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Abstract

Humans inhale, ingest, and touch thousands of fungi each day. The ubiquity and diversity of the fungal kingdom, reflected by its complex taxonomy, are in sharp contrast with our scarce knowledge about its distribution, pathogenic effects, and

Abbreviations: ABPA, allergic broncho-pulmonary aspergillosis; ABPM, allergic broncho-pulmonary mycosis; AFRS, allergic fungal rhinosinusitis; COPD, chronic obstructive pulmonary disease; DGGE, denaturing gradient gel electrophoresis; ECP, eosinophil cationic protein; ECRHS, European Community Respiratory Health Survey; EDN, eosinophil-derived neurotoxin; ERMI, environmental relative moldiness index; FeNO, fractional exhaled nitric oxide; HSP, hypersensitivity pneumonitis; Ig, immunoglobulin; PAMP, pathogen-associated molecular pattern; PCR, polymerase chain reaction; PRR, pattern recognition receptors; VOC, volatile organic compounds.

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effective interventions at the environmental and individual levels. Here, we present an overview of salient features of fungi as permanent players of the human exposome and key determinants of human health, through the lens of fungal allergy and other fungal hypersensitivity reactions. Improved understanding of the fungal exposome sheds new light on the epidemiology of fungal-related hypersensitivity diseases, their immunological substratum, the currently available methods, and biomarkers for environmental and medical fungi. Unmet needs are described and potential approaches are highlighted as perspectives.

KEYWORDS
allergic bronchopulmonary aspergillosis, allergy, exposome, fungus, hypersensitivity

1 | INTRODUCTION

The fungal kingdom is an intrinsic component of the global environment and a prominent contributor to planetary health.[1–3] Fungi are also a major cause of human diseases, including type I (IgE), type III (IgG), and type IV (T cells) hypersensitivity.[4] Long-known unmet needs in the field of fungal hypersensitivity are increasingly addressed by new technologies, as highlighted in EAACI position papers.[5–7] The aim of this position paper is to summarize our current understanding of fungi, their place in the human exposome, their involvement in hypersensitivity diseases, and to provide an overview of the tools available for their investigation and of the currently unmet needs.

The fungal kingdom comprises ubiquitous heterotrophic eukaryotes, which possess chitin in their cell walls, feed through osmotrophy, and are specialized in the decomposition and recycling of organic material.[8–10] The fungal and animal kingdoms share a common ancestor.[9] Fungi engage in intra- or interkingdom symbiotic interactions, for example, with algae in lichens or with plants in mycorrhizae.[10,11] Fungal systematics is complex,[12] but genomic technologies supported an extensive update of the classification and taxonomy of the fungal kingdom since 2011.[13,14] Fungi can be either unicellular, such as yeasts, or multicellular, forming filaments called hyphae (filamentous fungi). Filamentous fungi may present as mushrooms (e.g., *Coprinus*), polypores (e.g., *Ganoderma*), smuts (e.g., *Ustilago*), exhibit a fuzzy appearance typical of molds (e.g., *Penicillium*), and more. The ubiquity and diversity of fungi reflect those of the living world: spores of several fungal species have been detected in samples collected in arctic or desert areas,[15] and the number of fungal species has been estimated at 3.8 million,[16] most of them still unknown today. As a result, humans are exposed to fungi each day during their entire lifetime.

Fungi evolved to feed on virtually every complex molecule in any ecological niche, reducing it to simple units able to re-enter the nutrition chains. In doing so, fungi selected an evolutionary pathway characterized by complexity and mobility, through the efficient use of multiple developmental stages and enzyme secretion in the environment.[11] Fungi do not ingest the organisms they are feeding upon but release lytic enzymes able to process macromolecules into small nutrients. Ingested nutrients allow fungal growth and the production of mobile, airborne forms conveyed to new locations. Leftovers such as secreted components and previous developmental forms persist in the environment, explaining the ubiquity and abundance of fungal components.

Although fungi bear pathogen-associated molecular patterns (PAMPs), which are ligands for the host's innate immune pattern recognition receptors (PRRs), most interactions between fungi and the human host do not result in disease. Instead, a fungal disease usually manifests in susceptible hosts. Infection is often associated with immune deficiency, while type I hypersensitivity occurs mainly in atopic patients.[17,18] Depending on the ability of a given fungus to grow at human body temperature (thermotolerant fungi) or not (mesophilic fungi), the pathogenic threat posed to the human host is either both infectious and allergic, or allergic only. Typical examples of medically important thermotolerant fungi are *Aspergillus fumigatus* and *Candida albicans*, while *Alternaria alternata* and *Cladosporium herbarum* are typically mesophilic.[19]

The third form of fungi-related disease is caused by mycotoxins, small molecules produced by fungi as means to secure their feeding environment. Mycotoxins are potentially harmful when they are ingested from contaminated stored foods. As opposed to diseases related to airborne fungal forms, mycotoxin exposure other than through ingestion is not considered causal for mycotoxin-related diseases.[20]

Fungal antigens are characterized by intrakingdom specificity, with a relation between the fungal systematics and immunoglobulin (Ig) E sensitization pattern.[20] Many fungal proteins have evolved for specific functions associated with heterotroph nutrition. The degree of homology reflects phylogenetic distance.[21] There is extensive cross-recognition of fungal antigens, contributing to clinical cross-reactivity manifested as hypersensitivity symptoms related to exposure to various fungi, and biological cross-reactivity when skin or laboratory tests are performed for fungi-specific IgE and IgG.[22] Cross-reactivity between fungi and organisms from other domains of life is limited. A prominent exception is chitin, a carbohydrate component of the fungal cell wall, but also of insect and arachnid (house dust mites, crustaceans) exoskeleton.[20,23] However, other examples of medically

relevant cross-reactivity exist, for example, between the skin resident yeast *Malassezia (M.) sympodialis* and human host proteins.[24]

Fungal taxonomy is complex and still evolving. The main allergenic genera and species belong to three of the 10 fungal phyla currently described: *Ascomycota* (comprising *Candida*, *Alternaria*, *Aspergillus*, *Penicillium*, *Trichophyton*, and among other genera), *Basidiomycota* (e.g., *Rhodotorula* and *Ustilago*), and *Mucoromycota* (classified until recently as part of the former phylum *Zygomycota)* (e.g., *Mucor*)[10,25] (Table 1). Inside each phylum, phylogenetic relationship explains allergen cross-reactivity at the level of genera and species.[21] The following sections bring further detail to these topics.

2 | WHAT IS A FUNGUS?

Fungi are eukaryotic, heterotrophic, mainly aerobic organisms, possessing chitin in their cell walls, ergosterol in their plasma membranes, typical eukaryotic 80S ribosomes, that are able to produce lysine.[26–28] Fungi that display unicellular vegetative forms are called yeasts.[26] Most yeasts belong to the group of dimorphic fungi, defined on the basis of their ability to develop either as yeasts and related unicellular forms, for example, spherules and adiaspores, or as multicellular filamentous fungi, depending on physicochemical conditions and nutrient availability.[27,28] Yeasts reproduce through budding, while filamentous fungi display hyphae in a mycelium and produce spores.[26,27]

Dimorphism manifests in species across the fungal phylae, supporting the view that it evolved multiple times.[28] The main trigger of the dimorphic transition to yeast is a 37°C temperature, but yeasts may also be found in the environment.[29–31] In most cases, dimorphism allows fungi to develop both in the environment (free-living and saprotrophic) and in the host (parasitic).[28,32] Transition to either form may be associated with increased pathogenicity.[27,33] Like other fungal forms, yeasts can cause hypersensitivity reactions, infections, or both.[6,34,35] Severe infections may occur in immunocompetent hosts; conversely, yeasts may persist within the host, manifesting as opportunistic infections upon immune suppression or returning to the environment as saprotrophs after the host's death.[27] Examples of yeasts of medical importance in humans are shown in Table 2.

TABLE 1 Overview of the current classification of fungi at the phylum level

Fungal phylum	Examples of known allergenic genera and species
Ascomycota	*Alternaria*, *Aspergillus*, *Aureobasidium*, *Candida*, *Chaetomium*, *Chrysonilla*, *Cladosporium*, *Coccidioides*, *Curvularia*, dermatophytes (*Trichophyton*), *Drechslera*, *Epicoccum*, *Geotrichum*, *Penicillium*, *Stachybotrytis*, *Stemphyllium*, *Ulocladium*
Basidiomycota	*Coprinus*, *Malassezia*, *Psilocybe*, *Rhodotorula*, *Trichosporon*, *Ustilago*
Blastocladiomycota	None
Chytridiomycota	None
Cryptomycota	None
Microsporidia	None
Mucoromycota	*Mucor*, *Rhizopus*
Olpidiomycota	*None*
Sanchytriomycota	None
Zoopagomycota	None

Note: Phyla comprising established allergenic species are shown in bold font. References 9,11,12,19,25,74,79, allergen.org, and PubMed pubmed.ncbi.nlm.nih.gov/search for (phylum name) AND (hypersensitivity) OR (allergy) OR (IgE) OR (occupational) accessed July 24th, 2022.

Before 2011, the taxonomy of fungi was blurred by the coexistence of distinct names for the sexual (teleomorph) and asexual (anamorph) states of the same fungus. This dual nomenclature had hampered research on fungi for decades.[12] Since 2011, and as a fortunate sequel to the advent of DNA-base taxonomy genome, an initiative explicitly called "One fungus, one name" of the International Mycological Association resolved to use only one name per species.[14] To date, fungal taxonomy is still in progress and remains relatively unstable.

