International Journal of Medical Microbiology 301 (2011) 445–452

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

journal homepage: www.elsevier.de/ijmm

Mini review

Genetic susceptibility to *Aspergillus fumigatus* infections

Michael Ok, Hermann Einsele, Juergen Loeffler*

Julius-Maximilians-Universität Würzburg, Medizinische Klinik & Poliklinik II, Josef-Schneider-Str. 2, 97080 Würzburg, Germany

ARTICLE INFO

Keywords:
Single nucleotide polymorphism
Invasive aspergillosis
Cytokine
Toll-like receptor
Mannose-binding lectin
Plasminogen

ABSTRACT

Invasive aspergillosis mostly caused by the opportunistic mould *Aspergillus fumigatus* is characterized by high morbidity and mortality in risk group patients. Several ethno-pathological factors promote the development and the course of this fungal infection like neutropenia, T-cell depletion, CD34-selected stem cell products, corticosteroid therapy, or cytomegalovirus infections. Furthermore, a growing number of defined single nucleotide polymorphisms affiliated to genes affecting the innate immune response has been described which genetically determine susceptibility to *A. fumigatus*. Thereby, it concerns a broad band ranging from genes encoding for cytokines or chemokines, their respective receptors to those of toll-like receptors including further genes involved in recognition and defence of pathogens by the innate immune system. Here, we summarize in detail the current knowledge about genetic markers correlated with invasive aspergillosis and their relevance for the developing and outcome of infections with *A. fumigatus*.

Introduction

Moulds are ubiquitous inhabitants of the environment. The most clinically relevant mould is *Aspergillus fumigatus*. The fungus can cause different diseases in humans (Stevens, 2000), including invasive aspergillosis (IA). *A. fumigatus* mainly occurs in immunocompromised patients, where deficits in host defence render susceptibility to *A. fumigatus*. This risk of IA is related to the degree and duration of neutropenia (Gerson et al., 1984). Thus, IA often threatens patients receiving potent cytotoxic therapies for haematological malignancies or myeloablative allogeneic stem cell transplantation (alloSCT), and in a remarkable number of patients IA also occurs in the post-engraftment period, presumably due to further immunosuppression, such as prophylaxis or treatment of graft versus host disease. Furthermore, other groups of patients, such as patients after solid organ transplantation and critically ill patients receiving high-dose corticosteroid therapies are also at risk for IA.

Patients after alloSCT have a higher risk of IA compared to autologous transplant recipients as immunosuppression is administered with greater intensity (Einsele and Hebart, 2002). Furthermore, additional factors augment the risk of IA in this specific patient cohort. Upton et al. (2007) presented data from one of the largest stem cell transplant centers, the Fred Hutchinson Cancer Research Center in Seattle. Patient data were collected from 1st of January 1990 through 31st of December 2004 from a prospectively maintained database and by retrospective clinical chart review. They have shown that the probability of survival at 90 days after diagnosis was higher for patients identified as having IA from 2002 to 2004 than for patients whose IA was diagnosed in preceding years (45% vs. 22%; $p < 0.001$). Risk factors independently associated with all-cause mortality include impairment in pulmonary function before HCT, receipt of human leukocyte antigen-mismatched stem cells, neutropenia, elevated bilirubin and creatinine levels, receipt of corticosteroids at $\geq$2 mg/kg per day, disseminated and proven IA, and IA occurring >40 days after HCT. In addition, cytomegalovirus infections as well as respiratory virus infections are further risk factors for IA (Marr et al., 2002).

Morgan et al. (2005) presented data about the cumulative incidence of IA after 12 months. Based on a multi-center surveillance, incidence of IA after autologous SCT was 0.5%, 2.3% after alloSCT from a HLA-matched related donor, and 3.9% after alloSCT from an unrelated donor. Taken these numbers into account, IA is responsible for a relatively small number of hospitalizations; however, patients have long-term hospital stays and mortality rates of 50–90%, depending on the localization of the disease. In 2008, there were more than 10,000 patients hospitalized in the USA due to aspergillosis, resulting in 176,300 hospital days. The mean hospitalization was 17.6 days longer (compared to patients without IA) with mean additional costs of 96,000 USD (Tong et al., 2009).

Relevance and pathobiology of known genes influencing susceptibility to *A. fumigatus*

A. fumigatus is a saprotrophic fungus whose spores (conidia) can be found ubiquitously in nature. Colonies of the fungus produce

* Corresponding author. Tel.: +49 931 201 36412; fax: +49 931 201 36409.
E-mail address: loeffler_j@klinik.uni-wuerzburg.de (J. Loeffler).

1438-4221/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.ijmm.2011.04.013

thousands of grey-green conidia from conidiophores that easily become distributed by the air (Latgé, 1999). Every person inhales several hundred conidia each day that can reach the lung without causing clinical symptoms in healthy individuals. This observation is initially ascribed to an efficient first line of immune defence that consists of the mucosal barrier and mechanisms of the innate immune system, such as hydrophobic surfactant proteins, but also to the hydrophobic rodlet layer composed of RodA protein on the surface of resting conidia (Aimanianda et al., 2009). Pulmonary surfactant consists of a complex mixture of lipids (90%) and proteins (5–10%), for example, the surfactant proteins SP-A and SP-D, which can bind to *A. fumigatus* conidia in alveolar fluid (Allen et al., 1999). In consequence, allergic bronchopulmonary aspergillosis (ABPA) is inhibited in murine models by suppressing pulmonary cellular infiltration (Kishore et al., 2006).

Additionally, the serum protein mannose-binding lectin (MBL) is a key regulator during fungal infections that enhances innate immune mechanisms by binding to cell wall components of *A. fumigatus* (Vaid et al., 2007). Attachment to fungal cells is a strong signal for phagocytosis by immune effector cells and for activation of the lectin complement pathway as well as for release of proinflammatory cytokines (Takahashi et al., 2008).

Alveolar macrophages, monocytes, and polymorphonuclear leukocytes (PMNs) have been shown to be essential for clearance of fungal cells by phagocytosis and release of antimicrobial molecules, respectively. It is undisputed that efficient killing requires recognition of fungi by the use of different immune receptors. In the last few years, several families of immune receptors have been discovered that are involved in detection of *A. fumigatus*. These receptors comprise the families of the Toll-like receptors (TLRs), C-type lectins, and pentraxines.

TLRs are evolutionary conserved receptors that mediate an immune response after recognition of so-called pathogen-associated molecular patterns (PAMPs). For example, TLR2 is a known receptor for peptidoglycan, a major component of the cell wall of Gram-positive bacteria, whereas TLR4 is activated by lipopolysaccharide (LPS) derived from Gram-negative bacteria. TLR2 and TLR4 have been shown to represent important components of the initial host immune response to fungal pathogens (Romani, 2004). After internalization of fungi and upon direct contact, different immune-relevant genes are up-regulated, including *IL-1*, *IL-12*, and *TNF-α* (Roilides et al., 1999). In addition, TLR2- and TLR4-defective mice show a decreased recruitment of PMNs and a reduced cytokine response to *A. fumigatus* conidia and hyphae (Meier et al., 2003). Brown et al. (2003) demonstrated that the fungal cell wall component zymosan (β-1,3 glucan) triggers expression of proinflammatory cytokines via the C-type lectin Dectin-1, and a crosstalk between TLR2, TLR6, and Dectin-1 has been reported for induction of *TNF-α* and *IL-12* (Gantner et al., 2003). During swelling and germination of *A. fumigatus* conidia, β-1,3 glucans become exposed to the surface and can be targeted by cells of innate immune system expressing TLR2, TLR6, and Dectin-1, including macrophages and dendritic cells, which constitute part of the first line of defence (Hohl et al., 2005).

