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Emerging *Aspergillus* Species Almost Exclusively Associated With Primary Immunodeficiencies

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Invasive aspergillosis (IA) is the most serious mold infection encountered in patients with iatrogenic immunosuppression. IA is also a major cause of mortality and morbidity in individuals with primary immunodeficiency (PID). Although *Aspergillus fumigatus* is the most common etiologic agent of IA reported in PID patients, followed by *A. nidulans*, multiple poorly recognized *Aspergillus* species such as *A. udagawae, A. quadrilineatus, A. pseudoviridinutans, A. tanneri, A. subramanianii,* and *A. fumisynnematus* have been reported almost exclusively from patients with inborn defects in host antifungal defense pathways. Infection in PID patients exhibits patterns of disease progression distinct from those in iatrogenic immunosuppression. Specifically, the disease can be extrapulmonary and chronic with a tendency to disseminate in a contiguous manner across anatomical planes. It is also more refractory to standard antifungal therapy. This synopsis summarizes our understanding of emerging rare *Aspergillus* species that primarily affect patients with PIDs but not those with acquired immunodeficiencies.

Keywords. emerging non-*fumigatus Aspergillus* species; invasive aspergillosis; primary immunodeficiencies.

Aspergillus species are one of the most ubiquitously found saprophytic molds in soil and decaying vegetations, with a potential to cause opportunistic disease primarily in patients with defective immune systems [1]. Reports of aspergillosis have increased, mainly due to the increasing number of individuals with immunodeficiency and advances in life support systems [2]. Although invasive aspergillosis (IA) is most commonly recognized among the population with acquired immunodeficiency (secondary immunodeficiency), the number of primary immunodeficiencies (PIDs) recognized as underlying conditions predisposing to mycoses is increasing, most often in children and young adults [3]. PIDs are rare congenital genetic disorders, mostly due to abnormalities in a single gene that enhance susceptibility to autoimmunity or infectious disease [3]. Consequently, patients may encounter recurrent, protracted, or severe infections due to pathogens including fungi [4]. Among the known congenital primary immunodeficiencies at risk for IA discussed here are chronic granulomatous disease (CGD), autosomal-dominant (AD) hyper-IgE syndrome (HIES; also known as Job syndrome), AD deficiency in GATA2 (also known as MonoMAC, monocytopenia syndrome, B-cell and NK-cell lymphopenias), AD or AR

(autosomal-recessive) severe congenital neutropenia (SCN), LAD (AR type I leukocyte adhesion deficiency), also called CD18 deficiency, and caspase recruitment domain-containing protein 9 (CARD9) deficiency [3]. *Aspergillus fumigatus* is the primary cause of IA in individuals with PIDs, followed by *A. nidulans,* due to its propensity to cause infection in patients with CGD [5]. Other aspergilli also documented to infect PID patients include *A. pseudoviridinutans* [6], *A. tanneri* [7], *A. udagawae* [8], *A. flavus* [9], *A. niger* [10], *A. calidoustus* [11], *A. quadrilineatus* [12], *A. fumisynnematus* [13], and *A. subramanianii*. Without proper identification of the etiologic agents and adequate therapy, IA results in high mortality rates regardless of the species involved [14]. This paper focuses on the important mycological features of emerging non-*fumigatus Aspergillus* species reported from IA cases in PID patients.

DISTRIBUTION OF PATHOGENIC SPECIES IN DIFFERENT SECTIONS OF *ASPERGILLUS*

The genus *Aspergillus* contains more than 250 species, which are subdivided into 22 distinct sections. Of these, 13 sections, including *Candidi, Circumdati, Flavipides, Fumigati, Nidulantes, Nigri, Ornati, Restricti, Tanneri, Terrei, Usti, Versicolores,* and *Warcupi*, contain clinically relevant species (Figure 1) [15].