As stressed above, fungi are ubiquitous in the environment. Humans are exposed to fungi via inhalation in indoor and outdoor environments; they are also exposed via ingestion through the digestive tract and via contact with the skin and eyes. Exposure to fungi may result in infection (e.g., mucormycoses, coccidioidomycoses, invasive or noninvasive aspergillosis, or fusariosis) or hypersensitivity in predisposed individuals (such as conjunctivitis, asthma, rhinitis, allergic bronchopulmonary aspergillosis (ABPA), and hypersensitivity pneumonitis (HSP)).[6,35–37] In particular, fungal IgE sensitization is often present in patients with asthma, bronchiectasis, cystic fibrosis, and chronic obstructive pulmonary disease (COPD), contributing to the initiation or pathophysiology of these diseases, which rank among the most prevalent worldwide.[17,19,38] Fungal infections develop predominantly in immunocompromised hosts and may exceed 50% case-fatality rates.[17,39] HSP may occur in subjects without a previous condition and relates to a non-type 2 immune response with robust cellular and antigen-specific IgG responses.[35,40]

Moreover, fungi release mycotoxins and volatile organic compounds (VOC). Mycotoxin production aims to secure fungal nutrients, while VOC are defined as small molecules containing carbon and able to evaporate under ambient conditions, such as 0.01 kPa and 20°C.[41–43] Exposure to mycotoxins may occur through inhalation of airborne mycotoxins or ingestion of contaminated foods, giving rise to mycotoxicosis.[42,44]

Fungal VOC are produced by primary and/or secondary metabolic pathways as a species-specific profile subject to environmental changes. To date, more than 400 fungal VOC have been described, encompassing a wide variety of chemical compounds: simple hydrocarbons, heterocycles, aldehydes, ketones, alcohols, phenols, thioalcohols, thioesters and their derivatives, benzene derivatives, and cyclohexanes.[43,44] Although some fungal VOC have been shown to induce symptoms (fatigue, lethargy, headache, irritation of ocular and upper airway mucosae, and wheezing) and upregulate biomarkers of

TABLE 2 Examples of yeasts involved in hypersensitivity reactions and/or infections in humans

Phylum	Genus, species	Hypersensitivity/Allergy	Infection	Component of healthy human microbiome
A	*Candida* (e.g., *C. albicans*, *C. boidinii*)	Yes	Yes	Yes
A	*Saccharomyces* (e.g., *S. cerevisiae*)	Yes	Yes	Yes
A	*Geotrichum* (e.g., *G. candidum*)	Yes	Yes	Yes
B	*Malassezia* (e.g., *M. sympodialis*, *M. furfur*, *M. globosa*)	Yes	Yes	Yes
B	*Trichosporon* spp	Yes	Yes	Yes
B	*Rhodotorula* (e.g., *R. mucilaginosa*)	Yes	yes	Yes
M	*Rhizopus* (e.g., *R. microsporus*, *R. oryzae*, *R. stolonifer*)	Yes	Yes	Yes (infrequent)
M	*Mucor* (e.g., *M. racemosus*)	Yes	Yes	Yes
A	*Blastomyces dermatitidis*	No	Yes	No
A	*Coccidioides* (e.g., *C. immitis*, *C. posadasii*)	No	Yes	No
A	*Emmonsia* (e.g., *E. parva*)	No	Yes	Yes (infrequent)
A	*Emergomyces* (e.g., *E. africanus*)	No	Yes	No
A	*Histoplasma* (e.g., *H. capsulatum*)	No	Yes	No (reported in HIV-infected subjects)
A	*Paracoccidioides* (e.g., *P. brasiliensis*, *P. lutzii*)	No	Yes	No
A	*Sporothrix* (e.g., *S. schenckii*)	No	Yes	No
A	*Talaromyces marneffei*	No	Yes	No
B	*Cryptococcus* (e.g., *C. neoformans*, *C. gattii*)	No	Yes	Yes (infrequent)

Note: Yeasts are fungal unicellular vegetative forms. They are found as constituents of both the external exposome (natural and anthropic environment) and of the internal exposome (commensal mycobiome). Some yeasts are responsible for infections and/or hypersensitivity reactions. References 6,17,22,28–30,32,34,40,48,112,152,154 and PubMed pubmed.ncbi.nlm.nih.gov/search for (genus name) OR (species name) AND (hypersensitivity) OR (allergy) OR (IgE) OR (occupational) OR (infection) OR (mycobiota) accessed July 24th, 2022. A, *Ascomycota*, B, *Basidiomycota*, M, *Mucoromycota*.

inflammation in healthy volunteers[43,45] their impact on human health is still controversial.[41–43] Given their characteristics mentioned above, fungi are key constituents of the human exposome and further research is needed to evaluate and characterize the impact of fungal exposome on human health and use the data for risk assessment.

3 | FUNGAL EXPOSOME

Worldwide, fungi are an increasingly acknowledged part of the human exposome, both external[3,27] and internal as microbiome components collectively named mycobiota[33] (Figure 1). The external, or environmental, part of the fungal exposome may be divided into outdoor and indoor categories, with many shared features but also with many differences in terms of composition, variation, and potential interventions.

Indoor and outdoor fungal exposure shows geographical, seasonal, and urban versus rural variability[27,36,46,47] and induces immune responses and health effects, often respiratory and allergic, that may start in infancy.[48–51] In fact, human–fungus interactions extend from medicine and pharmacy to leisure activities, agriculture, food processing, industry, and even interplanetary travels.[3,4,11,31,52]

Currently, allergenic molecules from more than 40 fungal genera have been characterized (www.allergen.org, accessed July 25th, 2022), but molecular data are still lacking for many environmental fungi (Table 3). Cross-reactivity between fungal allergens may help to identify some, but not all, fungal sensitization outside of the available extracts and molecules.[53,54]

3.1 | External exposome

Airborne fungi and subcellular components originate from diverse sources, for example, soil, plants, animals, and water. Fungal spores are distributed by physical mechanisms of gravity, wind, water, and animals. Airborne particles originating from biological sources (viable and nonviable e.g., bacteria, fungi, pollen, mites, dead tissues, and pieces of these materials or their metabolic products including endotoxins and mycotoxins) are called bioaerosols.[55] Bioaerosols are ubiquitous; they originate mainly from soil and aquatic, animal, vegetal, and anthropogenic sources, become airborne and may travel long distances in the environment thanks to wind dispersal before sedimenting in so-called sinks, or settle, for example, on indoor surfaces or clothing.[46,55,56] Fungal spores, ranging from 1 to 30μm in size, are major components of bioaerosols, where their release, time of flight, survival, and hence, fitness for subsequent growth follow a variety of pathways.[56–58]

Culture-independent studies have demonstrated a very high diversity of airborne fungal taxa. As an example, each dust sample

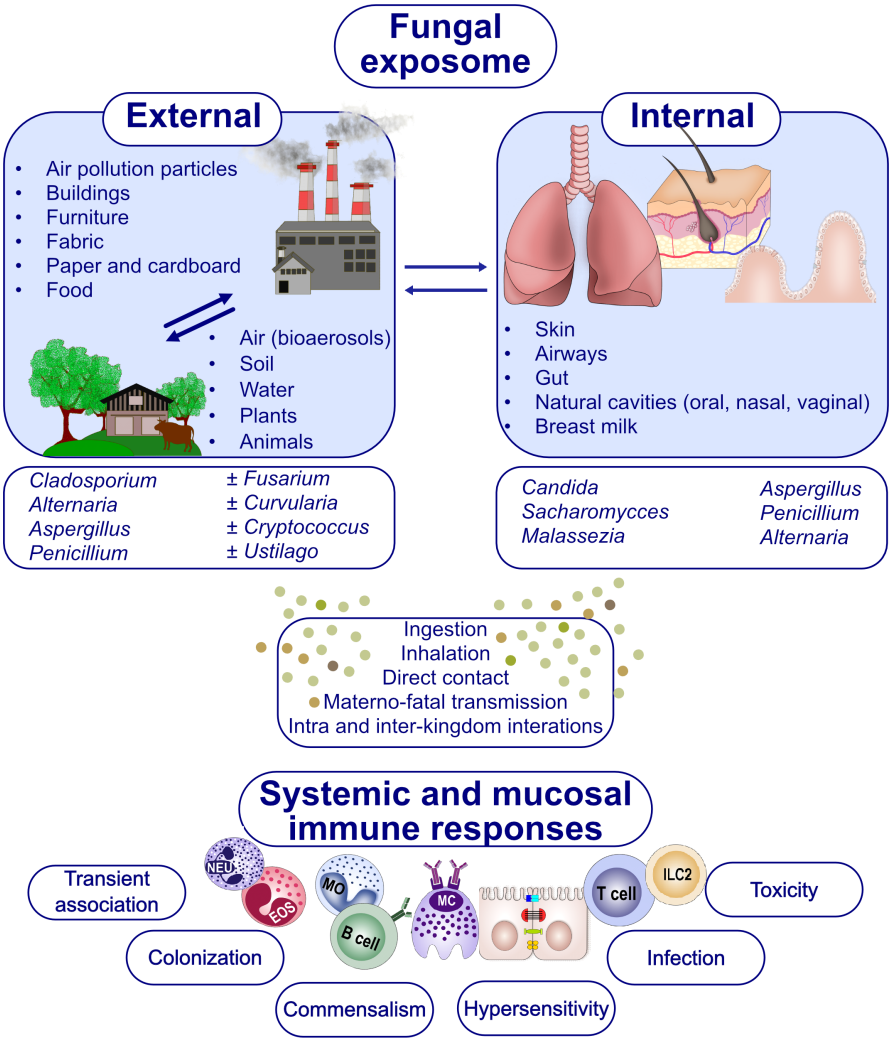


FIGURE 1 Fungal exposome components and their interaction with the human host. Fungi are ubiquitous in the outdoor and indoor environment, originating in both natural (e.g., soil, water, living or decaying plants, and animals), and anthropic (e.g., food, buildings, and furniture) sources, and are collectively denoted as "external fungal exposome." The internal fungal exposome consists of the fungal part of the microbiota, or mycobiota, comprising fungi found inside the human body or on its cutaneous and mucosal surfaces. The most prevalent fungal genera in the external and internal exposomes are shown. External and internal fungal exposomes interact, for example, daily inhalation and ingestion of fungi or fungal fragments. Fungi interact with other eukaryotes, such as *Protistae*, and with bacterial, viral and archaeal components of the mycobiota. The mucosal and systemic immune responses mounted by the host in presence of fungi may contribute to preserve health or induce fungal-related diseases. Lower left box: ± denotes inconstantly demonstrated airborne fungal genera (as opposed to ubiquitous ones).

taken from the outer surface of hundreds of homes in the United States contained more than 1000 fungal phylotypes, most of which belonging to taxa not described at the time of the study.[46] However, only *Cladosporium*, *Toxicocladosporium*, and *Alternaria* were found in this study in virtually all samples. These were also dominant taxa in terms of abundance, with *Cladosporium spp* often amounting to 75% of the whole fungal content of samples and *Alternaria spp* making up to 50%.[46] The results of a questionnaire sent to national and regional networks involved in the European Aeroallergen Network (EAN) and counting fungal spores have shown that most networks (44.7%) identified five or fewer fungal spore types, and only two networks (12.5%) identified more than 20 fungal spore types. On the contrary, all networks examined *Alternaria*,[16] most of them *Cladosporium*[14] and the third more cited spore was *Epicoccum*.[10] Forty-five other fungal spores were cited in the list, but only by one to six networks. Partly overlapping findings were reported from the European urban areas of Bratislava (Slovakia, temperate continental climate), Thessaloniki (Greece, Mediterranean climate), and Madrid (Spain, Mediterranean climate with semi-arid influence). The five top abundant Slovakian fungal spores were *Cladosporium*, *Leptosphaeria*, *Coprinus*, *Alternaria*, and *Ganoderma*,[59] while their Greek counterparts were *Cladosporium*, *Alternaria*, *Ustilago*, and Ascospores (*Aspergillus/Penicillium*).[60] *Cladosporium*, *Alternaria*, *Eurotium*, *Epicoccum*, *Penicillium*, and *Sporobolomyces* were detected in more than 90% of samples from Madrid.[61] In more arid climate types, such as Karachi (Pakistan) and Kuwait, *Cladosporium*, *Alternaria*, *Aspergillus*, and *Penicillium* were also found among the top frequent airborne fungal spores, together with *Curvularia* and *Periconia* in Karachi[62] and *Cryptococcus*, *Candida*, *Schizophyllum*, *Fusarium*, and *Gleotinia* in Kuwait.[63] Finally, under tropical climates, airborne fungal spores were dominated by *Cladosporium*, *Leptosphaeria*, *Coprinus*, *Aspergillus*, and *Penicillium* in Havana (Cuba) using a culture-independent direct identification method[64] and by *Penicillium* and *Aspergillus* in Nigeria using a culture-dependent approach.[65]