Hence, cytokines are important regulators for proinflammatory (Th1) and anti-inflammatory (Th2) immune reactions, for the orchestration of innate and adaptive immune responses and for successful clearance of *A. fumigatus*. Dysregulation of the Th1/Th2 cytokine balance and a switch to a Th2 immune response contribute to the development and unfavourable outcome of IA. Hebart and colleagues were able to demonstrate that patients with clinical evidence of IA were characterized by a higher ratio of *IFN-γ*/*IL-10* after stimulation with *A. fumigatus* (Hebart et al., 2002). These results underline the relevance of a well-balanced *TNF-α*/*IL-12* and *IL-10* stability in patients with IA.

As an opportunistic human pathogen in immunocompromised individuals, *A. fumigatus* can cause potentially lethal invasive infections. Once the spores entered the organism, conidia start germination resulting in germ tubes and later in hyphae that can become invasive and disseminated by the blood into various organs (Latgé, 2001). Localized lung tissue damage and local thrombosis of the lung are major complications after alloSCT. They occur in up to 50% of transplant patients and can account substantially for transplant-related mortality (Paterson and Singh, 1999). Among many factors, blood coagulation is controlled by plasminogen. Recently, it has also been demonstrated that plasminogen directly binds to *A. fumigatus* in a dose-dependent manner influencing pathogenesis of invasive fungal infections (Zaas et al., 2008; Behnsen et al., 2008).

Further complications that arise after exposure to *A. fumigatus* are allergic bronchopulmonary aspergillosis (ABPA) with hypersensitivity reactions that often occur in immunocompetent hosts with asthma or cystic fibrosis (Gibson, 2006). Subacute forms of pulmonary aspergillosis often result in multiple expanding cavities in the lung. Therefore, this disease is named chronic cavitary pulmonary aspergillosis (CCPA). It is distinguished histologically from the chronic necrotising pulmonary aspergillosis (CNPA) by the lack of visible hyphal invasion of tissue (Vaid et al., 2007).

Susceptibility to infections with *A. fumigatus* – general aspects

It is obvious that susceptibility to a very complex infectious disease, such as IA, involves multi-factorial events and is not related to one specified entity only. Three very recent reports underline this statement. Accordingly, resistance to IA is described to be dependent either on defects of the complement component C5, which plays an important role in chemotaxis and cell influx into the lung (Svirshchevskaya et al., 2009), reduced chemokine receptor 7 levels, which regulate myeloid cell reconstitution and thus influence susceptibility to IA (Hartigan et al., 2010), and impaired levels of surfactant protein SP-D (Madan et al., 2010). Furthermore, additional aspects have to be considered, such as the relevance of ethnicity for susceptibility to IA. Although *A. fumigatus* is a fungus with a worldwide distribution, it seems to be obvious that vulnerability is usually controlled by different genetic variants, which mostly show altered frequency and biological significance in different human populations (Asakura and Komatsu, 2009).

SNPs in cytokines, chemokines, and their receptor genes associated with an increased risk for infections caused by *A. fumigatus*

Currently, 13 immunorelevant genes were described, in which 22 defined single nucleotide polymorphisms (SNPs) have been found to influence the course and outcome of infections with *A. fumigatus*. Table 1 gives an overview about the identified genetic markers and the corresponding fungus-mediated diseases.

There are reports that cytokines (*IL-1β*) and cytokine receptors (tumor necrosis factor receptor 2 = *TNFR2*) are involved in immune response towards *A. fumigatus*. Sainz and colleagues identified rs1143627 in *IL-1β* and a variable number of tandem repeats (VNTR, at position −322) in the promoter region of *TNFR2* as possible risk factor for IPA in haematological patients, whereas SNPs in the *TNFR2* ligands *TNF-α* (position −308 and +489) and lymphotoxin α (LT-α, position +252) were not associated with invasive pulmonary aspergillosis (IPA) (Sainz et al., 2007a, 2008a). Additionally, one marker in *TNFR2* at position +676 showed no association with IPA. Recently, the same group investigated 3 SNPs in the *TNFR1* gene

Table 1
Association between defined genetic polymorphisms and an increased risk to suffer from diseases caused by *A. fumigatus*.