Aspergillus fumigatus in the section *Fumigati* is the primary etiologic agent of IA, and its inhaled conidia can cause multiple diseases depending on the host immune status. The spectrum of disease includes invasive pulmonary infection, disseminated disease, fungus ball (aspergilloma), and various hypersensitivity diseases such as allergic bronchopulmonary aspergillosis, allergic
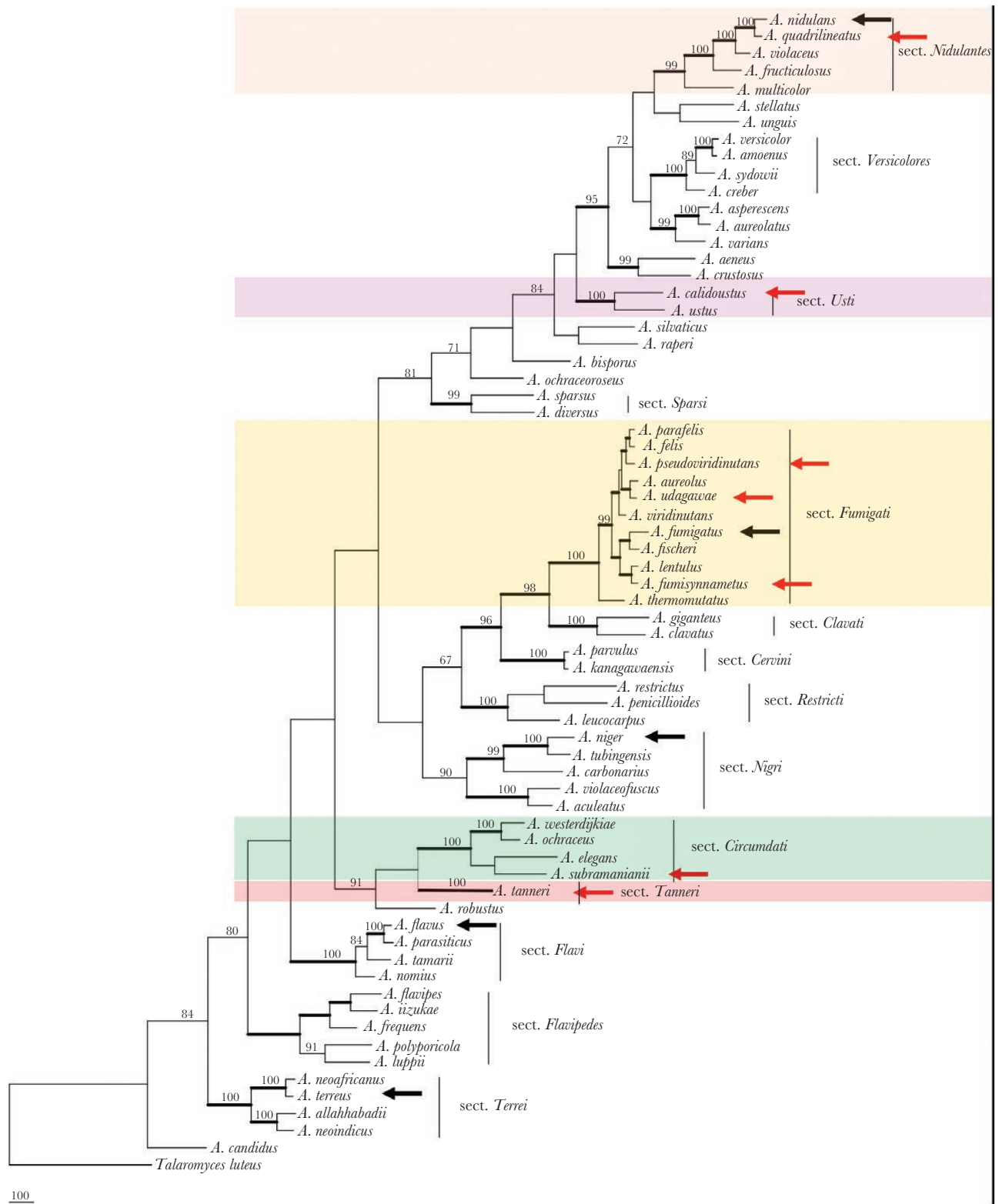
Figure 1. Phylogenetic tree inferred from combined β-tubulin, Calmodulin, MCM7, and RPB2 genome sequences. A black arrow indicates 5 well-known pathogenic species of *Aspergillus*, and a red arrow identifies other emerging aspergilli reported primarily in patients with underlying primary immunodeficiency. Thick branches indicate >95% bootstrap support; numbers above nodes are Bayesian probabilities greater than 0.90. Abbreviations: MCM7, Minichromosome Maintenance Protein 7; RPB2, RNA polymerase II second largest subunit.

asthma, and hypersensitivity pneumonitis [16]. However, aspergillosis caused by emerging poorly recognized species morphologically similar to *A. fumigatus* in patients with PID may exhibit differences in the clinical presentation, kinetics of tissue invasion, and susceptibility to antifungal therapy [17].

HUMAN PIDS ASSOCIATED WITH INVASIVE ASPERGILLOSIS

There are more than 200 different forms of PIDs, which are classified into 9 subclasses, depending on their underlying immunologic defect or predominant manifestations. Globally, a total of ~77 000 patients are registered as having a specific PID [18]. Table 1 summarizes the PIDs in which IA has been documented.

CGD
Ten *Aspergillus* species including *Aspergillus fumigatus*, *A. nidulans*, *A. calidoustus*, *A. flavus*, *A. niger*, *A. terreus*, *A. udagawae*, *A. pseudoviridinutans*, *A. quadrilineatus,* and *A. tanneri* have been documented from IA cases in CGD patients [3]. *A. fumigatus* is the primary etiologic agent (estimated ~55%), followed by *A. nidulans* (~35%), a species that is almost exclusively associated with IA in CGD but not encountered in patients with iatrogenic immunosuppression [5]. The reasons for the unique association of *A. nidulans* with CGD patients are not completely understood. It has been suggested that *A. nidulans* is less virulent than *A. fumigatus* due to the lower content of N-acetyl-galactosamine (GalNAc) in the galactosaminogalactan (GAG), an exopolysaccharide bound to the cell wall. GalNAc mediates adherence and is required for full virulence. The lower content of GalNAc in GAG was due to the low expression of the *Uge3* gene, which encodes glucose epimerase [19]. This may partly explain why *A. nidulans* rarely infects patients without CGD. However, the lower virulence level of *A. nidulans* does not explain the reason for the unique association with CGD and not with other PIDs.

