaneous candidiasis (CMC) were also reported to have increased IL-10 over interferon-γ (IFN-γ) production, possibly occurring in association with Asp299Gly.[53] However, the extent to which these polymorphisms contribute to the hyperactivation immune status of CMC patients with autoimmune regulator (*AIRE*) gene mutations[54] is not known. IL-10 has been reported to inhibit the action of human monocytes against *Candida albicans*, while in mice, the absence of IL-10 was associated with increased antifungal resistance. However, in experimental candidiasis, IL-10 exhibited both beneficial and detrimental effects depending on the degree of inflammation (reviewed in ref. 55). Therefore, despite the overall suppressive effect, IL-10 might be required to limit host damage under high levels of inflammation. This may explain why the Asp299Gly polymorphism does not appear to play a role in susceptibility and severity of human urogenital *C. albicans* infection.[56]

TLR2 is also known to recognize motifs from fungal pathogens and initiate immune responses. A TLR2-dependent IL-10 production was demonstrated in response to Candida and Aspergillus in experimental models of infection.[57,58] However, no association between the non-synonymous polymorphism in human *TLR2* (Arg753Gln) and susceptibility to IA, CCPA, or ABPA was found.[44,48,49] In candidiasis, the Arg753Gln polymorphism was shown to impact cytokine release, namely reduced IFN-γ and IL-8 and increased tumor necrosis factor-α (TNF-α) during Candida sepsis in intensive care unit (ICU) patients.[59] However, the extent to which the deregulated cytokine production contributes to susceptibility to candidiasis is not clear.

TLR9 is a receptor that detects unmethylated CpG motifs prevalent in bacterial and viral DNA.[60] Recently, *Aspergillus fumigatus* DNA was also shown to activate immune responses through TLR9-dependent recognition.[61] In humans, two variants were identified within the *TLR9* promoter, as well as a number of rare non-synonymous SNPs within the coding sequence.[62] An association of a promoter polymorphism, T−1237C, with asthma was reported, whereas no significant results were found regarding the other variants.[62] T−1237C was also demonstrated to be involved in susceptibility to atopic eczema, and an altered *TLR9* expression was considered to underlie this putative association.[63] Interestingly, we recently reported an association between T−1237C and the development of ABPA, but not severe asthma with fungal sensitization (SAFS)[44] or IA in allogeneic HSCT.[49] This was the first study to show that subtle genetic differences may occur between the two similar allergic groups of ABPA and SAFS patients.

Less well-characterized genetic variants in other TLRs, including *TLR1* and *TLR6*, have also been reported to influence susceptibility to acute IA after allogeneic HSCT.[48] Transplant recipients harboring either one polymorphism in *TLR1* (Arg80Thr) or a polymorphism combination in *TLR1* and *TLR6* (Asn248Ser and Ser249Pro) have an increased risk of invasive fungal disease.[48] Again, the fact that polymorphisms in HSCT recipients have been associated with aspergillosis suggests that other components in addition to hematopoietic cells play an important role in the recognition of Aspergillus ligands.

C-type lectin receptors (including dectin-1) are major mammalian PRRs for several fungal components and are the prototype of innate non-TLR signaling pathways for innate antifungal sensing.[64] Dectin-1 is a myeloid-expressed transmembrane receptor that specifically recognizes the cell wall carbohydrates β-1,3-glucans of many fungi, mediating myeloid cell activation, cytokine production, and a variety of antifungal responses.[64] Recently, a polymorphism in human dectin-1 that generates an early stop codon was identified and associated with an increased risk of mucocutaneous candidiasis (reviewed in ref. 65). This SNP was shown to result in impaired transport of dectin-1 to the cell surface as well as failure in mediating β-glucan binding.[65] In fact, individuals homozygous for this polymorphism lack expression of this receptor on the surface of their myeloid cells, and therefore these cells do not respond to β-glucan. Nevertheless, and although patients harboring this mutation presented defective cytokine production, the normal phagocytosis and killing capacity displayed by neutrophils and macrophages could explain why this dectin-1 deficiency was not associated with invasive fungal diseases.[65]

Genetic studies elucidating the role of membrane-bound PRRs have mainly focused on TLR4, and in particular, on a coding variant affecting the extracellular domain of the receptor (reviewed in Table 2). A recent study by Bochud et al. demonstrated that the *TLR4* variant Asp299Gly on the donor side was an independent predisposing factor to IA following HSCT.[47] In this regard, work from our group has also recently demonstrated that the same variant, while predisposing to fungal colonization, was nonetheless associated with resistance to fungal pneumonia of colonized patients.[49] The Asp299Gly polymorphism had been previously described to contribute to both increased susceptibility to CCPA[44] and Candida bloodstream infection.[52] Despite the increasing amount of data regarding genetic variability of TLRs, consideration must also be given to the role played by non-TLRs. In this regard, recent work evaluating polymorphisms in dectin-1,[64] together with the understanding of their functional impact, assures new research possibilities regarding the influence of the host genetic background in fungal susceptibility.