Gene	dbSNP number	SNP position	Asp pos.	Asp neg.	Statistics	Population	Disease	Reference
CXCL10 (4q21)	rs1554013	11101 **C**/T[a] [Downstream]	51	49	$p = 0.007$ OR = 2.2 CI = 1.2–3.8	Caucasian (retrospective)	IA after HSCT [EORTC/IFICG]	Mezger et al. (2008)
	rs3921	1642 C/**G**[a] [3′ UTR]	39	46	$p = 0.003$ OR = 2.6 CI = 1.4–5.0			
	rs4257674	−1101 A/**G**[a] [Promotor]	52	44	$p = 0.001$ OR = 2.8 CI = 1.6–5.2			
IFN-γ (12q14)	rs2069705	−1616 C/**T**[a] [Promotor]	69	56	$p = 0.010$ OR = 2.0 CI = 1.2–3.4			
IL-10 (1q31-q32)	rs1800896	−1082 A/**G** [Promotor]	58	55	$p = 0.046$ OR = 1.7 CI = 1.0–2.9			
	rs1878672	2068 C/**G**[a] [Intron]	67	57	$p = 0.025$ OR = 1.8 CI = 1.1–2.9			
	rs1800896	−1082 A/**G** [Promotor]	119 *Af* col. 27 ABPA	232	$p = 0.020$ OR = 1.7 CI = 1.1–2.5	Caucasian (retrospective)	colonization with *A. fumigatus* or ABPA after CF	Brouard et al. (2005)
	rs1800896 rs1800871 rs1800872 (haplotype)	−1082 A/**G** −819 C/**T** −592 **A**/C [Promotor]	9	96	$p = 0.012$ OR = 9.3 CI = 1.6–52.8	Korean (retrospective)	IPA after HSCT [EORTC/MSG]	Seo et al. (2005)
	rs1800896	−1082 A/**G** [Promotor]	59	61	$p = 0.052$ OR = 1.7 CI = 1.0–2.9	Caucasian (prospective)	IPA in haematological patients [EORTC/IFICG]	Sainz et al. (2007b)
IL-1β (2q14)	rs1143627	−511 C/**T** [Promotor]	59	51	$p = 0.095$ OR = 1.7 CI = 0.9–3.0	Caucasian (retrospective)	IPA in haematological patients [EORTC/IFICG]	Sainz et al. (2008a)
IL-4Rα (16p12.1-p11.2)	rs1805010	4679 A/C/G/T [75 I/L/F/V]	40	56	$p = 0.008$	Caucasian	ABPA	Knutsen et al. (2006)
MBL (10q11.2-q21)	rs5030737	868 C/**T** [52 C/R]	15	82	$p = 0.020$ OR = 3.3 CI = 1.2–8.9	Caucasian	CCPA	Vaid et al. (2007)
	rs36203921	1011 **A**/G [Intron]	11	84	$p < 0.003$ OR = 8.2 CI = 2.8–23.6	Indian	ABPA	Kaur et al. (2006)
	rs5030737	868 C/**T** [52 C/R]	10	82	$p = 0.015$ OR = 4.9 CI = 1.3–18.0	Caucasian	CNPA	Crosdale et al. (2001)
Plg (6q26)	rs4252125	28904 **A**/G[a] [472 N/D]	83	147	$p < 0.001$ OR = 5.6 CI = 1.9–16.5	Caucasian	IA after HSCT [EORTC/IFICG]	Zaas et al. (2008)
SFTPA2 (10q22.3)	rs17886221	1660 A/**G** [94 R/R]	10	11	$p = 0.058$ OR = 7.0 CI = .07–66.2	Indian	ABPA	Saxena et al. (2003) Madan et al. (2005)
	rs17886395	1649 C/**G** [91 A/P]			$p = 0.031$ OR = 4.2 CI = 1.1–15.7			
	rs17880349	1492 C/**T** [Intron]	7	46	$p = 0.090$ OR = 3.5 CI = 0.7–16.6	Caucasian	ABPA	Vaid et al. (2007)
TLR1 (4p14)	rs5743611	239 C/**G** [80 R/T]	22	105	$p < 0.001$ OR = 1.3 CI = 1.1–1.5	Caucasian (prospective + retrospective)	IA after HSCT [EORTC/IFICG]	Kesh et al. (2005)
	rs4833095	743 **A**/G [248 S/N]						
TLR4 (9q32-q33)	rs4986790 rs4986791 (haplotype)	1063 A/**G** [299 D/G] 1363 C/**T** [399 I/T]	103	263	$p = 0.020$ OR = 2.5 CI = 1.2–5.4	Caucasian	IA after HSCT [EORTC]	Bochud et al. (2008)
	rs4986790	1063 A/**G** [299 D/G]	40	80	$p = 0.003$ OR = 3.5 CI = 1.5–8.1	Caucasian	CCPA	Carvalho et al. (2008)
TLR6 (4p14)	rs5743810	745 **C**/T [249 S/P]	22	105	See *TLR1*	Caucasian (prospective + retrospective)	IA after HSCT [EORTC/IFICG]	Kesh et al. (2005)

Table 1 (*Continued*)

Gene	dbSNP number	SNP position	Asp pos.	Asp neg.	Statistics	Population	Disease	Reference
TLR9 (3p21.3)	rs5743836	−1237 **C**/T [Promotor]	22	80	$p = 0.043$ OR = 2.5 CI = 1.0–6.2	Caucasian	ABPA	Carvalho et al. (2008)
TNFR2 (1p36.3-p36.2)	VNTR	−322 [Promotor]	54	48	$p = 0.029$ OR = 2.5 CI = 1.1–5.0	Caucasian (prospective)	IPA in haematological patients [EORTC/IFICG]	Sainz et al. (2007a)

Significant p values, odds ratios (OR), and 95% confidence intervals (CI), as obtained by statistical tests, are indicated, respectively. In all markers, risk alleles are labelled in bold letters.

VNTR, variable number of tandem repeats; IA, invasive aspergillosis; IPA, invasive pulmonary aspergillosis; CF, cystic fibrosis; CCPA, chronic cavitary pulmonary aspergillosis; ABPA, allergic bronchopulmonary aspergillosis; CNPA, chronic necrotizing pulmonary aspergillosis; HSCT, haematopoietic stem cell transplantation; EORTC/IFICG/MSG, European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group/Mycoses Study Group.

[a] Positions of the SNPs have been determined at http://snpper.chip.org/. Please note that localization of SNPs might differ from specifications indicated in other databases. Furthermore, it has to be emphasized that more statistical data are available than Table 1 can provide. Interested readers are encouraged to have a closer look at the cited publications.

of 144 immunocompromised haematological patients and 131 healthy controls (rs2234649 at position −383 [A/C] and rs4149570 at position −609 [G/T] in the 5′UTR and rs767455 at position +36 [A/G] in the first exon of the gene). Seventy-seven patients developed invasive pulmonary aspergillosis, whereby rs767455 and rs4149570 were associated with IPA susceptibility ($p = 0.033$ and $p = 0.018$, respectively). These findings need to be confirmed in validation studies with larger samples of haematological patients (Sainz et al., 2010).

Further genetic and functional analyses have to follow to establish whether this VNTR alters expression level of *TNFR2* or whether this polymorphism is just in linkage disequilibrium with a nearby localized gene responsible for the observed association. In addition, the genetic variants at position −174 (C/G) and −634 (G/C) in the promoter of *IL-6* were not associated with susceptibility to IA in haematological patients (Sainz et al., 2008b).

Furthermore, alleles with a protective role in the pathogenesis of IA were discovered in the promoter region of the *IL-10* gene (Seo et al., 2005). Interleukin-10 is an anti-inflammatory cytokine, which down-regulates the expression of Th1 cytokines, MHC class II antigens, and costimulatory molecules on macrophages. It also enhances B cell survival, proliferation, and antibody production. Interleukin-10 can block NF-κB activity, leading to a predominant Th2 immune response. The promoter of *IL-10* contains several polymorphisms from which SNPs at positions −1082, −819, and −592 that have been intensively investigated (Turner et al., 1997). Haplotype analysis identified a protective role of the ACC haplotype in the development of IA after alloSCT (Seo et al., 2005). Confirming their findings, we found a statistical association between rs1800896 (−1082, G>A) and rs1878672 (2068, G>C) and the occurrence of IA (Mezger et al., 2008). The polymorphism at position −1082 has been reported to produce higher levels of *IL-10* if the G allele is present and lower levels if the A allele is present (Turner et al., 1997; Tagore et al., 1999).

In a large screening of 17 genes encoding for cytokines, chemokines and their receptors [*CCL2*, *CCR1*, *CCR5*, *CCR6*, *CCR7*, *CXCL10*, *ICAM-1*, *IFNG*, *IL-4*, *IL-6*, *IL-10*, *IL-12B*, *IL-18*, *SCYA20*, *TLR2*, *TLR4*, and *TNF-α*], 84 polymorphisms were analyzed for a possible association with the occurrence of IA (defined according to the EORTC-IFICG/NIAID-MSG diagnostic guidelines; Ascioglu et al., 2002) in patients after alloSCT (Mezger et al., 2008). The strongest genetic association was found for 3 markers (rs1554013, rs3921, rs4257674) in *CXCL10* (also called *IFN-γ-inducible protein of 10 kDa*, *IP-10 or small inducible cytokine subfamily B, member 10*, *SCYB10*) with p values of $p < 0.007$. CXCL10 is an inflammatory mediator, induced by IFN-γ which stimulates the directional migration of Th1 cells as well as increasing T cell adhesion to endothelium (Loetscher et al., 1996). Haplotype analysis for rs1554013 (C/T), rs3921 (C/G), rs4859588 (A/G), and rs4257674 (A/G) in *CXCL10* confirmed the single marker analysis and clearly identified 'CGAG' as the high-risk haplotype.