In CGD patients, aspergillosis is almost always subacute to chronic, regardless of the etiologic agent involved, and does not spread hematogenously because of the lack of angio-invasion. Unlike in patients with acquired immunosuppression, dissemination of *Aspergillus* to the lung, to the chest wall, and into the ribs or vertebrae is common [5].

AD and AR SCN Syndromes
IA caused by unspeciated *Aspergillus* has been reported in SCN patients harboring heterozygous mutations in the *ELA2* gene. Although profound neutropenia is the highest risk factor for aspergillosis in patients with acquired immunosuppression, fungal infection in general and aspergillosis in particular has rarely been documented for SCN patients [20], indicating that mononuclear phagocytes may compensate for the lack of neutrophils in these patients.

AR Type I LAD (CD18 Deficiency)
Though rare, IA due to unspeciated *Aspergillus* has been reported in patients with LAD type I. Aspergillosis has also been associated with LAD type II (genetic defect undetermined) [21], in which adherence and migration of granulocytes are diminished due to a carbohydrate fucosylation defect.

AD GATA2 Deficiency (MonoMAC Syndrome)
The incidence of IA in this population has been estimated to be ~17%, but the species distribution of the etiologic agents remains unknown [22].

AD Hyper-IgE Syndrome (AD-HIES)
In a recent French national survey of AD-HIES patients, 13 of 60 patients in the cohort (22%) had invasive aspergillosis and/or colonization [23]. Similarly, 25% of AD-HIES patients from the National Institutes of Health (NIH) cohort [24], particularly those with underlying structural lung disease, developed IA. The *Aspergillus* species causing infection in these cohorts of patients were not specified [24]. Recently, *A. fumisynnematus* was identified in a patient with underlying STAT3 haploinsufficiency caused by a novel *STAT3* splice site mutation. The patient had elevated IgE, allergic rhinitis, eosinophilic esophagitis, and fatal IA due to this species [13].

CARD9 Deficiency
In 2016, Rieber et al. [25] reported extrapulmonary *A. fumigatus* infection in 2 unrelated patients age 8 and 18 years with CARD9 deficiency. The younger patient died at age 12 with progressive intra-abdominal aspergillosis, whereas the second developed central nervous system IA at age 18 and intra-abdominal aspergillosis at age 25. Neither patient presented with pulmonary infection, and their neutrophils failed to migrate to the extrapulmonary site of infection. The mutations in these patients were identified as homozygous M1I *CARD9* and homozygous Q295X *CARD9,* respectively. Lack of pulmonary infection by *A. fumigatus* in these patients with PID is noteworthy as the primary disease caused by *A. fumigatus* in patients with secondary immunodeficiency is invasive pulmonary infection [2].

Cystic Fibrosis
Although cystic fibrosis is not in the general category of PID, it is worth mentioning that cystic fibrosis patients are predisposed to allergic bronchopulmonary aspergillosis and their respiratory secretions often contain various common *Aspergillus* species. Cystic fibrosis is a genetic disorder caused by mutations in the gene encoding the transmembrane conductance regulator protein [26].

MYCOLOGY

Poorly Recognized *Aspergillus* Species Documented From Cases of IA in Patients With PIDs
The exact *Aspergillus* epidemiology in patients with PIDs is not known because of infrequent culture isolation compounded by the lack of proper species identification. In many cases, the etiologic agents are reported simply as "*Aspergillus*." However, it is clear that *A. fumigatus* and *A. nidulans* are the most frequently reported *Aspergillus* species in patients with PIDs [3]. These 2 species and