Under temperate climates, seasonal variations usually increase fungal abundance with higher temperatures and rainfall, such as during summer and fall.[61] However, very high-temperature values may negatively affect the abundance of airborne fungal spores, as observed in Karachi and Lagos.[62,65] Significant interannual variations in rainfall are common and associated with variations in airborne fungal abundance.[61,64]

Depending on the considered fungi, spore release may occur preferentially during the daytime, as observed for *Alternaria*, *Cladosporium*, *Epicoccum*, and *Exosporium* or at night, for example,

TABLE 3 Examples of fungal molecular allergens among those currently included in the WHO/IUIS nomenclature

	Genus		Selected examples of protein families							
Phylum	Name	Number of allergens	Serine-proteases	Ribosomal proteins	Peroxisomal proteins	Enolases	Thioredoxin and Thioredoxin-like	Heat-shock proteins	Cyclophilins	Others
Ascomycota	*Alternaria*	12	Alt a 15	Alt a 5, Alt a 12		Alt a 6		Alt a 3		Alt a 1 (unknown function)
	Aspergillus	38	Asp f 13, Asp fl 13, Asp o 13, Asp v 13, Asp f 18, Asp n 13	Asp f 8, Asp f 23	Asp f 3	Asp f 22	Asp f 28, Asp f 29	Asp f 12, Asp f 19	Asp f 27	Asp f 1 (mitogillin family); Asp f 2 (unknown function), Asp f 4 (unknown function), Asp t 36 (triose phosphate isomerase)
	Candida	3			Cand a 3, Cand b 2					
	Cladosporium	10	Cla c 9, Cla h 9	Cla h 5, Cla h 12		Cla h 6				Cla h 8 (mannitol dehydrogenase)
	Curvularia	4	Cur l 1, Cur l 4			Cur l 2				Cur l 3 (cytochrome c)
	Epicoccum	1	Epi p 1							
	Fusarium	4	Fus p 9	Fus c 1			Fus c 2			
	Penicillium	17	Pen b 13, Pen c 13, Pen ch 13, Pen ch 18, Pen o 18	Pen b 26, Pen cr 26	Pen c 3	Pen c 22		Pen c 19		Pen c 32 (pectate lyase)
	Stachybotrys	1								Sta c 3 (extracellular desoxyribonuclease)
	Trichophyton	4	Tri r 4, Tri t 4							
	Ulocladium	1								Ulo c 1 (Alt a 1 homologue)
Basidiomycota	*Coprinus*	5					Cop c 2			
	Malassezia	13			Mala f 2, Mala f 3		Mala s 13	Mala s 10	Mala s 6	
	Psilocybe	2							Psi c 2	
	Rhodotorula	2	Rho m 2			Rho m 1				
	Schizophyllum	1								Sch c 1 (glucoamylase)
Mucoromycota	*Rhizopus*	2							Rhi o 2	Rhi o 1 (aspartate endopeptidase)
Total		120	21	9	6	6	5	5	4	

Note: There are 120 fungal molecular allergens included in the IUIS/WHO nomenclature (www.allergen.org accessed July 24th, 2022): 95 for the *Ascomycota* phylum, 23 for *Basidiomycota*, and 2 for *Mucoromycota* (one species, *Rhizopus oryzae*, formerly a member of the *Zygomycota* phylum). Allergen families with the greatest count of validated members are shown, together with examples of allergens. The table is not exhaustive for fungal molecular allergens, nor for biochemical families thereof.

Coprinus and *Leptosphaeria*.[59] Particulate air pollution such as PM10 is positively correlated with the fungal spore load.[59]

To sum up, the nature and abundance of the dominant fungal spores in the atmosphere exhibit marked variations related to climate (mean annual precipitation and temperature, soil pH, plant diversity, distance to coastal regions), season, time of the day, particulate air pollution, and include *Cladosporium* and *Alternaria* as consistently predominant genera, followed by *Aspergillus* and *Penicillium*, accompanied by locally important filamentous fungi and yeasts, such as *Fusarium*, *Curvularia*, *Cryptococcus*, and *Ustilago*.

Contact with the external fungal exposome may also occur through direct contact with soil and water. An international study reported on culturable fungi found in the sand of 91 marine, oceanic and freshwater beaches from Europe and Australia.[31] Notable differences were observed among European beaches, as well as between Europe and Australia. As an example, yeasts were found in abundance in sand samples from Sydney, Australia, but not in European samples, while *Aspergillus* spp were detected mainly in sand samples from the Mediterranean beaches and from Sydney, but not in samples from North European neither South-Western European beaches.[31]

Indoor exposure is paramount, as most people now spend most of their time indoors.[66] Fungi can be transported by dust particles, people, pets, and air ventilation systems into the indoor environment. The relative humidity and moisture content of building materials may also control to a certain level the fungal burden present on indoor materials. Water damage, defined as "a moisture problem caused by various leaks of water",[67] is an essential contributor to indoor mold growth, often related to climatic events (e.g., floods, storms, and rising ocean levels) or poor housing standards, including older homes.[3,66,68] Indoors, fungi can colonize virtually any material: walls, windows frames, furniture, carpets, books, wallpapers, and even spacecrafts. Biodeterioration due to fungal colonization poses additional health threats, both direct such as skin contact with fungi growing on documents from archives or libraries, and increased airborne spore and mycotoxin load, and indirect due to the toxicity of biocide treatments.[69–71] However, the effect of indoor exposure to certain fungi might be beneficial, and a protective effect against wheeze in infants has been reported for culturable yeasts from indoor dust.[72]

The abundance of indoor fungal spores shows geographic and seasonal variations related to exchanges between the outdoor and indoor environments, fungal growth, and meteorological conditions.[3] The degree of exposure to indoor molds was estimated at 5%–10% under cold-temperate climates, and up to 30% in warmer climates.[47] An environmental relative moldiness index (ERMI) may be used as a quantitative marker of indoor mold exposure.[68] The original ERMI, developed in the United States, is computed using polymerase chain reaction (PCR) quantification of 36 common indoor molds, of which 26 are related to water damage and 10 are not.[73] To acknowledge local fungal variability at the levels of species and abundance, the need for an adapted ERMI was demonstrated.[74]

Exposure to fungi also occurs at various workplaces. A distinction must be made between the intentional use of fungi and unintentional exposure to contaminated materials. The intentional use of fungi including molds and yeasts in workplaces is found in food production, pharmaceutical production, and microbiological laboratories. Representatives of the fungal genus *Penicillium*, for example, *Penicillium camemberti* and *Penicillium roqueforti*, are found in ripen cheese and salamis and produce antibiotics.[6,22,75] *Aspergillus niger* produces citric acid from residues of the sugar industry. The yeast *Saccharomyces cerevisiae* is used in brewery and bakery.[6,34] Multiple enzymes derived from *Aspergillus oryzae* or *Aspergillus niger* are employed during food processing, for example, α-amylase, cellulase, xylanase, glucoamylase in bakery, and pectinase and glucanase for fruit salad processing.[6] In microbiology laboratories, workers may be exposed to fungi when growing and multiplying microorganisms. Many more workers encounter fungi, including molds and yeasts, unintentionally: farmers working in fields or keeping animals, waste processors sorting by hand in waste management, wood processors handling moldy wood, metal workers inhaling contaminated cooling lubricants, renovating houses, to name just a few areas.[34,35] Exposure to mushrooms, members of the fungal kingdom which are beyond the scope of the present paper, is also recognized as a cause of occupational hypersensitivity.[6,22]

Despite the diversity of indoor molds, with more than 80 species currently described, and the fact that indoor air may be 70–100 times more contaminated than outdoor air,[76,77] there are only four genera of significant importance: *Aspergillus*, *Penicillium*, *Alternaria*, and *Cladosporium*.[66]

3.2 | Internal exposome

Animal and plant microbiota contain a fungal component, the mycobiota; conversely, fungi possess their own microbiota.[78] The diversity and make-up of fungal communities vary as a function of the considered anatomical site, of age, health status, lifestyle, and exposure.[33] Cross talk between fungal and bacterial components of the microbiota and between each of them and the host are essential for sustained commensalism.[33,78] The identification of fungal species associated with human mucosae and skin needs to be complemented by demonstrating their transient or resident status, the latter allowing recognition as genuine members of the mycobiota.[33] The most prevalent fungal genera in the healthy gut are the yeasts *Saccharomyces*, *Malassezia*, *Candida*, and *Cyberlindnera*.[33] In fact, the question of a gut mycobiota, defined as persistent commensal fungal species detected in stools but not in oral or food samples, is still open. Indeed, all gut fungal species were found to be transient in experiments performed with healthy Western adults, raising the hypothesis that, at least in this population, fungal colonization might be lacking.[79] Strikingly, frequent fungal taxa associated with oral, pulmonary, intestinal, or cutaneous locations, such as *Aspergillus*, *Cladosporium*, *Alternaria*, or *Penicillium*[33] overlap with environmental counterparts described in the previous section. On the contrary,

even if fungi do not colonize the healthy human host, their ubiquitous presence results in sustained contact and, therefore, the need for an adaptive immune response, often a Th17-oriented one.[80] A special case could be represented by breastmilk mycobiota, which comprises *Malassezia*, *Penicillium*, *Davidiella*, and *Sistotrema* genera, possibly explaining the abundance of yeasts from the *Malassezia* genus in the neonatal and young infant gut mycobiota.[33] It was suggested that the establishment of gut mycobiota could begin prior to birth, that fungal species in infant gut exhibit high variability during the first year of life, with *Saccharomyces* yeasts being the preponderant fungal component of gut mycobiota at the age of 1 year, and that altered abundance of certain gut fungi in infants was predictive of later development of allergic diseases.[51,81] Interestingly, in a multicentric birth cohort from Canada, decreased relative abundance of yeasts from the genera *Candida* and *Saccharomyces* but increased relative abundance of *Cladosporium* and *Aspergillus* was found in the gut of 1-year-old infants who later developed inhalant atopy.[51]

Among fungi associated with human skin, lipophilic yeasts of the *Malassezia* genus are the predominant constituent of skin mycobiota.[29] *Malassezia* species, mainly *M. sympodialis*, are probably the most important in terms of relationship to allergy and atopy especially in the development and progression of atopic dermatitis, explained by cross-reactivity between conserved eukaryotic proteins, such as thioredoxins (e.g., Mala s 13), manganese superoxide dismutase (Mala s 11), and cyclophilin (Mala s 6) being potential panallergens, found in fungi and humans.[24,33] Other constituents of the skin mycobiota include *Candida* spp and *Aspergillus* spp.[31,33]

3.3 | Tools for studying the fungal exposome

Collecting, storing, and conveying environmental or human samples for fungal assessment are critical steps. This preanalytical stage needs to be planned and performed according to the desired sample nature (bioaerosol, house dust, skin, and feces), to the environment (temperature, wind, relative humidity, and building material) or personal conditions (adult vs. child and professional vs. home exposure), and to the purpose of the study (e.g., epidemiological study vs. examination of a patient's case).[82–84] Sampling and analytical methods for external exposome assessment may vary in terms of sensitivity and specificity, depending on the environment, for example, indoor air sampling from hospital settings, homes, or working places.[1,85] So far, standardized collection methods are lacking, hindering comparison even for samples of the same nature.

