Human Genetic Susceptibility to Invasive Aspergillosis

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Introduction

Aspergillosis includes a wide spectrum of diseases caused by fungi of the genus Aspergillus with clinical manifestations that range from colonization (e.g., aspergilloma), to allergic bronchopulmonary aspergillosis, to disseminated forms of infection. Invasive aspergillosis (IA) has been estimated to occur in 5%–10% of acute myeloid leukemia patients during post-induction aplasia or consolidation therapy and after 5–15% of allogeneic hematopoietic stem cell transplants (HSCT) [1,2]. Additional persons at risk for IA include recipients of solid organ transplants and patients with chronic granulomatous disease (CGD). Despite the significant progress attained in the management of this severe infection, its prevention, diagnosis, and therapy remain extremely difficult, rendering it a leading cause of death among immunocompromised patients. Additionally, concerns over antimold prescription and the remarkably high healthcare costs owing to its chronic course and mortality rates have been diverting clinicians from universal prophylaxis to risk stratification and preemptive approaches. This has inspired the search for novel individual prognostic factors, particularly genetic, to apply in the categorization of those most vulnerable to infection.

Immune Recognition of Aspergillus: PAMPs, DAMPs, and Beyond

The physical barrier of the respiratory tract affords the first line of resistance against inhaled conidia of Aspergillus. In the event these escape the ciliated epithelium, conidia will then be challenged by cells of the innate immune system, including resident alveolar macrophages and dendritic cells (DCs), as well as recruited inflammatory cells, mainly polymorphonuclear neutrophils. These cells express a vast repertoire of pattern recognition receptors (PRRs) that sense pathogen-associated molecular patterns (PAMPs) and drive the secretion of proinflammatory cytokines and chemokines that arbitrate innate and adaptive immune responses. In the case of fungi, the cell wall is the main source of PAMPs owing to its dynamic composition and structural differences of components such as mannan, glucans, and nucleic acids. These PAMPs are sensed by PRRs on phagocytes, promoting phagocytosis and activation of neutrophils. Th1 and Th17 cytokines are secreted, which are known to be pro-inflammatory and lead to the production of secondary metabolites that promote lung inflammation, exacerbate infection, and influence subsequent host immune responses [3].

Genetic Variability of the Host and Susceptibility to IA

The inborn deficiency of the phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase leading to CGD is the best known example of primary immunodeficiency with predisposition to IA [7]. As a result of the impaired production of reactive oxygen species, patients with CGD often develop IA, typically within the first decade of life. Of interest, these patients are uniquely susceptible to IA due to defects in the innate immune system, in particular, the ability of Aspergillus to adapt to hypoxic microenvironments.