Table 1. Category of PIDs Associated With Aspergillosis

Category	Disease	Genetic Defect/Presumed Pathogenesis	Inheritance	Affected Immune Cells	Affected Immune Function Associated With Susceptibility to Aspergillosis	Susceptibility to Aspergillosis	Susceptibility to Other Fungal Infections	Reported Aspergillus Species
Congenital defects of phagocyte numbers and/or function	(a) CGD-gp91phox deficiency	Mutation in CYBB: electron transport protein (gp91phox)	XL	N+M	Killing (defective superoxide production)	High	Other mold infections (occasionally) Invasive candidiasis, (rarely)	A. calidoustus, A. flavus, A. fumigatus, A. fumisynnematus, A. quadrilineatus, A. nidulans, A. niger, A. pseudoviridinutans, A. tanneri, A. udagawae, A. terreus
	(b) CGD-p22phox deficiency	Mutation in CYBA: electron transport protein (p22phox)	AR	N+M	Killing (defective superoxide production)	High	Other mold infections (occasionally), invasive candidiasis (rarely)	
	(c) CGD-p47phox deficiency	Mutation in NCF1: adapter protein (p47phox)	AR	N+M	Killing (defective superoxide production)	High	Other mold infections (occasionally), Invasive candidiasis (rarely)	
	(d) CGD-p67phox deficiency	Mutation in NCF2: activating protein (p67phox)	AR	N+M	Killing (defective superoxide production)	High	Other mold infections (occasionally) Invasive candidiasis (rarely)	
	(e) CGD-p40phox deficiency	Mutation in NCF4: activating protein (p40phox)	AR	N+M	Killing (defective superoxide production)	High	Other mold infections (occasionally), invasive candidiasis (rarely)	
Defects of neutrophil numbers (congenital neutropenias)	(a) SCN1 (elastase deficiency)	Mutation in ELANE: misfolded protein response, increased apoptosis	AD	N	Myeloid differentiation	Rarely	Invasive candidiasis (rarely)	Aspergillus sp.
	(b) SCN3 (Kostmann disease)	Mutation in HAX1: control of apoptosis	AR	N	Myeloid differentiation	Rarely	Invasive candidiasis (rarely)	Aspergillus sp.
Defects of phagocyte motility	Leukocyte adhesion deficiencies type 1 (LAD1)	Mutation in ITGB2: B chain for adhesion proteins CD18	AR	N+M+L+NK	Adherence, chemotaxis, endocytosis, T/NK cytotoxicity	Rarely	Invasive candidiasis (rarely)	Aspergillus sp.
Defects of phagocyte number	GATA2 deficiency (Mono MAC syndrome)	Mutation in GATA2: loss of stem cells	AD	N+monocytes+peripheral DC+NK+B	Multilineage cytopenias	~20%	Histoplasmosis, cryptococcal meningitis	Aspergillus sp., A. udagawae
Combined immunodeficiencies with associated or syndromic features	AD-HIES (Job syndrome)	Dominant-negative heterozygous mutations in signal transducer and activator of transcription STAT3	AD, often de novo (spontaneous) defect	T cells, B cells	Structural lung disease as a result of prior pulmonary bacterial infections	High in patients with lung cavities	Histoplasmosis, cryptococcosis (rarely), mucosal candidiasis (~80%)	Aspergillus sp., A. psudoviridinutans
Defects in intrinsic and innate immunity	CARD9 deficiency	Mutations of CARD9	AR	N	Impaired neutrophil recruitment	Occasionally	Invasive candidiasis, specifically involving the central nervous system (high), deep dermatophytosis (high), phaeohyphomycosis	A. fumigatus

Abbreviations: AD, autosomal dominant inheritance; AR, autosomal recessive inheritance; B, B-lymphocytes; CGD, chronic granulomatous disease; CYBA, cytochrome b alpha subunit; CYBB, cytochrome b beta subunit; DC, dendritic cells; ELANE, elastase neutrophil-expressed; GATA2, GATA binding protein 2; HAX1, HLCS1-associated protein X1; HIES, hyper-IgE syndromes; ITGB2, integrin beta-2; L, lymphocytes; LAD1, leukocyte adhesion deficiencies type 1; M, monocytes–macrophages; Mono MAC syndrome, syndrome of monocytopenia, B cell, and NK cell lymphopenias; N, neutrophils; NCF1, neutrophil cytosolic factor 1; NCF2, neutrophil cytosolic factor 2; NCF4, neutrophil cytosolic factor 4; NK, natural killer cells; SCN, severe congenital neutropenias; T, T-lymphocytes; XL, X-linked inheritance.

their pathogenicity have been exhaustively reviewed. *Aspergillus flavus, A. terreus,* and *A. niger* are also well-characterized species that cause IA both in patients with PIDs and in those with iatrogenic immunosuppression [27]. The present discussion focuses largely on emerging, poorly recognized, non-*fumigatus Aspergillus* reported from patients with PIDs. In Figure 1, these species are marked with red arrows in the sections *Fumigati, Nidulantes, Usti, Circumdati,* and *Tanneri,* and Figure 2 shows conidial structures of the 5 least studied species. The well-known species are marked with black arrows. Table 2 summarizes the major characteristics and the outcome of IA caused by these species.

Aspergillus calidoustus

Aspergillus calidoustus belongs in the *Aspergillus* section *Usti* and is a relatively widespread species isolated from the indoor environment [28], although it is rarely reported from clinical cases. The species can be differentiated from other related species such as *A. ustus* or *A. granulosus* by its growth at ≥37°C and from *A. pseudodeflectus* by its low susceptibility to antifungal drugs [29].