Environmental samples may be analyzed by culture-dependent and culture-independent approaches. The former requires in vitro growth of fungal samples prior to identification, while the latter proceeds with spore and sub-spore fragments identification, either through microscopy analysis or through molecular methods. For most environmental fungal taxa, culture cannot be achieved.[11] On the contrary, for those growing in vitro, their growth rate will depend on the type of fungal culture and the nutrient media in use.[78] Microscopic examination allows for quantitative assessment of samples and low taxonomical detection of taxa which is a less precise approach. Immunological detection of molds using specific enzyme-linked immunoassay (ELISA) is also possible.[86] Alternatively, DNA-based approaches such as PCR targeting taxonomic marker sequences, or DNA metabarcoding, allow the identification of considerably higher taxonomic biodiversity within the collected samples.[11,87] However, this new technology also has some shortcomings, including primer bias which can heavily alter sequencing results,[88,89] or the fact that taxonomic marker sequences are not directly related to the identification of a fungal species, therefore introducing the need for operational taxonomic units.[11] The usage of DNA-based procedures for characterizing environmental fungi communities includes application of PCR amplification of ribosomal RNA genes and DNA fingerprinting methods such as denaturing gradient gel electrophoresis (DGGE), which uses a genetic fingerprinting method to examine microbial communities from environmental samples. These methods provide a broad quantification of fungi identified from the environment.[90,91] The metagenomic approach also allows the study of external fungal exposome.[5,11,33,83]

A third approach gaining momentum addresses fungal exposome using statistical modeling of airborne particles based on the study of air-mass movements categorized in spatio-temporal patterns of connectivity. This approach might alleviate the labor-intensive classical identification of airborne fungal spores, and eliminate the potential bias linked to the choice of the air sampling site.[92]

Studies on human mycobiota have taken advantage of the culturomics approach, which can be combined with molecular methods such as metagenomic deep sequencing, allowing the identification of more fungal taxa in patients and healthy controls.[5,33,93]

4 | OVERVIEW OF MAJOR FUNGAL-RELATED HYPERSENSITIVITY DISEASES

Fungus–human host interactions involve a combination of hypersensitivity, toxicity, and opportunistic infections[34,36,47,48,94–96] (Figure 2). Indoor and outdoor exposure to fungi is ubiquitous[31,66] and altered by climate change.[49,60,97] Referring to Gell and Coombs' classification, this section addresses type I (IgE), III (IgG) and IV (T-cell) fungal hypersensitivity,[4] and underlying immune mechanisms, for example, type 2 innate and adaptive immunity.[103,131]

4.1 | Upper airways: Allergic fungal rhinosinusitis (AFRS)

AFRS is a unique form of immune-mediated non-invasive fungal rhinosinusitis exhibiting a dysfunctional epithelium and prominent type 2 responses with eosinophilic inflammation and local production of IgE with broad cross-reactivity among fungal species.[37,98,99] Among AFRS patients, more than 50% displayed serum IgE directed to two or more fungal genera among *Aspergillus fumigatus*, *Alternaria alternata*, and *Candida albicans*.[99] Its prevalence is definitely higher in arid and tropical climates, such as in the Asia-Pacific region, Australia,

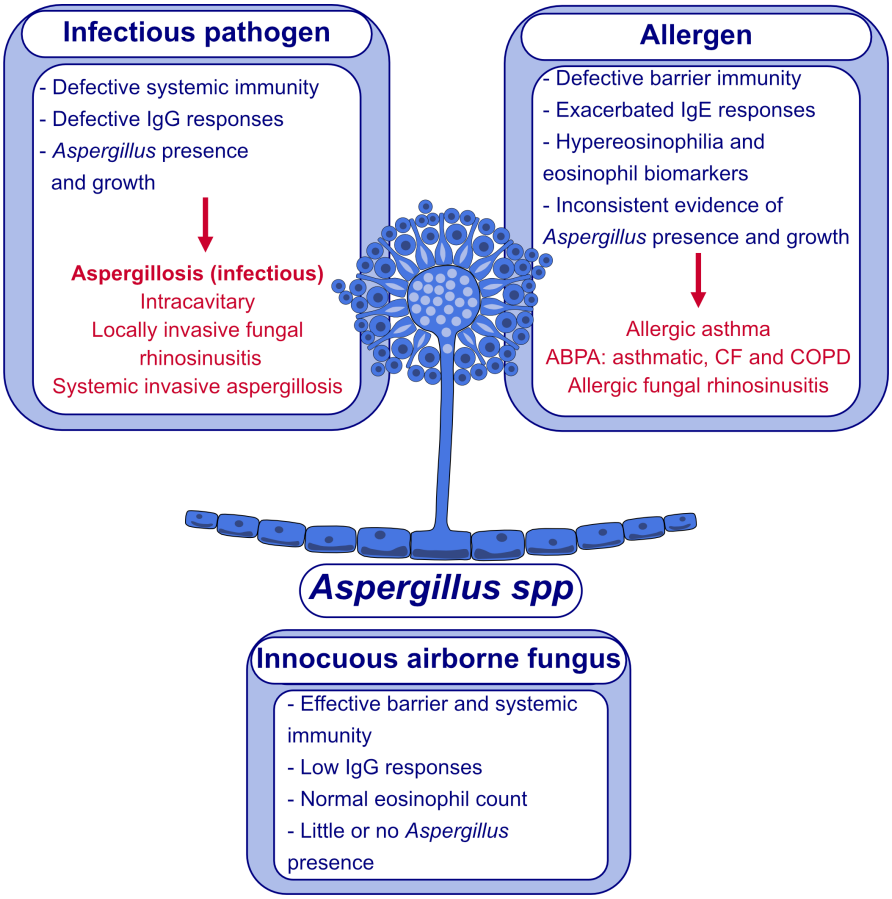


FIGURE 2 Normal versus pathological fungus–host interactions. Here, *Aspergillus fumigatus* is taken as an example of the balance between fungi and immune responses of the host, resulting in preserved health or development of allergic or infectious diseases. *Aspergillus fumigatus* is also one of the causal agents of hypersensitivity pneumonitis, an interstitial lung disease associated with antigen-specific IgG (not shown in the figure)

Thailand, Malaysia, India, the Middle East, Saudi Arabia, North Africa, and Southeastern and Southwestern parts of the United States, especially the Mississippi basin.[17,100,101] Climate influence is prominent, as demonstrated by a prevalence of 0.4% in Northern US states compared with over 10% in Southern ones. *Aspergillus fumigatus* is the most frequent fungus involved in AFRS.[17,100,101]

4.2 | Lower airways

4.2.1 | Asthma

Fungi possess a rich equipment of proteases, which may contribute to the disruption of bronchial epithelial tight junctions, an early step in the pathophysiology of asthma linked to epithelial dysfunction and increased risk of sensitization.[102] Subjects sensitized to thermotolerant fungi such as *Aspergillus* spp (filamentous) and *Candida* spp (yeast), which induce sensitization and can persist as colonizing, culturable organisms are at high risk of developing chronic severe lung disease, including life-threatening asthma, bronchiectasis, and lung fibrosis.[19] Conversely, mesophilic mold-related lung diseases typically manifest as respiratory exacerbations.[49,103] Examples are *Aspergillus fumigatus*-related fungal asthma, as opposed to *Alternaria alternata*-related asthma attacks in the aftermath of summer storms. With respect to *Aspergillus fumigatus*, it has been demonstrated that both specific IgE production (sensitization) and airway colonization (culturable mold present in bronchial samples) are associated with lung function deterioration.[19] In adults from the European Community Respiratory Health Survey (ECRHS), sensitization to *Alternaria* was associated with severe asthma[104] and a decrease in lung function, especially in women;[105] also, sensitization to molds (*Cladosporium* and *Alternaria*) was more prevalent in individuals living in damp dwellings and related to current asthma.[106] In pediatric asthma, mold sensitization related to impaired pulmonary function and increased airway hyperresponsiveness.[107] In adults with severe asthma, multiple fungal sensitizations are related to poorer asthma control.[108] Conversely, *Aspergillus fumigatus* specific IgG has not been associated with modified clinical outcomes in asthma, in the absence of ABPA or HSP.

In occupational settings, asthma has been associated with a variety of fungal exposures, for example, the yeast *Saccharomyces cerevisiae* and occupational baker's asthma,[34] or fungal enzymes employed in food processing.[6] Fungal dysbiosis may also influence the risk of asthma, as infants harboring an increased relative abundance of yeasts from the genera *Candida* and *Rhodotorula* in their gut were at higher risk of later development of asthma.[29]

4.2.2 | Allergic bronchopulmonary mycosis (ABPM)

ABPM is probably the most severe allergic fungal diseases.[94] It is typically induced by *Aspergillus fumigatus* (ABPA), often recognized

during an episode of respiratory exacerbation in asthma or cystic fibrosis patients and involving an exuberant anti-fungal type I and type III hypersensitivity response.[4,94,110] The allergic response comprises eosinophilic inflammation and high levels of total and *A. fumigatus*-specific IgE. The IgE response is a major diagnostic criterion for ABPA, often accompanied by high *A. fumigatus*-specific IgG responses as a minor diagnostic criterion, while the demonstration of *A. fumigatus* bronchial colonization is required in some diagnostic scores.[109–111]

ABPA natural history consists of sequential flare and remission episodes leading to irreversible lung damage with central bronchiectasis and fibrosis if not appropriately treated.