Functional analysis revealed that immature dendritic cell (iDCs) exposed to *A. fumigatus* germlings showed higher *CXCL10* expression, if carrying the wild-type genotype compared to the 'CGAG' high-risk haplotype. In addition, serum from patients with proven/probable IA showed increased serum levels of CXCL10, compared to immunocompromised patients without evidence of IA. Thus, these polymorphisms in *CXCL10* might have an impact on chemokine secretion upon exposure to *A. fumigatus* (Mezger et al., 2008).

In the last few years, it has become obvious that not only innate immune mechanisms contribute to fungal clearance, but also antigen-presenting cells and T lymphocytes play a pivotal role. CXCL10 was found to preferentially attract Th1 lymphocytes for mediation of an adaptive immune response (Loetscher et al., 1996). Hebart et al. (2002) previously showed that a significant antigen-specific proliferation of IFN-γ-producing T cells occurred in healthy individuals and in patients surviving IA. In the *CXCL10* promoter, a potential negative regulatory site for IFN-γ in the region between nucleotide positions −2002 and −930 has been reported. Interestingly, one of the markers analyzed (rs4257674, −1101, $p = 0.001$) is located in this gene region.

Association of SNPs in Toll-like receptor (tlr) genes and increased risk for *A. fumigatus* infections

Recognition of microbial products via TLRs and subsequent signalling is crucial for the innate immune system to initiate a response. Genetic alterations affect this response, e.g. by reduced cytokine levels. Kumpf et al. (2010) could recently demonstrate that the course of sepsis and pneumonia, but not the susceptibility to infections is dependent on a defined risk genotype in *TLR4*. This observation indicates that variants in *TLR* genes might not affect the capability of sensing invading microorganisms, but rather the appropriate initiation and modulation of the innate immune response.

For the interaction of *Aspergillus* with the vertebrate immune system, *TLR1*, *2*, *4*, and *6* have been described to be relevant innate immune receptors, despite the fact that *Aspergillus* ligands for these PRRs are still unknown. Kesh and colleagues revealed an association of IA after alloSCT with defined SNPs in *TLR1* and *TLR6*, whereas no involvement of markers (896 A/G and 1196 C/T) in *TLR4* was observable (Kesh et al., 2005). This observation is in accordance to our previous study, where no association to IA after alloSCT was detectable for the following 5 SNPs in *TLR4*: rs1927911 (C/T), rs4986790 (A/G), rs2737191 (A/G), rs5030728 (A/G), and rs1554973 (C/T) (Mezger et al., 2008). In addition, a recent study showed that glycogen synthase kinase 3 (GSK-3) is involved in the

TLR4-mediated signal transduction in human dendritic cells. We investigated the genetic markers rs334558 and rs6438552 in *GSK-3* gene and revealed no significant association with an increased risk for IA (Spinnler et al., 2010).

In contrast, Bochud et al. (2008) analyzed 2 patient cohorts and found an association of one haplotype in the third exon of *TLR4* consisting of rs4986790 (1063 A/G) and rs4986791 (1363 C/T). Their findings were supported by data of Carvalho and colleagues that revealed an association of the nucleotide substitution rs4986790 with CCPA (Carvalho et al., 2008). These results might be surprising as *TLR4* is considered to be the receptor for bacterial lipopolysaccharides (LPS). Thus, it can be speculated that *TLR4* may also bind other non-LPS molecules; however, despite of major efforts, the ligand of *A. fumigatus* has not been identified, yet.

Recently, the findings by Bochud et al. (2008) have been discussed controversially. As mentioned above, their data suggested an association between *TLR4* haplotypes in unrelated donors and an increased risk to IA among recipients of an alloSCT. The authors investigated a group of 336 patients from 1995 to 2003. Haplotype S4 with 4 SNPs related to *TLR4* showed an association with IA, whereby only donors and not recipients were relevant. Two SNPs (1063G and 1363T) resulted in changes of the amino acid sequence to the lipopolysaccharid-hyporesponsive form. Pamer (2008) raised the question whether differences in sensitivity of *TLR4* affect the strength of the innate immune response and this, more generally, increases resistance to infections by the high-affinity *TLR4* variant. Furthermore, Levitz et al. hypothesized that application of amphotericin B may activate phagocytic cells by stimulation of *TLR4*, which in turn contributes to their antifungal activity (Levitz et al., 2009; Sau et al., 2003). In addition, Cervera et al. (2009) suggested that HCMV infection could be an intermediate variable in the association with *TLR4* polymorphisms with aspergillosis. However, this hypothesis could not be supported by the multivariate analysis performed in the validation study of Bochud and colleagues. Finally, Asakura and Komatsu (2009) addressed the question of frequency of these markers in *TLR4*, because the relevant SNPs rs4986790 and rs4986791 are missing in the Asian population, where IA is also a common life-threatening complication after alloSCT (Asakura and Komatsu, 2009).

In parallel, there is also still conflicting data about a functional effect of the Asp299Gly polymorphism on sensibility of *TLR4* to LPS. Arbour et al. (2000) reported that this nucleotide exchange interrupts *TLR4*-mediated LPS signalling and leads to hyporesponsiveness to LPS (Arbour et al., 2000). By contrast, the investigations by Van der Graaf et al. (2005) could not deliver supportive data for a functional influence because the presence of the Asp299Gly (rs4986790) polymorphism did not result in hyporesponsivness to stimulation with *TLR4* stimuli and defective pro- or anti-inflammatory cytokine production after exposure of mononuclear cells to LPS or *A. fumigatus*. One possible reason for these differences might be the use of different forms of LPS, originating from various *Escherichia coli* strains (Lundberg et al., 2008). Future experiments must clarify if bearing the Asp299Gly polymorphism renders immune cells more sensitive to *A. fumigatus*.

Finally, a recent study was presented by Carvalho et al. (2008) which demonstrated a significant association between the presence of the cosegregating Asp299Gly/Thr399Ile polymorphisms (in *TLR4*) and fungal colonization ($p = 0.003$; OR = 10.6). However, susceptibility to fungal infections, predominantly fungal pneumonia, was significantly decreased in the presence of the same polymorphisms ($p = 0.03$; OR = 0.23).

Up to now, no involvement of *TLR2* polymorphisms for a genetic predisposition for fungal infections was demonstrated (Pamer, 2008). The SNP rs5743708 (Arg753Gln) was not linked with CCPA (Carvalho et al., 2008), and the markers rs1898830 (A/G), rs3804099 (C/T), and rs3804100 (C/T) were not associated with IA after alloSCT (Mezger et al., 2008).