A. *calidoustus* caused cerebral aspergillosis in an X-linked 17-year-old CGD patient who also underwent allogeneic hematopoietic stem cell transplantation (HSCT) [11]. The patient was treated with liposomal amphotericin B, but the brain abscess recurred; the patient died from a brain hemorrhage after a second operation. His underlying CGD combined with acquired immunodeficiency due to HSCT contributed to the susceptibility to this rare pathogen. Interestingly, *A. calidoustus* has been increasingly isolated from transplant populations, especially those who have had lung transplantation [28].
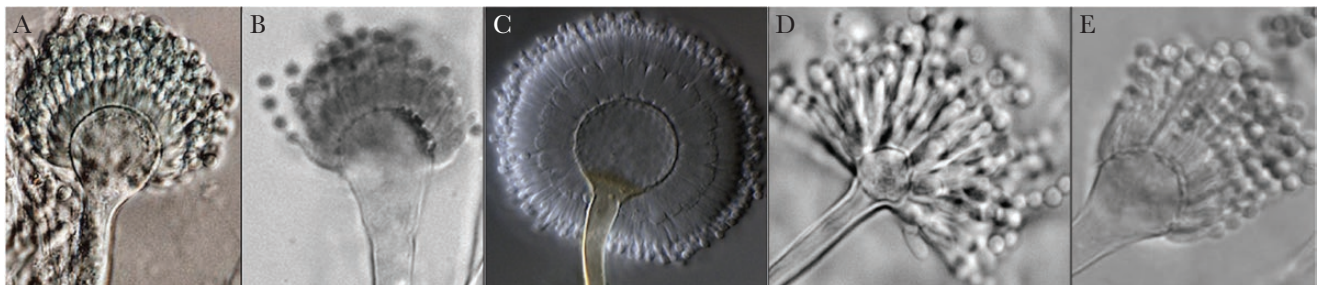
Aspergilus fumisynnematus

Aspergillus fumisynnematus, a member of section *Fumigati,* was first isolated from soil with the maximum growth temperature of 45°C [30]. The species is morphologically similar to other members of the section *Fumigati*, and identification requires a molecular method. However, synnemata production in aged culture on malt extract (ME) agar medium can be its unique feature within the section *Fumigati*.

Isolation of *A. fumisynnematus* from the bronchoalveolar lavage of a patient was tested for its susceptibility to antifungal agents, but patient information was lacking [31]. Recently, *A. fumisynnematus* was recovered from 2 PID patients with aspergillosis. In 1 case, a 37-year-old man with STAT3 haploinsufficiency caused by a *STAT3* splice site mutation had progressive infection of sinuses, orbital apex, and cavernous sinus [13]. The patient was treated with a combination of voriconazole, amphotericin B, posaconazole, micafungin, and terbinafine, in addition to surgical removal of the infected portions of the sinuses. However, the patient died 1 year after surgery due to uncontrollable brain invasion by the species [13]. The second case, a 4-year-old X-linked CGD patient hospitalized at the NIH, suffered from *A. fumisynnematus* infection in his spine and pelvis, with a probable primary lung lesion and possible dissemination to the brain. Radiographic evidence of scarring in his lung was suggestive of initial pulmonary infection. Amphotericin B therapy failed to arrest the disease, but aggressive therapy with voriconazole and granulocyte transfusions stabilized the brain lesion. *A. fumisynnematus* is susceptible to triazoles but is significantly more resistant to amphotericin B than *A. fumigatus* (Table 3).

Aspergillus pseudoviridinutans

Aspergillus viridinutans belongs to *Aspergillus* section *Fumigati,* first isolated from rabbit dung in Australia and subsequently documented in human clinical isolates around the world [32]. Recently, significant genetic diversity was found among strains of *A. viridinutans,* and some clinical strains have been reclassified to 4 closely related new cryptic species, *A. pseudoviridinutans, A. felis, A. pseudofelis*, and *A. parafelis* [33]. Among these 4 clinically relevant species, only *A. pseudoviridinutans* has been reported from patients with PID; 1 from a CGD patient and the other from a patient with AD-HIES syndrome [6]. Both cases were reported in 2009 using the old name of *A. viridinutans* [6]. The clinical manifestation of *A. pseudoviridinutans* infection was similar to that of *A. udagawae* and distinct from *A. fumigatus.* The infection was chronic and disseminated in a

Figure 2. Micromorphology of emerging rare *Aspergillus* species associated with primary immunodeficiencies. A, *A. fumisynnematus* (adopted from Samson et al. with permission). B, *A. pseudoviridinutans* (adopted from Sugui et al. with permission). C, *A. subramanianii* (adopted from Visagie et al. with permission). D, *A. tanneri* (adopted from Sugui et al. with permission). E, *A. udagawae* (adopted from Vinh et al. with permission).