3.3. Cytokines

Cytokines mediate the inflammatory and adaptive responses to fungi. The inflammatory response may serve to limit infection, but an overzealous or heightened inflammatory response may contribute significantly to the histological patterns and pathogenicity, as documented by the occurrence of severe fungal infections in patients with immune reconstitution disease.[4–6] Th1 cytokines

Table 2
Human genetic association studies evaluating polymorphisms in Toll-like receptors (TLRs) and susceptibility to fungal diseases

Reference	Association study	Gene	SNPs/haplotypes	Relevant findings
Carvalho et al.[44]	CCPA	*TLR4*	Asp299Gly[a]	A significant association was observed between Asp299Gly and CCPA ($n=40$) ($p=0.003$)
Bochud et al.[47]	IA	*TLR4*	Asp299Gly[a]	An association between donor Asp299Gly and risk of IA among recipients of HSCT from unrelated donors was observed, especially if combined with CMV positivity ($n=103$ IA cases)
Carvalho et al.[49]	IA	*TLR4*	Asp299Gly[a]	Donor Asp299Gly was associated with fungal colonization following HSCT ($p=0.003$), while susceptibility to fungal pneumonia was instead decreased in the presence of the same SNP ($p=0.03$)
Van der Graaf et al.[52]	BSI	*TLR4*	Asp299Gly[a]	The prevalence of Asp299Gly was higher in patients with Candida BSI ($n=43$) than in controls (26% vs. 10%)
Carvalho et al.[44]	ABPA	*TLR9*	T−1237C	Susceptibility to ABPA ($n=22$) was associated with T−1237C ($p=0.043$)
Kesh et al.[48]	IA	*TLR1*	Arg80Thr	Analysis of recipient SNPs showed that the presence of TLR1 Arg80Thr or the presence of both TLR1 Asn248Ser and TLR6 Ser249Pro was associated with IA ($n=22$ IA cases) ($p<0.001$)
		TLR6	Asn248Ser, Ser249Pro	

ABPA, allergic bronchopulmonary aspergillosis; BSI, bloodstream infection; CCPA, chronic cavitary pulmonary aspergillosis; CMV, cytomegalovirus; HSCT, hematopoietic stem cell transplantation; IA, invasive aspergillosis; SNP, single nucleotide polymorphism; TLR, Toll-like receptor.
[a] The Asp299Gly polymorphism was co-segregated with Thr399Ile.

(mainly IL-12 and IFN-γ), are central for protection against fungi. However, other cytokines and T cell-dependent pathways are also involved in antifungal immune responses.[42]

In terms of pro-inflammatory cytokine production, a polymorphism in the *TNF* promoter (G-308A) is responsible for variable cytokine levels. The high-producing A/A genotype was shown to be less common in patients with aspergillosis.[66] Accordingly, in vitro studies have demonstrated that TNF-α enhances specific phagocytic activity against conidia by pulmonary alveolar macrophages and augments the neutrophil damage of Aspergillus hyphae.[67] However, a lack of association between *TNF* polymorphisms and IA has also been described. In this study, a polymorphic site in the promoter of TNF receptor 2 (*TNFR2*) was instead reported to predispose to IA.[68]

The pro-inflammatory cytokine IL-1 is encoded by two separate genes, *IL1A* (IL-1α) and *IL1B* (IL-1β), located in a cluster that also contains the IL-1 receptor antagonist (*IL1RN*).[69] No association was found between the presence of IA and individual locus analysis of the *IL1A* (C−889T), *IL1B* (T−511C), and *IL1RN* (86-bp VNTR) polymorphisms.[70] Nevertheless, haplotype analysis revealed that VNTR2/−889C/−511T was strongly associated with susceptibility to develop IA, whereas a link between the VNTR2/−889C/−511C haplotype and resistance to IA was reported,[70] supporting the hypothesis that the *IL1* gene cluster may determine susceptibility to IA, although the precise mechanisms are yet unclear. IL-6 also contributes to the inflammatory response to fungi.[4] However, *IL6* polymorphisms failed to associate with IA.[71] Nevertheless, the *IL6* polymorphisms, together with those from the *IL1* gene cluster, appear to be involved in the modulation of C-reactive protein (CRP) levels, since a positive correlation with CRP values was found, although the authors were unable to link the circulating CRP values with the pathogenesis of IA infection.