In addition to *Aspergillus* spp, other filamentous fungi, for example, *Penicillium* spp, *Schizophyllum commune*, but also yeasts such as *Candida* spp may be responsible for ABPM (4110).

4.2.3 | Hypersensitivity pneumonitis (HSP)

HSP is an interstitial lung disease developing in susceptible individuals as a result of an immune-mediated response to inhaled environmental antigens.[35,40] Fungal antigens are among the most frequent culprits, for example, *Aspergillus* spp, *Alternaria* spp, *Cladosporium* spp, *Penicillium* spp, *Fusarium* spp, *Trichosporon* spp, *Saccharomycetes* spp, and *Mucor* spp, *Rhizopus* spp (water damage, hot tub, farming, gardening, food industry employment, wind musical instruments etc).[35,40] Fungal-related HSP usually presents as chronic HSP due to prolonged exposure to fungal material, in an occupational setting, at home, or during leisure activities and manifests as lung functional deterioration.[40,42] From a mechanistic viewpoint, the pathogenesis of HSP does not involve type 2 responses, but an inflammatory reaction with granuloma formation, exaggerated cellular responses and the production of antigen-specific IgG which can be measured in the serum during diagnostic work-up.[35,40]

4.3 | Skin

4.3.1 | Atopic dermatitis

The most notable connection between atopic dermatitis and fungi are *Malassezia sympodialis* and *Malassezia furfur*, frequent skin colonizers in healthy and affected individuals.[4] *Malassezia* spp are lipophilic yeasts evolutionarily adapted to growth on the skin, able to induce skin inflammation and type 2 immune responses including autoreactive T cells in susceptible, atopic patients, also behaving as opportunist infectious agents in rare cases.[4,29,112] Patients with atopic dermatitis exhibit skin bacterial and fungal dysbiosis, including greater abundance and distinct strains of *Malassezia* as compared with healthy controls.[29,113] Moreover, increased skin abundance of *Malassezia* has been associated with increasing severity in atopic dermatitis.[29] *Malassezia furfur* may be an exacerbating factor in patients with head/neck atopic dermatitis, and elevated *Malassezia*-specific IgE levels have been reported in these patients.[114] Clinically, *Malassezia* allergy may be suspected in adolescents or young adults with severe atopic dermatitis lesions of the head and neck that are recalcitrant to conventional therapy. These patients usually benefit from antifungal therapy (including daily itraconazole or ketoconazole followed by long-term weekly treatment).

Some *Malassezia* allergens, for example, manganese superoxide dismutase Mala s 11 and thioredoxin Mala s 13, share structural epitopes and IgE reactivity with human proteins, suggesting that molecular mimicry may play a role in the pathophysiology of some autoimmune processes.[24,115]

4.3.2 | Cholinergic urticaria

In patients suffering from cholinergic urticaria, sensitization to *Malassezia globosa* was associated to IgE to sup_MGL1304, an allergenic protein cross-reactive between *Malassezia* and human sweat.[116] Cosensitization to *Malassezia globosa* and to other skin resident fungi such as *Malassezia furfur*, *Candida albicans*, and *Trichophyton mentagrophytes* was frequently observed.[116]

5 | OVERVIEW OF DIAGNOSTIC TOOLS FOR FUNGAL HYPERSENSITIVITY DISEASES

Diagnostic work-up of fungal hypersensitivity diseases is often complex, requiring a multistep investigation with a combination of clinical, imaging, and laboratory tools sometimes complemented by therapeutic response analysis.

5.1 | Clinical and imaging tools

Lung function test in ABPA often shows the features of uncontrolled asthma with airflow obstruction with or without airway bronchodilator reversibility. In HSP, a restrictive pattern is often found in combination with a decrease in the carbon monoxide transfer test[117] (Table 4).

Computed tomography of the chest is used to diagnose both ABPA and HSP. In ABPA, central bronchiectasis is a common finding of relatively late onset and high attenuation mucus is pathognomonic,[118] while in HSP, the most common finding is a patched ground glass pattern.[117] The limited studies on magnetic resonance imaging (MRI) did not provide evidence for any added diagnostic value of these investigations.[119] Imaging, in particular, tomographic assessment of thoracic fungal diseases has proven specificity, with patterns and particular signs well described and adopted in clinical practice.[155]

Exhaled nitric oxide, a marker of type 2 inflammation, appears to be elevated in cystic fibrosis patients suffering from ABPA compared with *Aspergillus*-sensitized cystic fibrosis patients. Therefore FeNO (fractional exhaled nitric oxide) might have a role as a diagnostic test in the context of cystic fibrosis.[120]

The study of exhaled air led to the emergence of "breathomics," which provides noninvasive analysis of exhaled air signatures.[121]

Using an electronic nose technology, chronic lung diseases comprising asthma and COPD were phenotyped as a function of their inflammatory profile.[121] Applications to exhaled fungal VOC have been proposed for fungal-related diseases, such as *Aspergillus* infections and volatile profiles for *Candida* species, but applications in the field of fungal hypersensitivity are lacking.[43]

5.2 | Laboratory tools

Evidence of IgE responses and eosinophil involvement support a type I hypersensitivity mechanism, while mycological evidence of fungal persistence is more challenging to obtain.[17,19,105,107] Particularly in the case of HSP and ABPA, the determination of specific antifungal IgG (*A. fumigatus*-specific IgG in the case of ABPA) is an additional useful diagnostic tool.[22]

5.2.1 | Mycology

The detection of fungi at various human body sites, their identification at the species level, the quantification of the fungal burden and the assessment of their in vitro susceptibility or resistance to anti-fungal drugs are collectively denoted as a mycological diagnosis in the clinical laboratory. The demonstration of fungi in clinical samples requires a variety of methods to address the diversity of this kingdom, for example, unicellular (yeasts) vs. multicellular, filamentous hyphae, their geographical diversity, and regional differences in medical and laboratory practice.[112,122]

Conventional mycological diagnosis relies on the macroscopic and microscopic assessment of fresh and culture samples.[122,123] Direct examination of fresh samples aims at recognizing characteristic features, such as fungal hyphae. Inoculation on fungal growth media, followed by 5–7 days of incubation yields colonies further identified at the species level using MALDI-TOF Mass Spectrometry and a specialized fungal reference spectra database.[124] Additional examination and analysis can be achieved by microscopic (direct, optical, or electronic) and molecular methods.

Soluble fungal antigens can be detected in fluid samples. The most widely used are (1-3)-β-D-glucan, considered a pan-fungal cell wall marker, and galactomannan, mainly released during *Aspergillus* spp hyphal growth.[125]

High levels of Asp f 1, a major and marker allergen of Aspergillus fumigatus, in resected nasal polyps from patients with chronic rhinosinusitis with nasal polyps, were associated with increased markers of type 2 immune responses and might contribute to the diagnosis of AFRS.[126]

5.2.2 | Cytology and pathology

From a pathophysiological viewpoint, direct evidence of an eosinophilic type 2 inflammation associated with and attributed to a fungus is compelling evidence for ongoing fungal allergic disease. Direct microscopic examination of naso-sinusal, bronchial, or sputum samples may be performed in search of eosinophilic inflammation with fungal non-invasive colonization of thick mucus plugs.[111] Eosinophils, eosinophilic inflammation markers such as Charcot-Leyden crystals, and fungal hyphae were recently proposed as pathognomonic for ABPA/ABPM.[110]

5.2.3 | Hematology

Systemic or local eosinophilia is a hallmark of fungal allergic diseases.[127] Eosinophils are readily counted and interpreted through a basic white blood cell count. The upper normal for the blood eosinophil count is set at 0.4×10^9/L to 0.5×10^9/L (500 elements/mm^3)[128,129] but often used at lower cutoffs, such as 0.3×10^9/L or 0.15×10^9/L for type 2/non-type 2 stratification and therapeutic management.[130,131] Eosinophilia is an inconsistent marker of a predominantly type 2 response, without specificity for ongoing fungal-related atopic disease. It is subject to variations related to comorbidities and ongoing treatments, especially corticosteroids.[128]

5.2.4 | Immunology

Immunoglobulin IgE or IgG responses and inflammatory mediators are markers of the fungal-host interaction.[132–137] Such biomarkers are valuable endpoints of individual susceptibility because fungal

TABLE 4 Lung function and fungal diseases

	Obstructive syndrome	Restrictive syndrome
Diagnosis	Allergic bronchopulmonary mycosis/aspergillosis	Paracoccidioidomycosis with ground-glass opacities
	Severe asthma with fungal sensitization	Fungal pneumonia with consolidation: paracoccidioidomycosis, histoplasmosis, accute coccidioidomycosis, aspergillosis, mucormycosis, candidiasis
	Occupational fungi-related asthma	Pleural effusion, for example, in mucormycosis
	Hypersensitivity pneumonitis (infrequent)	Hypersensitivity pneumonitis

Note: Fungal hypersensitivity and fungal infections may alter lung function parameters and associate with a restrictive or obstructive syndrome. References 17,19,35,110,130.

exposure is not a direct predictor of health effects at the individual level.[97] Eosinophil biomarkers such as eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN) may assist with the stratification and therapeutic management of eosinophilic fungal-related diseases.[138]

In the ECRHS cohort of general adult population, indoor mold exposure was associated with a higher risk of adult-onset asthma, oculo-respiratory and general symptoms, as well as increased asthma severity scores, predicted by higher levels of IgE and of the eosinophil biomarker ECP.[95–97,139] Preexisting sensitization to airborne molds was associated with a higher risk of mold-related disease.[95]

Determination of fungal-specific antibody responses: IgE, IgG

Fungal sensitization: anti-fungal specific IgE. Sensitization is defined as detectable specific IgE using either skin prick tests or laboratory methods. According to current literature, the diagnosis of ABPA is possible when the level of specific IgE anti-*A. fumigatus* is greater than 0.35 kUA/L and probable when the value is equal to or greater than 20 kUA/L.[109,132,133,140–142]

Quantitative laboratory IgE methods allow dynamic monitoring and cross-reactivity assessment.[94,133,134,136,143] Given the variations in fungal extract preparation and intermethod variability (Figure 3), dynamic monitoring must rely on the same method.

Identifying the primary fungal sensitizer is an essential step because of the extensive cross-reactivity among whole fungal extracts for skin and IgE tests. It is performed using molecular allergens for in vitro diagnostics, which is currently limited to a handful of molecules, mainly from the filamentous fungi *Aspergillus fumigatus*, *Aspergillus oryzae*, *Alternaria alternata*, *Cladosporium herbarum*, and the yeast *Malassezia sympodialis*.