Table 2. Characteristics and Outcome of Primary Immunodeficiency Patients With Emerging Rare *Aspergillus* Invasive Aspergillosis

Case	Causative Agent	Underlying Disease	Sex	Age	Clinical Signs During Course of Illness	Signs of Disease in Autopsy	Antifungal Used	Surgery	Duration of Course of Illness	Outcome (Alive/Died)	Reference
1	A. fumisynnematus	STAT3 haploinsufficiency	M	37	Sinuses, orbital apex, cavernous sinus, temporal lobe	-	AmB, VRC, POS, MFG, TRB,	Sinus surgeries, orbital and cavernous sinus tissue debridements	1 y	Died	[13]
2	A. pseudoviridinutans	CGD (p47phox)	M	14	Liver abscesses, pneumonia, right middle lobe nodule, mediastinal lymphadenopathy mediastinal with necrosis	-	ITC, VRC, VRC+CAS, POS, POS+CAS	-	6 y	Alive	[6]
3	A. pseudoviridinutans	AD-HIES	M	8	Bilateral pulmonary lesions, left pleural mass and effusion, and a right upper lung cavity with nodule, Guillain-Barré syndrome, progressive respiratory failure with aspiration of abscess cavity content	Extensive fungal abscesses in the lungs and left pleural space that extended into the airways, diaphragm, and spleen	VRC, AmB, POS+CAS+5FC, granulocyte infusions, adjunctive granulocyte colony stimulating factor, interferon-γ	Debridements of lung, pleural, diaphragmatic, and udpulmonic abscesses, left lower lobe segmentectomy	<1 y	Died	[6]
4	A. tanneri	CGD (gp91phox)	M	17	Osteomyelitis, recurrent pneumonias, with pleural effusion, mediastinal abscess, and extension to liver and spleen, massive pulmonary infection	Innumerable abscesses in lung, liver, mediastinum, and abdominal cavity specimens, with multiple adhesions, intense inflammatory infiltrates, and necrosis	Gamma interferon, AmB, ITC, VRC, CAS, granulocyte transfusions	Appendectomy, excision of liver abscess and splenectomy	1 y	Died	[7]
5	A. tanneri	CGD (gp91phox)	M	19	Mediastinal adenopathy, pneumonia of the right upper lobe, cervical adenopathy, hepatosplenic abscesses	Bilateral fungal pneumonia with cavities, ocular vein involvement, hepatomegaly, periaortic and mesenteric lymphadenopathy	VRC, VPOS, AmB, MFG, L-AmB, TBF	Dilation of a ureter and stent placement	2 y	Died	[7]
6	A. subramanianii	Work-up ongoing	M	49	Thoracic spine, mediastinum	-	VRC, POS	Several thoracic spine laminectomies	10 y	Alive	Present study
7	A. udagawae	CGD (gp91phox)	M	27	Cough, hemoptysis, left lower lobe nodule, pneumonia	-	AmB, VRC, CAS	Partial lobectomy, thoracoplasty, vertebral resection with rod lacement	<1 y	Alive	[8]
8	A. udagawae	CGD (p47phox)	F	36	Cough, dyspnea, lingular consolidation, scattered nodules, persistent mediastinal lymphadenopathy	-	ITC, VRC, POS	-	<1 y	Alive	[8]
9	A. udagawae	CGD (gp91phox)	M	29	Diffuse pulmonary interstitial thickening, infiltrates in the left pulmonary apex and right lower lobe, apical consolidation involving the pleura, lingula, fissure and left lower lobe, thyroid lesions, neurological deficits, and bilateral cerebral hypodensities	Septated bulbous hyphae were identified diffusely in the lungs with multiple pleural adhesions, in the left ventricular and septal myocardium with fibrinous pericardium along the tricuspid valve, in both thyroid lobes, and in the brain	L-AmB, POS, VRC+CAS, AmB, TRB, 5-FC	Mediastinal and hilar lymphadenopathy	14 y	Died	[8]

Abbreviations: 5-FC: flucytosine; AFG, anidulafungin; AmB, amphotericin B; CAS, caspofungin; CGD, chronic granulomatous disease; ITC, itraconazole; L-AmB, liposomal amphotericin B; POS, posaconazole; TRB: terbinafine; VRC, voriconazole.

Table 3. Antifungal Susceptibility Profile of Non-*fumigatus Aspergillus* Species Causing Invasive Aspergillosis in Patients With Primary Immunodeficiencies, Using CLSI Broth Microdilution Method

Aspergillus spp.	MIC/MEC, µg/mL			
	AmB	VRC	ITC	POS
A. calidoustus	2	8 to 16	4 to 16	8 to16
A. fumigatus	0.5	0.5	0.5	0.125
A. fumisynnematus	4	0.25	0.5	0.25
A. nidulans	2	0.25	0.5	0.25
A. pseudoviridinutans	2 to 8	1 to 8	1 to 16	<0.016 to 0.5
A. quadrilineatus	0.5	0.5	0.125	0.25
A. subramanianii	2	0.5	0.25	0.25
A. tanneri	>16	4	4	0.5
A. udagawae	0.5 to 2	1 to 4	1 to 4	0.25 to 0.5