IFN-γ is able to potentiate the antifungal activity of human phagocytic cells,[72] with a higher IFN-γ/IL-10 ratio contributing to a favorable response to antifungal therapy in patients with clinical evidence of IA.[73] Although high levels of IFN-γ resulting from homozygous carriage of the T+874A polymorphism in the *IFNG* gene were associated with aspergillosis,[66] patients with inborn errors of the IL-12/IL-23/IFN-γ-mediated immunity were reported to be susceptible to disseminated paracoccidioidomycosis[74] and histoplasmosis.[75]

IL-4 has been suggested as the first determinant of T cell differentiation into Th2 cytokine-producing cells during fungal diseases.[72] Frequent polymorphisms in the IL-4 receptor (*IL4R*) of ABPA patients, in particular Ile75Val, were associated with IL-4-induced up-regulation of CD23 expression.[76] The increased sensitivity of ABPA patients to IL-4 was proposed as a contributing factor to increased B-cell activity eventually leading to Aspergillus-specific Th2 responses.[77] In candidiasis, a promoter polymorphism of *IL4* (C−589T) was identified, and homozygous carriage of the T allele, linked with increased IL-4 production, was associated with rVVC.[78] Since IL-4 blocks macrophage-mediated anti-Candida responses, at least in part by the inhibition of NO production, elevated IL-4 levels were suggested to compromise immune responses to Candida in rVVC. Accordingly, a common haplotype in the *IL4* promoter (−1098T/−589C/−33C) was also associated with CDC.[79] Nonetheless, a protective effect from another haplotype (−1098T/−589T/−33T) was also reported, suggesting that *IL4* variants may differently affect susceptibility to candidiasis.

As already mentioned, IL-10 may have positive and negative effects on immune responses to fungi.[55] Elevated IL-10 levels were associated with IA and linked to unfavorable outcome in non-neutropenic immunocompromised patients.[80] A common polymorphism in the *IL10* promoter (G−1082A) affecting transcriptional activity has been associated with altered IL-10 serum levels. The high-producing G/G genotype has been linked with both increased Aspergillus colonization and ABPA in patients with cystic fibrosis.[81] In contrast, the common *IL10* promoter haplotype ACC (−1082A/−819C/−592C) has been shown to protect from IA after HSCT, although no correlation with IL-10 serum levels was estimated.[82] Additionally, despite being protective against IA in allogeneic HSCT,[83] the low-producing A/A genotype was also linked with CCPA,[66] suggesting that failure to control the inflammatory response could underlie the development of CCPA. As a matter of fact, CCPA patients were shown to be lower producers of transforming growth factor-β (TGF-β) as compared with those with allergic disease, mostly due to lower frequencies of the high-producing T/T genotype (T+869C) of the *TGFB1* gene.[66]

Recently, data has also pointed to the relevance of genetic variants in chemokine genes in host susceptibility to fungal diseases. A large-scale screening of polymorphisms led to the finding of three markers in CXC chemokine ligand 10 (*CXCL10*; C+11101T, C+1642G, and A−1101G) resulting in increased susceptibility to IA after HSCT.[84] Furthermore, immature wild-type dendritic cells exposed to *A. fumigatus* showed a markedly increase in CXCL10 expression, in contrast to those harboring the risk haplotype. In this regard, it is also interesting to note that patients who survived IA had significantly higher CXCL10 levels in comparison to healthy controls.

In general, polymorphisms affecting cytokine function and expression have been mostly studied regarding aspergillosis. In fact, genetic variants leading to defects in levels of several pro-inflammatory and anti-inflammatory cytokines have been linked with susceptibility to aspergillosis in its various forms (reviewed in Table 3). Particular emphasis has been given to the understanding of genetic variation in the *IL10* gene. In this case, consistent

Table 3
Human genetic association studies evaluating polymorphisms in cytokines and susceptibility to fungal diseases