A complementary approach for improving the specificity of fungal sensitization assessment relies on functional assays similar to an ex vivo provocation test, carried out using a sample of the patient's circulating basophils, the culprit allergens, and flow cytometry.[144,145] Good sensitivity and specificity were reported for select indications of fungal involvement, such as ABPA or cholinergic urticaria, in small population samples.[135,146,147]

Fungal serology: anti-fungal specific IgG. Since fungi are ubiquitous, detecting antifungal-specific IgG in the blood of individuals free of fungal disease is common. Fungi of the commensal microbiota, for example, from gut or skin, induce detectable systemic IgG, which contribute to protection against pathogenic fungi.[148] High concentrations of antifungal-specific IgG may support the diagnosis of ABPA/ABPM and HSP. In contrast to specific IgE, IgG concentrations in healthy individuals vary greatly depending on the antigen; therefore, reference values have to be established for each antigen.[149] The diagnostic performance varies as a function of culprit organism, reference population, exposure, and diagnostic methods.[35,94,150] The latter comprise multiple technical solutions with heterogeneous diagnostic performance, for example, in-house and automated ELISA and EIA, Western blot, lateral flow assays, immunoprecipitation.[94,150] In-house assays and "precipitin" detection are gradually discontinued in clinical laboratories and replaced with new methods.[151] As an example, a rapid lateral flow (immunochromatographic) test exhibited sensitivity of 79% for serological ABPA and 93% for ABPA with bronchiectasis and specificity of 81% or higher for the detection of anti-*Aspergillus* IgG.[150]

6 | UNMET NEEDS

The diversity and abundance of the fungal exposome stands in contrast with the unmet needs in knowledge and methods related to fungal effects on human health.

6.1 | Unmet scientific needs

1. Little is known about the prevalence, specificity, patterns, and temporal changes of sensitization to most airborne fungi

We suggest tackling the fungal sensitization landscape, defined as the detectable IgE sensitization, its molecular targets and the putative clusters of relevant allergen families among a panel of fungal allergens. This panel should aim to fully represent the fungal exposome and its longitudinal evolution. Harnessing expertise in fungal ecology, fungal culture requirements, fungal allergen biochemistry including further characterization of allergens, cohort studies, fungus-induced immune responses, large-scale analysis of allergen investigations, and big data analysis will be necessary.

2. Species of the airborne fungal exposome are differently distributed under different climate conditions

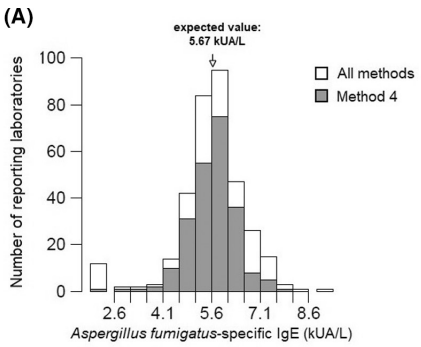
Probing the climate-related variations of the fungal sensitization landscape through replication in sister cohorts would contribute to the translation of cohort data to personalized prediction of lung function evolution.

3. Fungal sensitization affects lung function, but data are available only for a small number of fungal species and with heterogeneous methodology

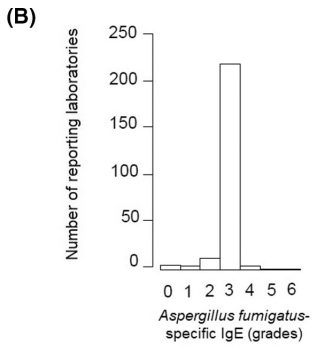
This scientific barrier relates to translating mycological and immunological data into clinically relevant profiles and endotypes. An effective approach could be taken by assessing the fungal sensitization landscape in existing cohorts, for example, general population, specific allergic populations, and severe asthma.

4. Therapeutic options for fungal hypersensitivity diseases are limited

Therapeutic management of fungal hypersensitivity diseases is outside the scope of the current work but deserves being mentioned



	n	Mean	SD	CV(%)
All methods	347	5.67	0.79	13.9
Method 1	37	6.77	0.86	12.8
Method 2	14	5.22	0.75	14.3
Method 3	43	5.62	0.51	9.1
Method 4	226	5.64	0.64	11.4
Other methods	7	1.82	2.19	120.8



	n	0	1	2	3	4	5	6
All methods	214	4	3	9	193	3	1	1
Method 1	25	0	0	2	19	3	0	1
Method 2	12	0	0	0	12	0	0	0
Method 3	19	0	0	0	19	0	0	0
Method 4	146	1	0	3	141	0	1	0
Other methods	12	3	3	4	2	0	0	0

FIGURE 3 Determination of *Aspergillus fumigatus*-specific IgE in serum. (A) quantitative assays; (B) semi-quantitative assays. An example of external quality assessment for *Aspergillus fumigatus*-specific IgE determination is shown, performed with academic and privately-held clinical laboratories in the European Union and the United Kingdom reporting (A) quantitative or (B) semi-quantitative results for the same sample shipped the same day and assessed during a defined, limited time frame. The expected result is determined as (A) the mean expressed in kilounits of allergen-specific IgE per liter (kUA/L) of all quantitative results or (B) the grade reported by most participants on a discrete scale from 0 (undetectable) to 6 (very high). Intra- and intermethod variability are monitored as the dispersion of reports vs. the expected result. While intermethod variability is inherent to the lack of intermethod standardization, intramethod variability is an indicator of poor performance and hence reliability of a given method. CV, coefficient of variation, n, number of participating laboratories, SD, standard deviation.

as the end point of a medically oriented approach. Besides avoiding exposure to culprit fungi, corticosteroids, allergen immunotherapy, biologicals, and antifungals may be used, but sound validation and guidelines are often lacking.

6.2 | Unmet technical needs

1. Large-scale fungal identification, culture, and production

A major technical barrier hampering the study of fungal sensitization is the lack of stable, well-defined fungal material in sufficient amounts. A further issue is the high diversity of fungal spore morphology and subsequent difficulties in identification, which is more complex when compared with pollen. Involving highly specialized laboratories and networks with expertise in the discovery, identification, and optimal culture of environmental fungal genera and fungal allergy investigation is needed.

2. High-throughput, standardized, sensitive, and specific methods for investigating fungal sensitization

An obvious need is the standardization of the existing diagnostic tools. A miniaturized allergen multiplex assay would optimally address the highly diverse fungal exposome and allow comparison between research, translational, and clinical levels.

3. Investigating the relation between clinical and exposure data through an exposome approach requires high-power statistical analysis and multiple comparisons

Special computational and statistical software and skills are needed, such as environment-wise association studies, trajectory analyses and artificial intelligence.

7 | CONCLUSION AND PERSPECTIVES

The vast and largely uncharted field of the fungal exposome calls for a multidisciplinary approach including environmental science, allergology, immunology, mycology, pulmonary medicine, epidemiology, and biostatistics. The unmet needs in the domain of fungal exposome health effects and personalized medicine should be addressed with three concurrent front lines:

- Thorough clinical and exposure characterization aiming at the identification of further pathophysiologically relevant species and molecules and a better understanding of their interaction with the host's immune responses.
- Innovative biomarker assays allowing the personalized profiling of immune responses to fungal species and molecules.
- Advanced statistical analyses and epidemiological interpretation able to predict the health effects of ongoing fungal exposure and climate-related changes in the fungal exposome.

The results of this research would improve our understanding of the health effect of the fungal exposome, paving the way for improved diagnostic and therapeutic management of hypersensitivity to fungi, comprising allergic and nonallergic conditions, in the context of the current climate change and global need for sustainable housing.

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CONFLICT OF INTEREST

JV reports speaker and consultancy fees in the past 5 years from Meda Pharma (Mylan), Novartis, Sanofi, Thermo Fisher Scientific, Astra Zeneca, outside the submitted work. MR reports speaker fees in the past 5 years from Leti and Thermo Fisher Scientific, outside the submitted work. The other authors declare no competing interests in relation to this study.

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REFERENCES

1. Raulf M, Buters J, Chapman M, et al. Monitoring of occupational and environmental aeroallergens – EAACI position paper. *Allergy*. 2014;69:1280-1299.
2. Prescott SL, Logan AC, Bristow J, et al. Exiting the Anthropocene: achieving personal and planetary health in the 21st century. *Allergy*. 2022. doi:10.1111/all.15419
3. Cecchi L, D'Amato G, Annesi-Maesano I. External exposome and allergic respiratory and skin diseases. *J Allergy Clin Immunol*. 2018;141:846-857.
4. Crameri R, Garbani M, Rhyner C, Huitema C. Fungi: the neglected allergenic sources. *Allergy*. 2014;69:176-185.
5. Radzikowska U, Baerenfaller K, Cornejo-Garcia JA, et al. Omics technologies in allergy and asthma research: An EAACI position paper. *Allergy*. 2022. doi:10.1111/all.15412
6. Jeebhay MF, Moscato G, Bang BE, et al. Food processing and occupational respiratory allergy – an EAACI position paper. *Allergy*. 2019;74:1852-1871.
7. Papadopoulos NG, Agache I, Bavbek S, et al. Research needs in allergy: an EAACI position paper, in collaboration with EFA. *Clin Transl Allergy*. 2012;2:21.
8. Cole GT. Basic biology of fungi. In: Baron S, ed. *Medical Microbiology*. University of Texas Medical Branch at Galveston; 1996. Accessed August 24, 2021. http://www.ncbi.nlm.nih.gov/books/NBK8099/
9. Lücking R, Aime MC, Robbertse B, et al. Fungal taxonomy and sequence-based nomenclature. *Nat Microbiol*. 2021;6:540-548.
10. James TY, Stajich JE, Hittinger CT, Rokas A. Toward a fully resolved fungal tree of life. *Annu Rev Microbiol*. 2020;74:291-313.
11. Peay KG, Kennedy PG, Talbot JM. Dimensions of biodiversity in the earth mycobiome. *Nat Rev Microbiol*. 2016;14:434-447.
12. Hibbett DS, Taylor JW. Fungal systematics: is a new age of enlightenment at hand? *Nat Rev Microbiol*. 2013;11:129-133.
13. Stengel A, Stanke KM, Quattrone AC, Herr JR. Improving taxonomic delimitation of fungal species in the age of genomics and Phenomics. *Front Microbiol*. 2022;13:847067. Accessed July 24, 2022. https://www.frontiersin.org/articles/10.3389/fmicb.2022.847067
14. Taylor JW. One fungus = one name: DNA and fungal nomenclature twenty years after PCR. *IMA Fungus*. 2011;2:113-120.
15. Cantrell SA, Dianese JC, Fell J, Gunde-Cimerman N, Zalar P. Unusual fungal niches. *Mycologia*. 2011;103:1161-1174.
16. Hawksworth DL, Lücking R. Fungal diversity revisited: 2.2 to 3.8 million species. *Microbiol Spectr*. 2017;5:1-17. doi:10.1128/microbiolspec.FUNK-0052-2016
17. Denning DW, Chakrabarti A. Pulmonary and sinus fungal diseases in non-immunocompromised patients. *Lancet Infect Dis*. 2017;17:e357-e366.
18. Lionakis MS, Iliev ID, Hohl TM. Immunity against fungi. *JCI Insight*. 2017;2:93156.
19. Welsh KG, Holden KA, Wardlaw AJ, et al. Fungal sensitization and positive fungal culture from sputum in children with asthma are associated with reduced lung function and acute asthma attacks respectively. *Clin Exp Allergy*. 2021;51:790-800.
20. Barnes C. Fungi and atopy. *Clin Rev Allergy Immunol*. 2019;57:439-448.
21. Soeria-Atmadja D, Onell A, Borgå A. IgE sensitization to fungi mirrors fungal phylogenetic systematics. *J Allergy Clin Immunol*. 2010;125:1379-1386.e1.
22. Quirce S, Vandenplas O, Campo P, et al. Occupational hypersensitivity pneumonitis: an EAACI position paper. *Allergy*. 2016;71:765-779.
23. Naranjo-Ortiz MA, Gabaldón T. Fungal evolution: diversity, taxonomy and phylogeny of the fungi. *Biol Rev Camb Philos Soc*. 2019;94:2101-2137.
24. Roesner LM, Ernst M, Chen W, et al. Human thioredoxin, a damage-associated molecular pattern and Malassezia-crossreactive autoallergen, modulates immune responses via the C-type lectin receptors Dectin-1 and Dectin-2. *Sci Rep*. 2019;9:11210.
25. Home – Taxonomy – NCBI. Accessed September 20, 2021. https://www.ncbi.nlm.nih.gov/taxonomy
26. Palková Z, Váchová L. Yeast cell differentiation: lessons from pathogenic and non-pathogenic yeasts. *Semin Cell Dev Biol*. 2016;57:110-119.
27. Van Dyke MCC, Teixeira MM, Barker BM. Fantastic yeasts and where to find them: the hidden diversity of dimorphic fungal pathogens. *Curr Opin Microbiol*. 2019;52:55-63.