Association of polymorphisms in *mannose-binding lectin* (*mbl*) and surfactant proteins with an increased susceptibility to *A. fumigatus*

A defined SNP (rs36203921, 1011 A/G) in the *MBL* gene was identified to contribute to allergic bronchopulmonary aspergillosis (ABPA) by influencing MBL plasma level and protein activity (Kaur et al., 2006, 2007). Despite a small cohort of 11 Indian patients with ABPA, this finding seems to be remarkable because functional analysis demonstrated that patients homozygous for the 1011 A allele showed significantly higher plasma MBL levels and activity in comparison to patients homozygous for the G allele. Due to the intronic localization of this polymorphism, it seems to be possible that further SNPs in exons or the promoter region of the *MBL* gene might be in linkage disequilibrium and account to elevated MBL levels.

Supporting data for the relevance of MBL in the context of *A. fumigatus*-mediated diseases were delivered by Vaid et al. (2007) and Crosdale et al. (2001) who observed an association of the marker rs5030737 (868 C/T) to CCPA or CNPA in the Caucasian population, respectively. The non-synonymous amino acid exchange from arginin to cystein at position 52 results in low levels of functional MBL in the serum.

Besides *MBL*, further SNPs in C-type lectins were intensively investigated leading to the identification of an association between ABPA and the variants rs17886395 (1649 C/G) and rs17886221 (1660 A/G) of the surfactant protein A2 (*SFPTPA2*) gene (Madan et al., 2005; Saxena et al., 2003). However, it has to be emphasized that only a small cohort of patients (10 with ABPA and 11 without ABPA) was analyzed belonging to the Indian population. The SNP rs17886221 simply encodes for a synonymous amino acid exchange at position 94. Database analysis revealed that this marker is very rare in the European population (NCBI). Thus, it remains to be shown why it might be important in the Caucasian population.

In contrast, there are supporting data that the SNP rs17880349 (1492 C/T) in *SFPTPA2* might be regarded as a risk factor for ABPA due to observations by Vaid et al. (2007). Despite a small cohort of patients with ABPA ($n = 7$), the authors found an association in the Caucasian population. Further work has to be done to confirm their results with larger number of patients.

Other investigations about the role of surfactant proteins in the context of allergic airway responses proposed that a polymorphism (Met11Thr) in the surfactant protein D gene (*SFTPD*) is associated with atopy in the black population and potentially with lower asthma susceptibility in white subjects (Brandt et al., 2008).

Influence of defined plasminogen alleles to susceptibility to IA

Coagulopathies are common clinical observations in patients suffering from IA mainly after liver and renal organ transplantations; these diseases are regularly related with high mortality. However, the underlying mechanisms are still unclear; normal haemostasis involves a complex interaction of primary (platelet-vascular) and secondary (coagulation factors) pathways (Lai et al., 2007). Recently, Zaas et al. (2008) identified a non-synonymous SNP (rs4252125; Asp472Asn) in the human plasminogen gene. Their association study with a cohort of 236 allogeneic stem cell transplant recipients revealed that alleles at this SNP significantly affected the risk of developing IA. In addition, these authors showed that plasminogen directly binds to *A. fumigatus*. For subsequent activation of surface-bound plasminogen to plasmin, host or micro-

bial activators are mandatory (Behnsen et al., 2008). It can be hypothesized that plasminogen bound to the fungal cell wall might favour its activation to plasmin. Then, plasmin might contribute to local destruction of the lung tissue, thereby promoting pathogen invasion and pulmonary haemorrhage (Sun et al., 2004).

Risk of aspergillosis in non-immunocompromised patients

Infections with *A. fumigatus* also occur in non-immunocompromised patients, such as patients with chronic granulomatous disease (CGD) or in patients with allergic bronchopulmonary aspergillosis.

CGD is an inherited disorder characterized by the inability of phagocytes to generate normal amounts of superoxide, leaving patients susceptible to life-threatening infections, including infections with *Aspergillus* species. The frequency of aspergillosis in these patients depends on different factors, including the age of the patients. Rösen-Wolff et al. (2001) showed that age-related acquired skewing of the lionization ratio can result in an increased susceptibility to *A. fumigatus* infections in X-CGD carriers.

Sambatakou et al. (2006) investigated the genetic risk of patients with allergic bronchopulmonary aspergillosis for chronic cavitary pulmonary aspergillosis (CCPA). CCPA is a slowly destructive form of pulmonary aspergillosis occurring in non-immunocompromised patients. Within the aspergillosis patients, CCPA was associated with lower frequency of the *IL-10*-1082G allele (OR = 0.38, $p = 0.0006$) and G/G genotype [chi(2) = 22.45, $p < 0.001$] and with a lower frequency of the TGF-beta1 +869T allele (OR = 0.42, $p < 0.0029$) and T/T genotype [chi(2) = 17.82, $p < 0.001$] compared with non-CCPA patients and normal controls.

These 2 studies highlight the fact that the relevance of genetic factors for *Aspergillus* infections is difficult to define in these inhomogeneous patient cohorts.

Phenotypic impact of significant SNPs

As described in the paragraphs above, we know numerous genetic markers in immune-relevant genes, which are significantly associated with an altered risk for *Aspergillus* infections. Such significant genetic markers were found in genes encoding *IL1-β* (rs1143627), *TNFR1* (rs767455 and rs4149570), *TNFR2* (VNTR, at position −322 in the promoter region of *TNFR2*), *IL-10* [rs1800896 (influencing also CCPA in patients with ABPA) and rs1878672], *CXCL10* [haplotype CGAG composed of rs1554013 (C/T), rs3921 (C/G), rs4859588 (A/G), and rs4257674 (A/G)], and *TLR4* (rs4986790 and rs4986791). However, unfortunately, most of these studies lack any functional data, and the authors did not provide any information whether the respective mutation leads to increased or decreased protein levels. Therefore, it can only be speculated about the phenotypic consequences, especially as cytokines and chemokines have multiple, very complex tasks in vivo. As an example of the diverse functions of cytokines, *IL1-β* (rs1143627) is an important mediator of the inflammatory response and involved in a variety of cellular activities including cell proliferation, differentiation, and apoptosis. *IL-10* (rs1800896, rs1878672) is a cytokine, which can influence numerous immune-relevant processes, it down-regulates the expression of Th1 cytokines, MHC class II antigens, and costimulatory molecules. *IL-10* blocks NF-κB activity and is capable of inhibiting synthesis of proinflammatory cytokines like *IFN-γ*, *IL-2*, *IL-3*, and *TNF-α*. Finally, it also displays potent abilities to suppress the antigen presentation capacity of antigen-presenting cells. Both examples demonstrate the broad and complex impact of altered cytokine levels in vivo and thus underline the need of functional analyses of identified genetic markers.

Phenotypic consequences on the function or expression related to IA are only described for *TLR4*, *IL10*, *CXCL10*, and MBL. Variation of sensitivity, biological activity, or plasma level concentration was determined in appropriate association studies (Bochud et al., 2008; Turner et al., 1997; Tagore et al., 1999; Mezger et al., 2008; Kaur et al., 2006, 2007; Crosdale et al., 2001; Vaid et al., 2007).