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| Gene(s) | SNP(s) | Amino acid change | Type of patients | Cases (total patients) | Association [OR (95% CI), P value] | Probable mechanism(s) | Ref. |
|---------|--------|-------------------|------------------|-----------------------|------------------------------------|-----------------------|------|
| AGER    | rs1800624 | -                 | HSCT (D/R)       | 41 (223)              | 2.0 (1.0–3.8), P = 0.04 (D)        | Enhanced expression of RAGE       | [18] |
|         |         |                   |                  |                       | 2.0 (1.0–4.1), P = 0.05 (R)        |                       |      |
| CLEC7A  | rs16910526 | Y238X             | HSCT (D/R)       | 39 (205)              | 2.5 (1.0–6.5), P = 0.05 (D)        | Defective cell surface expression of HLA-DR    | [15,16] |
|         |         |                   | Hematological    | 21 (138)              | 3.9 (1.5–10.0), P = 0.005 (D+R)    |                       |      |
|         |         |                   |                  |                       | n.a., P = 0.02                     |                       |      |
| CLEC7A  | rs7309123 | -                 | Hematological    | 57 (182)              | 5.5 (1.9–16.4), P = 0.001          | Impaired expression of HLA-DR        | [17] |
| CXCL10  | rs1554013 | -                 | HSCT (D)         | 81 (139)              | 2.2 (1.2–3.8), P = 0.007           | Impaired expression of CXCL10       | [19] |
|         | rs1800403 | -                 |                  | 21 (138)              | 2.6 (1.4–5.0), P = 0.003           |                       |      |
|         | rs1800406 | -                 |                  | 2.8 (1.6–5.2), P = 0.001 |                       |                       |      |
|         |         |                   |                  |                       |                                     |                       |      |
|         | rs7309123 |                   |                  |                       |                                     |                       |      |
| IL1A    | rs1800587 | -                 | Hematological    | 59 (110)              | 15.4 (1.4–171.2), P = 0.02         | Unknown                | [25] |
| IL1B    | rs16944  | -                 |                  |                       |                                     |                       |      |
| IL1RN   | VNTR 86-bp(n) | -                 |                  |                       |                                     |                       |      |
| CLEC7A  | rs7309123 | -                 | Hematological    | 59 (120)              | 4.5 (1.6–12.9), P = 0.001          | Unknown                | [27] |
| IL10    | rs1800896 | -                 | HSCT (R)         | 9 (105)               | 9.3 (1.6–52.8), P = 0.01           | Unknown                | [26] |
|         | rs1800871 | -                 |                  |                       |                                     |                       |      |
|         | rs1800872 | -                 |                  |                       |                                     |                       |      |
| MBL2    | 'MBL-low genotypes' | -           | D120G | 15 (106)              | 7.3 (1.9–27.3), P = 0.003          | Unknown                | [22] |
| MASP2   | rs72550870 | D120G             | HSCT (R)         | 59 (194)              | 3.0 (1.5–6.1), P < 0.001           |                       |      |
|         |         |                   |                  | 5.6 (1.9–16.5), P < 0.001         |                       |                       |      |
| PLG     | rs4252125 | D472N             | HSCT (R)         | 59 (194)              | 6.4 (2.0–20.6), P = 0.002          |                       |      |
| S100B   | rs9722   | -                 | HSCT (D)         | 41 (223)              | 3.15 (1.6–6.1), P < 0.001          | Enhanced secretion of S100B        | [18] |
| TLR1    | rs5743611 | R80T              | HSCT (R)         | 22 (127)              | 1.2 (1.0–1.5), P = 0.04            | Unknown                | [28] |
|         | rs4833095 | N248S             |                  |                       | 1.2 (1.0–1.5), P = 0.02            |                       |      |
|         | rs5743810 | S249P             |                  |                       | 1.3 (1.1–1.5), P < 0.001           |                       |      |
| TLR3    | rs3775296 | -                 | HSCT (D)         | 42 (223)              | 2.4 (1.3–4.6), P = 0.007           | Defective antigen presentation and activation of CD8+ T-cell responses | [14] |
| TLR4    | rs4916790 | D299G             | HSCT (D)         | 33 (336)              | 6.2 (2.0–19.3), P = 0.002 (discovery study) | Unknown                | [11] |
|         | rs4916791 | T399I             |                  | 103 (366)             | 2.5 (1.2–5.4), P = 0.02 (validation study) |                       |      |
| TNFR1   | rs4149570 | -                 | Hematological    | 77 (144)              | n.a., P = 0.02                     | Impaired expression of TNFR1 mRNA   | [29] |
| TNFR2   | rs574596  | -                 | Hematological    | 54 (102)              | 2.5 (1.1–5.0), P = 0.03            | Unknown                | [30] |

SNP – single nucleotide polymorphism; OR – odds ratio; HSCT – hematopoietic stem cell transplantation; D – donor; R – recipient; RAGE – receptor for advanced glycation end products; CXCL – chemokine (C-X-C motif) ligand; MBL – mannose-binding lectin; MASP – MBL-associated serine protease; PLG – plasminogen; TLR – toll-like receptor; TNFR – tumor necrosis factor receptor; n.a. – not available; VNTR – variable number of tandem repeats.

1 For patients that underwent HSCT, the source of the variant (donor, recipient, or both) associated with susceptibility to IA is indicated.
2 The controls for the association reported were not hematological patients, but healthy subjects of comparable Dutch ancestry.
3 Association with increased susceptibility to IA was observed for a haplotype comprising SNPs in the IL-1 gene cluster, namely IL1A rs1800587/IL1B rs16944/IL1RN VNTR 86-bp(n), but not for single loci.
4 Association with increased susceptibility to IA was observed for the absence of the ACC haplotype in rs1800896, rs1800871, and rs1800872, respectively.
5 MBL-low genotypes correspond to a group of genotypes denoted by letters (O/O and LXA/O). LX represents an MBL promoter haplotype comprising SNPs of the MBL2 gene at positions 2550 (H/L) and 221 (Y/X), known to influence transcription rates and to result in low concentrations of serum MBL. Nonsynonymous variants are collectively named O (including amino acid replacements at codons 52, 54, or 57) and cause a reduction of the MBL levels due to impaired assembly of MBL monomers into functional oligomers. A indicates the wild type.
6 Association results regard the comparisons DD vs. DN and DD vs. NN, respectively.
7 Association results regard R80T, N248S and S249P, and R80T or N248S and S249P, respectively.
8 Association with increased susceptibility to IA was observed for a TLR4 haplotype (termed S4) that included both D299G and T399I.
susceptible to diseases with the *A. nidulans* complex, which are weakly virulent molds that rarely cause infection in immunocompromised patients. For most individuals however, genetic propensity to aspergillosis has a polygenetic source. A polygenic variant by itself has a negligible effect on phenotype; only in combination with other remarkable predisposing variants (e.g., profound immunosuppression) do sizable phenotypic effects arise.