Abbreviations: AFG, anidulafungin; AmB, amphotericin B; CAS, caspofungin; MIC, minimum inhibitory concentration; MEC, minimum effective concentration; ITC, itraconazole; POS, posaconazole; VRC, voriconazole.

contiguous spread and appeared to be refractile to antifungal therapy. *A. pseudoviridinutans* grows at 37ºC, with an intermediate growth rate between *A. fumigatus* (fast) and *A. viridinutans* (slow). The *A. psuedoviridinutans* colonies attain a diameter of 5.5 to 6.5 cm on ME agar, as well as on Czapek's solution agar, in 5 days. Colonies on ME agar are white with dense aerial hyphae and scant conidiation with yellow colony reverse. The *A. pseudoviridinutans* growth patterns at 42ºC and 37ºC are similar, and it fails to grow at 50ºC [15]. The species has decreased susceptibility in vitro to both amphotericin B and azoles compared with *A. fumigatus* (Table 3) [6].

Aspergillus quadrilineatus

Aspergillus quadrilineatus (syn. = *Emericella quadrilineata*) is a soil fungus commonly isolated in tropical countries [34]. It belongs to the *Aspergillus* section *Nidulantes* and is a sibling species of *A. nidulans. A. quadrilineatus* grows faster on both Czapek's and ME agar media at 30°C and 37°C than at 25°C. Conidial structures of *A. nidulans* and *A. quadrilineatus* can not be distinguished, but the length of conidiophores tends to be slightly shorter in *A. quadrilineatus.* The 2 species can be differentiated with certainty by their number of ascospore crests depicted in scanning electron microscopy: 4 crests in *A. quadriliniatus* vs 2 crests in *A. nidulans* ascospores. The 2 species can also be readily distinguished by DNA sequencing (Figure 1) [12]. Despite the close morphologic and genetic relatedness, the 2 species differ significantly in their in vitro susceptibility to amphotericin B and caspofungin (Table 3). However, the triazoles are active in vitro against both species [12].

Although *A. nidulans* causes IA primarily in CGD patients, infection due to *A. quadrilineatus* has been documented in both CGD and cancer patients [12, 34]. In fact, IA caused by *A. quadrilineatus* in 2 of 3 CGD patients was originally reported as *A. nidulans* based only on the conidial structures [12].

Aspergillus subramanianii

Aspergillus subramanianii belongs to the *Aspergillus* section *Circumdati* and produces yellow to ochre colonies owing to the yellow-colored conidia and sclerotia that do not turn black. The section *Circumdati* members can be distinguished from each other based on morphological characteristics, along with DNA sequences and extrolite profiles [35]. Unlike some species in the section *Circumdati*, *A. subramanianii* produces ochratoxin A inconsistently and/or only in trace amounts. It has been isolated from house dust samples in Mexico, air from Panama, and shelled Brazil nuts in Canada [35].

Aspergillus subramanianii produces faster-growing colonies on Czapek's yeast autolysate agar at 37°C (4.5–5 cm in diameter after 7 days) compared with its phylogenetically close species in the section *Circumdati*. On ME agar, the colony surface is floccose to somewhat velutinous, and mycelial areas are white with light yellow sporulated spots and light brown colony reverse [35].

IA caused by *A. subramanianii* was diagnosed in a 49-year-old man without CGD or CARD9 deficiency but with a PID yet to be precisely identified (NIH–Fungal Pathogenesis Lab, unpublished data, 2018). The patient presented with chronic disseminated infection of the thoracic spine and adjacent mediastinal tissue. Several thoracic spinal laminectomies, followed by therapy with voriconazole and posaconazole, gradually resolved the infection over a 10-year period (Eric Meissner, MD, unpublished data, 2018; initial identification of *A. subramanianii* by Nathan Wiederhold, Pharm D, 2018).

Aspergillus tanneri

Aspergillus tanneri was described in 2012 [7], based on 2 strains isolated from fatal cases of IA in X-linked CGD patients treated at the NIH. Both patients came from the southwestern part of the United States, California and New Mexico, but the specific environmental source of the species remains unknown.

Aspergillus tanneri was originally classified as a non-ochratoxin-producing member of the *Aspergillus* section *Circumdati* [7]. Subsequently, its unique attributes have led to creation of a new *Aspergillus* section *Tanneri* to accommodate the species [36]. *A. tanneri* is a slow-growing species at 37°C and requires 2 weeks to attain a colony size of 3 cm and 4–5 cm on Czapek's agar and ME agar, respectively. Growth is slightly faster at 30°C, and colonies are white at the beginning but grow yellow due to the maturation of conidia with age. *A. tanneri* has significantly lower in vitro susceptibility to most antifungals than other pathogenic *Aspergillus* species (Table 3).

Unlike *A. fumigatus*, *A. tanneri* causes chronic disease with primary pulmonary foci but has disseminated to almost every organ of the body [7]. Both patients with *A. tanneri* died despite intensive antifungal therapy supplemented by gamma interferon and granulocyte transfusions.