Requirements for genetic association studies

There are various relevant issues to be considered in genetic association studies. These include the appropriate sample-recruitment strategy, rationale variant selection, minimum genotyping error, relevant data analysis, and valid interpretation of robust data. Inappropriate control groups, genotyping errors, investigator biases, and over-elaborate data exploration have to be prevented (Hattersley and McCarthy, 2005). For studies investigating susceptibility to IA, this requires consideration of an appropriate number of cases and controls which could be difficult due to the limited number of patients with IA, well-defined cases (proven and probable IA only) and controls (according to the criteria of the EORTC, De Pauw et al., 2008) and homogeneous patient cohorts (patients receiving an alloSCT, leukaemia patients, ABPA patients).

Conclusions

Stratification of high-risk patients (e.g. patients after allo-SCT), based on their individual genetic profiles can be beneficial and might lead to a reduction of the incidence of IA. The consequences of an early identification and characterization of patients at high risk for *A. fumigatus* infection comprise (i) the individualization of diagnostic procedures (e.g. prospective screening of patients using highly sensitive assays such as PCR), (ii) the usage of antifungal prophylactic regimes, and (iii) the individualization of antifungal therapy regimes including the administration of newly developed therapeutic drugs. Functional studies of identified SNPs are essential. Moreover, it will be relevant to determine the contribution of cross-talks, antagonistic, and/or synergistic effects of the so far identified SNPs on the risk for fungal infections.

Conflict of interest statement

The material submitted for publication has not been previously reported and is not under consideration for publication elsewhere. The authors concur with the publication of the manuscript. We do not have any conflicts regarding financial interests.

References

Aimanianda, V., Bayry, J., Bozza, S., Kniemeyer, O., Perruccio, K., Elluru, S.R., Clavaud, C., Paris, S., Brakhage, A.A., Kaveri, S.V., Romani, L., Latgé, J.P., 2009. Surface hydrophobin prevents immune recognition of airborne fungal spores. Nature 460, 1117–1121.

Allen, M.J., Harbeck, R., Smith, B., Voelker, D.R., Mason, R.J., 1999. Binding of rat and human surfactant proteins A and D to *Aspergillus fumigatus* conidia. Infect. Immun. 67, 4563–4569.

Arbour, N.C., Lorenz, E., Schutte, B.C., Zabner, J., Kline, J.N., Jones, M., Frees, K., Watt, J.L., Schwartz, D.A., 2000. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. Nat. Genet. 25, 187–191.

Asakura, Y., Komatsu, T., 2009. Toll-like receptor 4 polymorphisms and aspergillosis. N. Engl. J. Med. 360 (635), 635, author reply 635–636.

Ascioglu, S., Rex, J.H., de Pauw, B., Bennett, J.E., Bille, J., Crokaert, F., Denning, D.W., Donnelly, J.P., Edwards, J.E., Erjavec, Z., Fiere, D., Lortholary, O., Maertens, J., Meis, J.F., Patterson, T.F., Ritter, J., Selleslag, D., Shah, P.M., Stevens, D.A., Walsh, T.J., 2002. Invasive Fungal Infections Cooperative Group of the European Organization for Research and Treatment of Cancer; Mycoses Study Group of the National Institute of Allergy and Infectious Diseases Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. Clin. Infect. Dis. 34, 7–14.

Behnsen, J., Hartmann, A., Schmaler, J., Gehrke, A., Brakhage, A.A., Zipfel, P.F., 2008. The opportunistic human pathogenic fungus *Aspergillus fumigatus* evades the host complement system. Infect. Immun. 76, 820–827.

Bochud, P.Y., Chien, J.W., Marr, K.A., Leisenring, W.M., Upton, A., Janer, M., Rodrigues, S.D., Li, S., Hansen, J.A., Zhao, L.P., Aderem, A., Boeckh, M., 2008. Toll-like receptor 4 polymorphisms and aspergillosis in stem-cell transplantation. N. Engl. J. Med. 359, 1766–1777.

Brandt, E.B., Mingler, M.K., Stevenson, M.D., Wang, N., Khurana Hershey, G.K., Whitsett, J.A., Rothenberg, M.E., 2008. Surfactant protein D alters allergic lung responses in mice and human subjects. J. Allergy Clin. Immunol. 121, 1140–1147.

Brouard, J., Knauer, N., Boelle, P.Y., Corvol, H., Henrion-Caude, A., Flamant, C., Bremont, F., Delaisi, B., Duhamel, J.F., Marguet, C., Roussey, M., Miesch, M.C., Chadelat, K., Boule, M., Fauroux, B., Ratjen, F., Grasemann, H., Clement, A., 2005. Influence of interleukin-10 on *Aspergillus fumigatus* infection in patients with cystic fibrosis. J. Infect. Dis. 191, 1988–1991.

Brown, G.D., Herre, J., Williams, D.L., Willment, J.A., Marshall, A.S., Gordon, S., 2003. Dectin-1 mediates the biological effects of beta-glucans. J. Exp. Med. 197, 1119–1124.

Carvalho, A., Pasqualotto, A.C., Pitzurra, L., Romani, L., Denning, D.W., Rodrigues, F., 2008. Polymorphisms in toll-like receptor genes and susceptibility to pulmonary aspergillosis. J. Infect. Dis. 197, 618–621.

Cervera, C., Moreno, A., Lozano, F., 2009. Toll-like receptor 4 polymorphisms and aspergillosis. N. Engl. J. Med. 360, 634–635, author reply.

Crosdale, D.J., Poulton, K.V., Ollier, W.E., Thomson, W., Denning, D.W., 2001. Mannose-binding lectin gene polymorphisms as a susceptibility factor for chronic necrotizing pulmonary aspergillosis. J. Infect. Dis. 184, 653–656.

De Pauw, B., Walsh, T.J., Donnelly, P., Stevens, D.A., Edwards, J.E., Calandra, T., Pappas, P.G., Maertens, J., Lortholary, O., Kauffman, C.A., Denning, D.W., Patterson, T.F., Maschmeyer, G., Bille, J., Dismukes, W.E., Herbrecht, R., Hope, W.W., Kibbler, C.C., Kullberg, B.J., Marr, K.A., Muñoz, P., Odds, F.C., Perfect, J.R., Restrepo, A., Ruhnke, M., Segal, B.H., Sobel, J.D., Sorrell, T.C., Viscoli, C., Wingard, J.R., Zaoutis, T., Bennett, J.E., 2008. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin. Infect. Dis. 46, 1813–1820.

Einsele, H., Hebart, H., 2002. Cellular immunity to viral and fungal antigens after stem cell transplantation. Curr. Opin. Hematol. 9, 485–489.

Gantner, B.N., Simmons, R.M., Canavera, S.J., Akira, S., Underhill, D.M., 2003. Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. J. Exp. Med. 197, 1107–1117.

Gerson, S.L., Talbot, G.H., Hurwitz, S., Strom, B.L., Lusk, E.J., Cassileth, P.A., 1984. Prolonged granulocytopenia: the major risk factor for invasive pulmonary aspergillosis in patients with acute leukemia. Ann. Intern. Med. 100, 345–351.

Gibson, P.G., 2006. Allergic bronchopulmonary aspergillosis. Semin. Respir. Crit. Care Med. 27, 185–191.