28. Sil A, Andrianopoulos A. Thermally dimorphic human fungal pathogens—polyphyletic pathogens with a convergent pathogenicity trait. *Cold Spring Harb Perspect Med*. 2014;5:a019794.
29. Lunjani N, Satitsuksanoa P, Lukasik Z, Sokolowska M, Eiwegger T, O'Mahony L. Recent developments and highlights in mechanisms of allergic diseases: microbiome. *Allergy*. 2018;73:2314-2327.
30. Green BJ. Emerging insights into the occupational mycobiome. *Curr Allergy Asthma Rep*. 2018;18:62.
31. Brandão J, Gangneux JP, Arikan-Akdagli S, et al. Mycosands: fungal diversity and abundance in beach sand and recreational waters - relevance to human health. *Sci Total Environ*. 2021;781:146598.
32. Muñoz JF, McEwen JG, Clay OK, Cuomo CA. Genome analysis reveals evolutionary mechanisms of adaptation in systemic dimorphic fungi. *Sci Rep*. 2018;8:4473.
33. Santus W, Devlin JR, Behnsen J. Crossing kingdoms: how the Mycobiota and fungal-bacterial interactions impact host health and disease. *Infect Immun*. 2021;89:e00648-20.
34. Levetin E, Horner WE, Scott JA. Environmental allergens workgroup. Taxonomy of allergenic fungi. *J Allergy Clin Immunol Pract*. 2016;4:375-385.e1.
35. Barnes H, Troy L, Lee CT, Sperling A, Strek M, Glaspole I. Hypersensitivity pneumonitis: current concepts in pathogenesis, diagnosis, and treatment. *Allergy*. 2022;77:442-453. doi:10.1111/all.15017
36. Caillaud D, Leynaert B, Keirsbulck M, Nadif R, Mould ANSES Working Group. Indoor mould exposure, asthma and rhinitis: findings from systematic reviews and recent longitudinal studies. *Eur Respir Rev*. 2018;27:170137.
37. Kato A, Peters AT, Stevens WW, Schleimer RP, Tan BK, Kern RC. Endotypes of chronic rhinosinusitis: relationships to disease phenotypes, pathogenesis, clinical findings, and treatment approaches. *Allergy*. 2022;77:812-826.
38. Kwizera R, Bongomin F, Olum R, et al. Prevalence of aspergillus fumigatus skin positivity in adults without an apparent/known atopic disease in Uganda. *Ther Adv Infect Dis*. 2021;8:20499361211039040.
39. Baxi SN, Portnoy JM, Larenas-Linnemann D, Phipatanakul W. Environmental allergens workgroup. Exposure and health effects of fungi on humans. *J Allergy Clin Immunol Pract*. 2016;4:396-404.
40. Costabel U, Miyazaki Y, Pardo A, et al. Hypersensitivity pneumonitis. *Nat Rev Dis Primer*. 2020;6:65.
41. Morath SU, Hung R, Bennett JW. Fungal volatile organic compounds: a review with emphasis on their biotechnological potential. *Fungal Biol Rev*. 2012;26:73-83.
42. Borchers AT, Chang C, Eric GM. Mold and human health: a reality check. *Clin Rev Allergy Immunol*. 2017;52:305-322.
43. Inamdar AA, Morath S, Bennett JW. Fungal volatile organic compounds: more than just a funky smell? *Annu Rev Microbiol*. 2020;74:101-116.
44. Fromme H, Gareis M, Völkel W, Gottschalk C. Overall internal exposure to mycotoxins and their occurrence in occupational and residential settings – An overview. *Int J Hyg Environ Health*. 2016;219:143-165.
45. Wålinder R, Ernstgård L, Norbäck D, Wieslander G, Johanson G. Acute effects of 1-octen-3-ol, a microbial volatile organic compound (MVOC)—An experimental study. *Toxicol Lett*. 2008;181:141-147.
46. Barberán A, Ladau J, Leff JW, et al. Continental-scale distributions of dust-associated bacteria and fungi. *Proc Natl Acad Sci USA*. 2015;112:5756-5761.
47. Jaakkola MS, Quansah R, Hugg TT, Heikkinen SAM, Jaakkola JJK. Association of indoor dampness and molds with rhinitis risk: a systematic review and meta-analysis. *J Allergy Clin Immunol*. 2013;132:1099-1110.e18.
48. Knutsen AP, Bush RK, Demain JG, et al. Fungi and allergic lower respiratory tract diseases. *J Allergy Clin Immunol*. 2012;129:280-291. quiz 292–293.
49. D'Amato G, Chong-Neto HJ, Monge Ortega OP, et al. The effects of climate change on respiratory allergy and asthma induced by pollen and mold allergens. *Allergy*. 2020;75:2219-2228.
50. Ward TL, Knights D, Gale CA. Infant fungal communities: current knowledge and research opportunities. *BMC Med*. 2017;15:30.
51. Boutin RCT, Sbihi H, McLaughlin RJ, et al. Composition and associations of the infant gut fungal microbiota with environmental factors and childhood allergic outcomes. *mBio*. 2021;12:e0339620.
52. Cortesão M, de Haas A, Unterbusch R, et al. Aspergillus Niger spores are highly resistant to space radiation. *Front Microbiol*. 2020;11:560.
53. Bowyer P, Fraczek M, Denning DW. Comparative genomics of fungal allergens and epitopes shows widespread distribution of closely related allergen and epitope orthologues. *BMC Genomics*. 2006;7:251.
54. Tham R, Vicendese D, Dharmage SC, et al. Associations between outdoor fungal spores and childhood and adolescent asthma hospitalizations. *J Allergy Clin Immunol*. 2017;139:1140-1147.e4.
55. Xie W, Li Y, Bai W, et al. The source and transport of bioaerosols in the air: a review. *Front Environ Sci Eng*. 2021;15:44.
56. Lymperopoulou DS, Adams RI, Lindow SE. Contribution of vegetation to the microbial composition of nearby outdoor air. *Appl Environ Microbiol*. 2016;82:3822-3833.
57. Kakde U. *Fungal Bioaerosols: Global Diversity, Distribution and Its Impact on Human Beings and Agricultural Crops*. Bionana Frontier; 2012:323-329.
58. Lagomarsino Oneto D, Golan J, Mazzino A, Pringle A, Seminara A. Timing of fungal spore release dictates survival during atmospheric transport. *Proc Natl Acad Sci USA*. 2020;117:5134-5143.
59. Ščevková J, Hrabovský M, Kováč J, Rosa S. Intradiurnal variation of predominant airborne fungal spore biopollutants in the central European urban environment. *Environ Sci Pollut Res Int*. 2019;26:34603-34612.
60. Damialis A, Vokou D, Gioulekas D, Halley JM. Long-term trends in airborne fungal-spore concentrations: a comparison with pollen. *Fungal Ecol*. 2015;13:150-156.
61. Núñez A, García AM, Moreno DA, Guantes R. Seasonal changes dominate long-term variability of the urban air microbiome across space and time. *Environ Int*. 2021;150:106423.
62. Hasnain SM, Akhter T, Waqar MA. Airborne and allergenic fungal spores of the Karachi environment and their correlation with meteorological factors. *J Environ Monit JEM*. 2012;14:1006-1013.
63. Al Salameen F, Habibi N, Uddin S, et al. Spatio-temporal variations in bacterial and fungal community associated with dust aerosol in Kuwait. *PloS ONE*. 2020;15:e0241283.
64. Almaguer-Chávez M, Aira MJ, Rojas T-I, Fernández-González M, Rodríguez-Rajo F-J. New findings of airborne fungal spores in the atmosphere of Havana, Cuba, using aerobiological non-viable methodology. *Ann Agric Environ Med AAEM*. 2018;25:349-359.
65. Odebode A, Adekunle A, Stajich J, Adeonipekun P. Airborne fungi spores distribution in various locations in Lagos, Nigeria. *Environ Monit Assess*. 2020;192:87.
66. Michel M, Sereme Y, Mezouar S, Vitte J. Indoor environmental allergens. *Encyclopedia of Respiratory Diseases*. Elsevier; 2019. doi:10.1016/B978-0-12-801238-3.11492-8
67. Heseltine E, Rosen J, World Health Organization. *WHO Guidelines for Indoor Air Quality: Dampness and Mould*. WHO; 2009.
68. Vesper S, Wymer L, Cox D, et al. The environmental relative moldiness index reveals changes in mold contamination in United States homes over time. *J Occup Environ Hyg*. 2021;18:35-41.
69. Romani M, Warscheid T, Nicole L, et al. Current and future chemical treatments to fight biodeterioration of outdoor building materials and associated biofilms: moving away from ecotoxic and towards efficient, sustainable solutions. *Sci Total Environ*. 2022;802:149846.