Reference	Association study	Gene	SNPs/haplotypes	Relevant findings
Sainz et al.[70]	IA	*IL1RN, IL1A, IL1B*	VNTR2/−889C/−511T VNTR2/−889C/−511C	The VNTR2/−889C/−511T haplotype was associated with IA, whereas VNTR2/−889C/−511C was shown to be protective against the development of IA ($n=59$ IA cases)
Seo et al.[82]	IA	*IL10*	−1082A/−819C/−592C	The *IL10* ACC haplotype had an apparent protective role in the development of IA after allogeneic transplantation ($n=105$ HSCT patients; 9.9% IA cases)
Sainz et al.[83]	IA	*IL10*	G−1082A	The *IL10* A/A low-producing genotype was associated with resistance to develop IA ($n=59$ IA cases) ($p=0.001$)
Brouard et al.[81]	ABPA	*IL10*	G−1082A	A significant relationship was found between the *IL10* G/G high-producing genotype and both colonization with *Aspergillus fumigatus* and ABPA ($n=378$ CF patients)
Sambatakou et al.[66]	CCPA	*IL10*	G−1082A	CCPA ($n=24$) was associated with lower frequency of the G allele ($p=0.0006$) and G/G genotype ($p<0.001$)
Sainz et al.[68]	IA	*TNFR2*	VNTR at −322	Susceptibility to IA was associated with VNTR at position −322 in the promoter region of *TNFR2* ($n=54$ IA cases) ($p=0.029$)
Knutsen et al.[76]	ABPA	*IL4R*	Ile75Val	*IL4R* SNPs were observed in 95% of ABPA patients, with the Ile75Val polymorphism observed in 80% of ABPA patients ($n=40$)
Babula et al.[78]	rVVC	*IL4*	C−589T	The T/T genotype was detected in 59.5% of patients with rVVC ($n=42$), with a correlation between homozygosity and increased vaginal IL-4 levels ($p<0001$)
Choi et al.[79]	CDC	*IL4*	−1098T/−589C/−33C −1098T/−589T/−33T	The TCC haplotype was overrepresented in patients with CDC ($n=40$) ($p=0.01$), whereas the TTT haplotype appeared to be protective against CDC ($p=0.018$)
Sambatakou et al.[66]	ABPA/CCPA	*IL15*	A+13689T	Both ABPA ($n=9$) and CCPA ($n=24$) were associated with a higher frequency of the high-producing A allele ($p=0.0028$) and A/A genotype ($p<0.001$)
Sambatakou et al.[66]	ABPA/CCPA	*TNF*	A−308G	Both ABPA ($n=9$) and CCPA ($n=24$) were associated with a lower frequency of the high-producing A/A genotype ($p<0.01$)
Sambatakou et al.[66]	ABPA/CCPA	*IFNG*	T+874A	A higher frequency of the high-producing T/T genotype, although not with the T allele, and CCPA ($n=24$) and ABPA ($n=9$) was found
Sambatakou et al.[66]	CCPA	*TGFB1*	T+869C	CCPA ($n=24$) was associated with a lower frequency of the high-producing T allele ($p<0.0029$) and T/T genotype ($p<0.001$)
Mezger et al.[84]	IA	*CXCL10*	C+11101T, C+1642G and A−1101G	*CXCL10* SNPs were associated with IA in allogeneic HSCT recipients ($n=81$ IA cases) ($p=0.007$, 0.003, 0.001, respectively)

ABPA, allergic bronchopulmonary aspergillosis; CCPA, chronic cavitary pulmonary aspergillosis; CDC, chronic disseminated candidiasis; CF, cystic fibrosis; HSCT, hematopoietic stem cell transplantation; IA, invasive aspergillosis; IFN, interferon; IL, interleukin; rVVC, recurrent vulvovaginal candidiasis; SNP, single nucleotide polymorphism; TNF, tumor necrosis factor; TGF, transforming growth factor; VNTR, variable number of tandem repeats.

associations between low IL-10-producing genotypes and increased susceptibility to aspergillosis have been reported.[66,81–83] In contrast, polymorphisms in *IL4* appear instead to have a specific impact on susceptibility to Candida diseases.[78,79] In addition to cytokines, chemokines are also thought to play an important role in the host antifungal defense. The fact that polymorphisms affecting *CXCL10* were recently reported to predispose to IA following HSCT[84] further reinforces the need to look beyond cytokines and to explore new avenues of research, leading to a more integrated view of immune defects and their contribution to susceptibility to fungal diseases.

4. Conclusions

Although the dissection of complex traits of host immune genetics into susceptibility to fungal diseases is complex, the contribution of host genetics may hold the key to elucidation of new risk factors for these severe, often fatal diseases. Although the overall functional significance of the findings discussed in this review cannot be underestimated, there is an obvious need to independently replicate some of the studies with smaller sample power. In addition, considering the key role played by many of these polymorphisms at the host–fungus interface, further large-scale clinical and translational studies are needed to confirm the data obtained from human cohorts.

Understanding host–pathogen interactions at the level of host genetic susceptibility, together with the molecular and cellular bases affected, will allow the identification of potential therapeutic targets and the design of prophylactic strategies exerting control over the outcome of innate immune pathways. The genomic screening of at-risk patients may ultimately be used to individualize treatment through the formulation of new targeted and patient-tailored antifungal therapeutics, which are likely to improve the management and outcome of fungal diseases.

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