Hartigan, A.J., Kallal, L.E., Hogaboam, C.M., 2010. CCR7 impairs hematopoiesis following hematopoietic stem cell transplantation increasing susceptibility to invasive aspergillosis. Blood August (Epub ahead of print).

Hattersley, A.T., McCarthy, I.T., 2005. What makes a good genetic association study? Lancet 366, 1315–1323.

Hebart, H., Bollinger, C., Fisch, P., Sarfati, J., Meisner, C., Baur, M., Loeffler, J., Monod, M., Latgé, J.P., Einsele, H., 2002. Analysis of T-cell responses to *Aspergillus fumigatus* antigens in healthy individuals and patients with hematologic malignancies. Blood 100, 4521–4528.

Hohl, T.M., Van Epps, H.L., Rivera, A., Morgan, L.A., Chen, P.L., Feldmesser, M., Pamer, E.G., 2005. *Aspergillus fumigatus* triggers inflammatory responses by stage-specific beta-glucan display. PLoS Pathog. 1, e30.

Kaur, S., Gupta, V.K., Shah, A., Thiel, S., Sarma, P.U., Madan, T., 2006. Elevated levels of mannan-binding leptin (MBL) and eosinophilia in patients of bronchial asthma with allergic rhinitis and allergic bronchopulmonary aspergillosis associate with a novel intronic polymorphism in MBL. Clin. Exp. Immunol. 143, 414–419.

Kaur, S., Gupta, V.K., Thiel, S., Sarma, P.U., Madan, T., 2007. Protective role of mannan-binding lectin in a murine model of invasive pulmonary aspergillosis. Clin. Exp. Immunol. 148, 382–389.

Kesh, S., Mensah, N.Y., Peterlongo, P., Jaffe, D., Hsu, K., van den Brink, M., O'Reilly, R., Pamer, E., Satagopan, J., Papanicolaou, G.A., 2005. TLR1 and TLR6 polymorphisms are associated with susceptibility to invasive aspergillosis after allogeneic stem cell transplantation. Ann. N. Y. Acad. Sci. 1062, 95–103.

Kishore, U., Greenhough, T.J., Waters, P., Shrive, A.K., Ghai, R., Kamran, M.F., Bernal, A.L., Reid, K.B., Madan, T., Chakraborty, T., 2006. Surfactant proteins SP-A and SP-D: structure, function and receptors. Mol. Immunol. 43, 1293–1315.

Knutsen, A.P., Kariuki, B., Consolino, J.D., Warrier, M.R., 2006. IL-4 alpha chain receptor (IL-4Ralpha) polymorphisms in allergic bronchopulmonary aspergillosis. Clin. Mol. Allergy 4, 3.

Kumpf, O., Giamarellos-Bourboulis, E.J., Koch, A., Hamann, L., Mouktaroudi, M., Oh, D.Y., Latz, E., Lorenz, E., Schwartz, D.A., Ferwerda, B., Routsi, C., Skalioti, C., Kullberg, B.J., van der Meer, J.W., Schlag, P.M., Netea, M.G., Zacharowski, K., Schumann, R.R., 2010. Influence of genetic variations in *TLR4* and *TIRAP*/*Mal* on the course of sepsis and pneumonia and cytokine release: an observational study in three cohorts. Crit. Care 14, R103.

Lai, C.C., Liaw, S.J., Lee, L.N., Hsiao, C.H., Yu, C.J., Hsueh, P.R., 2007. Invasive pulmonary aspergillosis: high incidence of disseminated intravascular coagulation in fatal cases. J. Microbiol. Immunol. Infect. 40, 141–147.

Latgé, J.P., 1999. *Aspergillus fumigatus* and aspergillosis. Clin. Microbiol. Rev. 12, 310–350.

Latgé, J.P., 2001. The pathobiology of *Aspergillus fumigatus*. Trends Microbiol. 9, 382–389.

Levitz, S.M., Shoham, S., Cleary, J.D., 2009. Toll-like receptor 4 polymorphisms and aspergillosis. N. Engl. J. Med. 360, 634, author reply.

Loetscher, M., Gerber, B., Loetscher, P., Jones, S.A., Piali, L., Clark-Lewis, I., Baggiolini, M., Moser, B., 1996. Chemokine receptor specific for IP10 and mig: structure, function, and expression in activated T-lymphocytes. J. Exp. Med. 184, 963–969.

Lundberg, A., Wikberg, L.A., Ilonen, J., Vaarala, O., Böttcher, M.F., 2008. Lipopolysaccharide-induced immune responses in relation to the TLR4(Asp299Gly) gene polymorphism. Clin. Vaccine Immunol. 15, 1878–1883.

Madan, T., Kaur, S., Saxena, S., Singh, M., Kishore, U., Thiel, S., Reid, K.B., Sarma, P.U., 2005. Role of collectins in innate immunity against aspergillosis. Med. Mycol. 43, 155–163.

Madan, T., Reid, K.B., Clark, H., Singh, M., Nayak, A., Sarma, P.U., Hawgood, S., Kishore, U., 2010. Susceptibility of mice genetically deficient in SP-A or SP-D gene to invasive pulmonary aspergillosis. Mol. Immunol. 47, 1923–1930.

Marr, K.A., Carter, R.A., Boeckh, M., Martin, P., Corey, L., 2002. Invasive aspergillosis in allogeneic stem cell transplant recipients: changes in epidemiology and risk factors. Blood 100, 4358–4366.

Meier, A., Kirschning, C.J., Nikolaus, T., Wagner, H., Heesemann, J., Ebel, F., 2003. Toll-like receptor (TLR) 2 and TLR4 are essential for *Aspergillus*-induced activation of murine macrophages. Cell. Microbiol. 5, 561–570.

Mezger, M., Steffens, M., Beyer, M., Manger, C., Eberle, J., Toliat, M.R., Wienker, T.F., Ljungman, P., Hebart, H., Dornbusch, H.J., Einsele, H., Loeffler, J., 2008. Polymorphisms in the chemokine (C-X-C motif) ligand 10 are associated with invasive aspergillosis after allogeneic stem-cell transplantation and influence CXCL10 expression in monocyte-derived dendritic cells. Blood 111, 534–536.

Morgan, J., Wannemuehler, K.A., Marr, K.A., Hadley, S., Kontoyiannis, D.P., Walsh, T.J., Fridkin, S.K., Pappas, P.G., Warnock, D.W., 2005. Incidence of invasive aspergillosis following hematopoietic stem cell and solid organ transplantation: interim results of a prospective multicenter surveillance program. Med. Mycol. 43, 49–58.

Pamer, E.G., 2008. TLR polymorphisms and the risk of invasive fungal infections. N. Engl. J. Med. 359, 1836–1838.

Paterson, D.L., Singh, N., 1999. Invasive aspergillosis in transplant recipients. Medicine (Baltimore) 78, 123–138.

Roilides, E., Dimitriadou-Georgiadou, A., Sein, T., Kadiltsoglou, I., Walsh, T.J., 1999. Tumor necrosis factor alpha enhances antifungal activities of polymorphonuclear and mononuclear phagocytes against *Aspergillus fumigatus*. Infect. Immun. 66, 5999–6003.