In conformity with the crucial requirement of innate immunity for effective antifungal host defense, several studies have uncovered associations between genetic variants in components of the innate immune system and risk for IA [8–10] (Table 1). One classical example regards a donor haplotype in TLR4 reported to increase susceptibility to infection after HSCT, especially if combined with cytomegalovirus seropositivity [11]. Despite TLR4 polymorphisms having also been linked with chronic aspergillosis in immunocompetent individuals [12] and fungal colonization in HSCT recipients [13], their prognostic significance remains disputed. The fact that TLR4 ligands in fungi are still unknown and the limited knowledge of the biological consequences of human TLR4 deficiency to antifungal immunity have been hampering the employment of TLR4 genotyping in risk stratification approaches. Most importantly, genetic variants affecting the function of innate receptors other than TLR4 have also been deemed relevant. Indeed, alongside the discovery of TLR3-mediated activation of protective memory CD8(+) T cell responses in experimental aspergillosis, a donor polymorphism impairing the expression of the human receptor was found to predispose to IA due to the inability of human DCs to efficiently prime memory CD8(+) responses to the fungus [14]. Additionally, and given the pivotal role of dendin-1 in fungal sensing, it is also not surprising that human dendin-1 deficiency has been reported to contribute to susceptibility to IA [15–17]. Interestingly, a stop codon polymorphism compromising the surface expression and dendin-1-mediated cytokine production displayed a cumulative effect toward risk for infection after HSCT when present concurrently in donors and recipients of stem cell grafts [16], a finding emphasizing the contribution of non-hematopoietic dendin-1 to antifungal immunity. As host damage perception is also fundamental for resolution of infection [5], genetic variants triggering hyperactive DAMP signaling, and presumably leading to uncontrolled inflammatory response to the fungus, were recently found to increase risk for IA [18]. Finally, and although positive associations between genetic variants in cytokine genes and vulnerability to IA have been reported [8], the lack of functional validation and the underpowered design of most studies precludes definite conclusions about the contribution of polymorphisms affecting cytokine production. One exception is the link proposed between a haplotype in CXCL10 and risk for IA in HSCT recipients [19]. Mechanistically, this haplotype was correlated with the inability of DCs to express CXCL10 and, interestingly, patients impaired antifungal mechanisms of the host may be a turning point toward innovative stratification strategies based on genetic screening or immune profiling to predict risk and severity of disease, efficacy of antifungal prophylaxis and therapy, and eventually contribute to the successful design of antifungal vaccines.

**Unveiling Human Susceptibility to IA: What Might the Future Hold?**

Our existing knowledge of the genetic bases of susceptibility to IA derives from studies screening single variants in candidate genes using small patient cohorts. In addition, statistical issues with multiple comparisons and the lack of validation in larger, independent cohorts or via biological studies of disease mechanisms are further limitations of candidate gene association studies. As cutting-edge “omics” techniques are becoming affordable, multidisciplinary integrative approaches targeting variability in genome-wide association studies or expression in whole-transcriptomics studies may help to identify novel susceptibility signatures in otherwise unsuspected genes or pathways besides confirming those currently acknowledged. As “omics” have contributed to the identification of genetic susceptibility traits in cancer research, these techniques could be ultimately extrapolated with success to the field of invasive fungal diseases. Furthermore, only now are we beginning to fully grasp the significance of the microbiota and its interactions with the mammalian immune system in defining susceptibility to infection. Indeed, the structure and composition of lung microbial communities in patients at-risk was found to diverge significantly from that of healthy individuals [24], thus suggesting a likely susceptibility signature to IA that may involve a host–fungus–microbiota triad. All these state-of-the-art approaches however do not weaken the weight of functional validation. Given that “omics” studies by nature disregard all preceding knowledge about disease pathobiology, studies unveiling useful mechanistic insights into the relevant signatures found, be them genetic or biological, are still essential.

**Concluding Remarks**

The identification of patient-specific prognostic signatures of susceptibility to IA in high-risk patients is currently one major priority in the fields of hematology and microbiology. Ultimately, the discovery of reliable markers of susceptibility consistently associated with risk for IA and functionally correlated with impaired antifungal mechanisms of the host may be a turning point toward innovative stratification strategies based on genetic screening or immune profiling to predict risk and severity of disease, efficacy of antifungal prophylaxis and therapy, and eventually contribute to the successful design of antifungal vaccines.

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