Aspergillus udagawae

Aspergillus udagawae (teleomorph = *Neosartorya udagawae*), a member of *Aspergillus* section *Fumigati*, was first isolated from the soil of a sugar cane plantation in Brazil [37]. Like *A. fumigatus*, *A. udagawae* is heterothallic, but *A. udagawae* produces the teleomorph much more readily on conventional mycological media, whereas strains of *A. fumigatus* require particular growth conditions to complete the sexual life cycle. *A. udagawae* fails to grow at a temperature above 42°C, whereas *A. fumigatus* grows at ≥45°C [8]. The 2 species also differ in their minimal and optimal growth temperatures. *A. udagawae* grows slowly at 10°C and optimally at 30–35°C, but *A. fumigatus* fails to grow at 10°C and grows optimally at 37°C [38]. Because *A. udagawae* is more resistant than *A. fumigatus* to various antifungals, the *A. udagawae* infections documented are primarily refractory to standard antifungal therapy [38].

Cases of aspergillosis due to *Aspergillus udagawae* have been reported sporadically in CGD patients [8], in a patient with myelodysplastic syndrome [8], and in a patient with diabetes mellitus [39]. The 3 cases of IA caused by *A. udagawae* reported in CGD patients were all chronic disease and disseminated in a contiguous manner [38]. The median duration of infection was approximately 7 times longer (35 weeks vs 5.5 weeks) than that typically observed for IA caused by *A. fumigatus* in CGD.

Species Identification of Non-*Fumigatus Aspergillus* Species Primarily Associated With PIDs

Species identification of those poorly recognized *Aspergillus* isolated from patients with PID can be challenging. Because of their decreased susceptibility to widely used antifungal agents compared with *A. fumigatus*, identification at the species level is critical for proper patient management. Identification schemes include culture followed by polymerase chain reaction (PCR) sequencing of representative genes. Although ITS and partial ß-tubulin have been most commonly amplified and sequenced genes for species identification, the value of ITS sequencing is minimal for the speciation of emerging non-*fumigatus Aspergillus* species. The ß-tubulin gene, by using the primers currently reported, may amplify the benA or the tubC alleles, and hence could yield different results between tests [40]. For a diagnostic laboratory to complete its mission in a timely and accurate manner, we view caM as the best gene choice for PCR sequencing. It is sufficiently variable to detect several haplotypes in a species, is relatively easy to amplify, and produces a clean, easy to read sequence. In addition, the caM sequence of nearly every *Aspergillus* species is now accessible, thus making this locus very effective in assessment of species identity.

Innate Resistance of Pathogenic *Aspergillus* Species to Antifungal Agents

Table 3 summarizes the antifungal susceptibility profile of the rare *Aspergillus* species recovered from PID patients with IA. The antifungal triazoles are preferred agents for the treatment and prevention of IA in immunocompromised patients [14]. However, with wide application of azoles in clinical practice and agriculture, the emergence of triazole resistance in *A. fumigatus* due to mutations in the *Cyp51A* gene is a global public health concern [17].

Recent changes in the taxonomy of *Aspergillus* have also had major implications for our understanding of drug susceptibility profiles. For instance, the new cryptic species of *Aspergillus* section *Fumigati* exhibits significantly different drug susceptibilities than *A. fumigatus* in vitro, except for those that have acquired resistance against azoles [17]. *A. calidoustus* has decreased susceptibility to all triazoles, and *Aspergillus tanneri* is a species notorious for its decreased susceptibility to amphotericin B and triazoles (Table 3). Using the CLSI broth microdilution method, we also tested the antifungal susceptibility of *A. subramanianii* and *A. fumisynnematus* isolates recovered from PID patients. Both isolates showed elevated minimum inhibitory concentrations for amphotericin B but were susceptible to azole drugs.

CONCLUDING REMARKS

The present work summarizes the clinical, diagnostic, mycological, and treatment features of emerging *Aspergillus* species, which are almost exclusively reported from cases of IA in patients with various congenital immune defects. The species discussed here have not been well recognized, partly due to their rarity in clinical settings compounded by taxonomic changes of the species, but more importantly, due to the lack of species identification in diagnosis. In many reviews or case reports of aspergillosis in patients with PIDs, the etiologic agent is often listed as "*Aspergillus.*" Because these species cause subacute to chronic infection in patients with PID and are generally more difficult to treat due to their lower susceptibility to available antifungal agents than *A. fumigatus*, correct and prompt identification at the species level is critical for appropriate therapy to improve patient outcomes. Notably, DNA sequence-based species identification is more accurate than using morphological characteristics to differentiate the emerging *Aspergillus* species. It is critical that physicians who care for PID patients with aspergillosis are aware of these emerging non-*fumigatus Aspergillus* species and their unique features. It is also critical to develop new drugs and/ or treatment strategies that will be effective against these species.

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