Romani, L., 2004. Immunity to fungal infections. Nat. Rev. Immunol. 4, 1–23.

Rösen-Wolff, A., Soldan, W., Heyne, K., Bickhardt, J., Gahr, M., Roesler, J., 2001. Increased susceptibility of a carrier of X-linked chronic granulomatous disease (CGD) to *Aspergillus fumigatus* infection associated with age-related skewing of lyonization. Ann. Hematol. 80, 113–115.

Sainz, J., Pérez, E., Hassan, L., Moratalla, A., Romero, A., Collado, M.D., Jurado, M., 2007a. Variable number of tandem repeats of TNF receptor type 2 promoter as genetic biomarker of susceptibility to develop invasive pulmonary aspergillosis. Hum. Immunol. 68, 41–50.

Sainz, J., Hassan, L., Perez, E., Romero, A., Moratalla, A., López-Fernández, E., Oyonarte, S., Jurado, M., 2007b. Interleukin-10 promoter polymorphism as risk factor to develop invasive pulmonary aspergillosis. Immunol. Lett. 109, 76–82.

Sainz, J., Pérez, E., Gómez-Lopera, S., Jurado, M., 2008a. IL1 gene cluster polymorphisms and its haplotypes may predict the risk to develop invasive pulmonary aspergillosis and modulate C-reactive protein level. J. Clin. Immunol. 28, 473–485.

Sainz, J., Pérez, E., Gómez-Lopera, S., López-Fernández, E., Moratalla, L., Oyonarte, S., Jurado, M., 2008b. Genetic variants of IL6 gene promoter influence on C-reactive protein levels but are not associated with susceptibility to invasive pulmonary aspergillosis in haematological patients. Cytokine 41, 268–278.

Sainz, J., Salas-Alvarado, I., López-Fernández, E., Olmedo, C., Comino, A., García, F., Blanco, A., Gómez-Lopera, S., Oyonarte, S., Bueno, P., Jurado, M., 2010. TNFR1 mRNA expression level and TNFR1 gene polymorphisms are predictive markers for susceptibility to develop invasive pulmonary aspergillosis. Int. J. Immunopathol. Pharmacol. 23, 423–436.

Sambatakou, H., Pravica, V., Hutchinson, I.V., Denning, D.W., 2006. Cytokine profiling of pulmonary aspergillosis. Int. J. Immunogenet. 33, 297–302.

Sau, K., Mambula, S.S., Latz, E., Henneke, P., Golenbock, D.T., Levitz, S.M., 2003. The antifungal drug amphotericin B promotes inflammatory cytokine release by a Toll-like receptor- and CD14-dependent mechanism. J. Biol. Chem. 278, 37561–37568.

Saxena, S., Madan, T., Shah, A., Muralidhar, K., Sarma, P.U., 2003. Association of polymorphisms in the collagen region of SP-A2 with increased levels of total IgE antibodies and eosinophilia in patients with allergic bronchopulmonary aspergillosis. J. Allergy Clin. Immunol. 111, 1001–1007.

Seo, K.W., Kim, D.H., Sohn, S.K., Lee, N.Y., Chang, H.H., Kim, S.W., Jeon, S.B., Baek, J.H., Kim, J.G., Suh, J.S., Lee, K.B., 2005. Protective role of interleukin-10 promoter gene polymorphism in the pathogenesis of invasive pulmonary aspergillosis after allogeneic stem cell transplantation. Bone Marrow Transplant. 36, 1089–1095.

Spinnler, K., Mezger, M., Steffens, M., Sennefelder, H., Kurzai, O., Einsele, H., Loeffler, J., 2010. Role of glycogen synthase kinase 3 (GSK-3) in innate immune response of human immature dendritic cells to *Aspergillus fumigatus*. Med. Mycol. 48, 589–597.

Stevens, D., 2000. Aspergillosis. In: Goldman, L., Bennett, J.C. (Eds.), Cecil Textbook of Medicine. , 21st ed. Elsevier Science, Philadelphia, pp. 1875–1877.

Sun, H., Ringdahl, U., Homeister, J.W., Fay, W.P., Engleberg, N.C., Yang, A.Y., Rozek, L.S., Wang, X., Sjöbring, U., Ginsburg, D., 2004. Plasminogen is a criti-

cal host pathogenicity factor for group A streptococcal infection. Science 305, 1283–1286.

Svirshchevskaya, E.V., Shevchenko, M.A., Huet, D., Femenia, F., Latge, J.P., Boireau, P., Berkova, N., 2009. Susceptibility of mice to invasive aspergillosis correlates with delayed cell influx into the lungs. Int. J. Immunogen. 36, 289–299.

Tagore, A., Gonsalkorale, W.M., Pravica, V., Hajeer, A.H., McMahon, R., Whorwell, P.J., Sinnott, P.J., Hutchinson, I.V., 1999. Interleukin-10 (IL-10) genotypes in inflammatory bowel disease. Tissue Antigens 54, 386–390.

Takahashi, M., Iwaki, D., Kanno, K., Ishida, Y., Xiong, J., Matsushita, M., Endo, Y., Miura, S., Ishii, N., Sugamura, K., Fujita, T., 2008. Mannose-binding lectin (MBL)-associated serine protease (MASP)-1 contributes to activation of the lectin complement pathway. J. Immunol. 180, 6132–6138.

Tong, K.B., Lau, C.J., Murtagh, K., Layton, A.J., Seifeldin, R., 2009. The economic impact of aspergillosis: analysis of hospital expenditures across patient subgroups. Int. J. Infect. 13, 24–36.

Turner, D.M., Williams, D.M., Sankaran, D., Lazarus, M., Sinnott, P.J., Hutchinson, I.V., 1997. An investigation of polymorphisms in the interleukin-10 gene promoter. Eur. J. Immunogenet. 24, 1–8.

Upton, A., Kirby, K.A., Carpenter, P., Boeckh, M., Marr, K.A., 2007. Invasive aspergillosis following hematopoietic cell transplantation: outcomes and prognostic factors associated with mortality. Clin. Infect. Dis. 44, 531–540.

Vaid, M., Kaur, S., Sambatakou, H., Madan, T., Denning, D.W., Sarma, P.U., 2007. Distinct alleles of mannose-binding lectin (MBL) and surfactant proteins A (SP-A) in patients with chronic cavitary pulmonary aspergillosis and allergic bronchopulmonary aspergillosis. Clin. Chem. Lab. Med. 45, 183–186.

Van der Graaf, C., Kullberg, B.J., Joosten, L., Verver-Jansen, T., Jacobs, L., Van der Meer, J.W., Netea, M.G., 2005. Functional consequences of the Asp299Gly Toll-like receptor-4 polymorphism. Cytokine 30, 264–268.

Zaas, A.K., Liao, G., Chien, J.W., Weinberg, C., Shore, D., Giles, S.S., Marr, K.A., Usuka, J., Burch, L.H., Perera, L., Perfect, J.R., Peltz, G., Schwartz, D.A., 2008. Plasminogen alleles influence susceptibility to invasive aspergillosis. PLoS Genet. 4, e1000101.