70. Pinheiro AC, Sequeira SO, Macedo MF. Fungi in archives, libraries, and museums: a review on paper conservation and human health. *Crit Rev Microbiol*. 2019;45:686-700.
71. Gomoiu I, Chatzitheodoridis E, Vadrucci S, Walther I, Cojoc R. Fungal spores viability on the international Space Station. *Orig Life Evol Biosphere J Int Soc Study Orig Life*. 2016;46:403-418.
72. Behbod B, Sordillo JE, Hoffman EB, et al. Wheeze in infancy: protection associated with yeasts in house dust contrasts with increased risk associated with yeasts in indoor air and other fungal taxa. *Allergy*. 2013;68:1410-1418.
73. Vesper S, McKinstry C, Haugland R, et al. Development of an environmental relative moldiness index for US homes. *J Occup Environ Med*. 2007;49:829-833.
74. Täubel M, Karvonen AM, Reponen T, Hyvärinen A, Vesper S, Pekkanen J. Application of the environmental relative moldiness index in Finland. *Appl Environ Microbiol*. 2016;82:578-584.
75. Banjara N, Suhr MJ, Hallen-Adams HE. Diversity of yeast and Mold species from a variety of cheese types. *Curr Microbiol*. 2015;70:792-800.
76. Kotzias D. Indoor air and human exposure assessment – needs and approaches. *Exp Toxicol Pathol*. 2005;57(Suppl 1):5-7.
77. Yassin MF, Almouqatea S. Assessment of airborne bacteria and fungi in an indoor and outdoor environment. *Int J Environ Sci Technol*. 2010;7:535-544.
78. Bonfante P, Venice F, Lanfranco L. The mycobiota: fungi take their place between plants and bacteria. *Curr Opin Microbiol*. 2019;49:18-25.
79. Auchtung TA, Fofanova TY, Stewart CJ, et al. Investigating colonization of the healthy adult gastrointestinal tract by fungi. *mSphere*. 2018;3:e00092-18.
80. Kirchner FR, LeibundGut-Landmann S. Tissue-resident memory Th17 cells maintain stable fungal commensalism in the oral mucosa. *Mucosal Immunol*. 2021;14:455-467.
81. Sbihi H, Boutin RC, Cutler C, Suen M, Finlay BB, Turvey SE. Thinking bigger: how early-life environmental exposures shape the gut microbiome and influence the development of asthma and allergic disease. *Allergy*. 2019;74:2103-2115.
82. Barnes CS, Horner WE, Kennedy K, Grimes C, Miller JD. Environmental allergens workgroup. Home assessment and remediation. *J Allergy Clin Immunol Pract*. 2016;4:423-431.e15.
83. Quince C, Walker AW, Simpson JT, Loman NJ, Segata N. Shotgun metagenomics, from sampling to analysis. *Nat Biotechnol*. 2017;35:833-844.
84. Kabir E, Azzouz A, Raza N, et al. Recent advances in monitoring, sampling, and sensing techniques for bioaerosols in the atmosphere. *ACS Sens*. 2020;5:1254-1267.
85. Méheust D, Le Cann P, Reboux G, Millon L, Gangneux J-P. Indoor fungal contamination: health risks and measurement methods in hospitals, homes and workplaces. *Crit Rev Microbiol*. 2014;40:248-260.
86. Zahradnik E, Kespohl S, Sander I, et al. A new immunoassay to quantify fungal antigens from the indoor mould aspergillus versicolor. *Environ Sci Process Impacts*. 2013;15:1162-1171.
87. de Groot GA, Geisen S, Wubs ERJ, et al. The aerobiome uncovered: multi-marker metabarcoding reveals potential drivers of turn-over in the full microbial community in the air. *Environ Int*. 2021;154:106551.
88. Elbrecht V, Leese F. Can DNA-based ecosystem assessments quantify species abundance? Testing primer bias and biomass—sequence relationships with an innovative metabarcoding protocol. *PLoS ONE*. 2015;10:e0130324.
89. Elbrecht V, Vamos EE, Meissner K, Aroviita J, Leese F. Assessing strengths and weaknesses of DNA metabarcoding-based macroinvertebrate identification for routine stream monitoring. *Methods Ecol Evol*. 2017;8:1265-1275.
90. Borneman J, Hartin RJ. PCR primers that amplify fungal rRNA genes from environmental samples. *Appl Environ Microbiol*. 2000;66:4356-4360.
91. Landeweert R, Leeflang P, Kuyper TW, et al. Molecular identification of ectomycorrhizal mycelium in soil horizons. *Appl Environ Microbiol*. 2003;69:327-333.
92. Choufany M, Martinetti D, Soubeyrand S, Morris CE. Inferring long-distance connectivity shaped by air-mass movement for improved experimental design in aerobiology. *Sci Rep*. 2021;11:11093.
93. Hamad I, Ranque S, Azhar EI, et al. Culturomics and amplicon-based metagenomic approaches for the study of fungal population in human gut microbiota. *Sci Rep*. 2017;7:16788.
94. Carsin A, Romain T, Ranque S, et al. Aspergillus fumigatus in cystic fibrosis: An update on immune interactions and molecular diagnostics in allergic bronchopulmonary aspergillosis. *Allergy*. 2017;72:1632-1642.
95. Norbäck D, Zock J-P, Plana E, et al. Mould and dampness in dwelling places, and onset of asthma: the population-based cohort ECRHS. *Occup Environ Med*. 2013;70:325-331.
96. Zhang X, Sahlberg B, Wieslander G, Janson C, Gislason T, Norback D. Dampness and moulds in workplace buildings: associations with incidence and remission of sick building syndrome (SBS) and biomarkers of inflammation in a 10 year follow-up study. *Sci Total Environ*. 2012;430:75-81.
97. Valkonen M, Täubel M, Pekkanen J, et al. Microbial characteristics in homes of asthmatic and non-asthmatic adults in the ECRHS cohort. *Indoor Air*. 2018;28:16-27.
98. Dykewicz MS, Rodrigues JM, Slavin RG. Allergic fungal rhinosinusitis. *J Allergy Clin Immunol*. 2018;142:341-351.
99. Haruna S, Takeda K, El-Hussien MA, et al. Local production of broadly cross-reactive IgE against multiple fungal cell wall polysaccharides in patients with allergic fungal rhinosinusitis. *Allergy*. 2022;77:3147-3151. doi:10.1111/all.15413
100. Panjabi C, Shah A. Allergic aspergillus sinusitis and its association with allergic bronchopulmonary aspergillosis. *Asia Pac Allergy*. 2011;1:130-137.
101. Chakrabarti A, Kaur H. Allergic aspergillus rhinosinusitis. *J Fungi Basel Switz*. 2016;2:E32.
102. Heijink IH, Kuchibhotla VNS, Roffel MP, et al. Epithelial cell dysfunction, a major driver of asthma development. *Allergy*. 2020;75:1902-1917.
103. Caillaud D, Keirsbulck M, Leger C, Leynaert B, of the Outdoor Mould ANSES Working Group. Outdoor Mold and respiratory health: state of science of epidemiological studies. *J Allergy Clin Immunol Pract*. 2021;10(3):768-784.e3.
104. Neukirch C, Henry C, Leynaert B, Liard R, Bousquet J, Neukirch F. Is sensitization to Alternaria alternata a risk factor for severe asthma? A population-based study. *J Allergy Clin Immunol*. 1999;103:709-711.
105. Sunyer J, Soriano J, Antó JM, et al. Sensitization to individual allergens as risk factors for lower FEV1 in young adults. European Community respiratory health survey. *Int J Epidemiol*. 2000;29:125-130.
106. Norbäck D, Björnsson E, Janson C, Palmgren U, Boman G. Current asthma and biochemical signs of inflammation in relation to building dampness in dwellings. *Int J Tuberc Lung Dis*. 1999;3:368-376.
107. Byeon JH, Ri S, Amarsaikhan O, et al. Association between sensitization to Mold and impaired pulmonary function in children with asthma. *Allergy Asthma Immunol Res*. 2017;9:509-516.
108. Masaki K, Fukunaga K, Matsusaka M, et al. Characteristics of severe asthma with fungal sensitization. *Ann Allergy Asthma Immunol*. 2017;119:253-257.
109. Agarwal R, Chakrabarti A, Shah A, et al. Allergic bronchopulmonary aspergillosis: review of literature and proposal

of new diagnostic and classification criteria. *Clin Exp Allergy*. 2013;43:850-873.
110. Asano K, Hebisawa A, Ishiguro T, et al. New clinical diagnostic criteria for allergic bronchopulmonary aspergillosis/mycosis and its validation. *J Allergy Clin Immunol*. 2021;147:1261-1268.e5.
111. Moss RB. Diagnosing allergic bronchopulmonary aspergillosis/mycosis: return to lost horizons. *J Allergy Clin Immunol*. 2021;147:1212-1214.
112. Chen SC-A, Perfect J, Colombo AL, et al. Global guideline for the diagnosis and management of rare yeast infections: an initiative of the ECMM in cooperation with ISHAM and ASM. *Lancet Infect Dis*. 2021;21:e375-e386.
113. Stefanovic N, Flohr C, Irvine AD. The exposome in atopic dermatitis. *Allergy*. 2020;75:63-74.
114. Darabi K, Hostetler SG, Bechtel MA, Zirwas M. The role of Malassezia in atopic dermatitis affecting the head and neck of adults. *J Am Acad Dermatol*. 2009;60:125-136.
115. Palomares O, Elewaut D, Irving PM, Jaumont X, Tassinari P. Regulatory T cells and immunoglobulin E: a new therapeutic link for autoimmunity? *Allergy* Published Online First: 19 July. 2022. doi:10.1111/all.15449
116. Altrichter S, Schumacher P, Alraboni O, et al. Sensitization against skin resident fungi is associated with atopy in cholinergic urticaria patients. *Clin Transl Allergy*. 2020;10:18.
117. Ojanguren I, Ferraro V, Morisset J, Muñoz X, Fink J, Cruz MJ. Assessment and management of occupational hypersensitivity pneumonitis. *J Allergy Clin Immunol Pract*. 2020;8:3295-3309.
118. Kanj A, Abdallah N, Soubani AO. The spectrum of pulmonary aspergillosis. *Respir Med*. 2018;141:121-131.
119. Sodhi KS, Gupta P, Shrivastav A, et al. Evaluation of 3 T lung magnetic resonance imaging in children with allergic bronchopulmonary aspergillosis: pilot study. *Eur J Radiol*. 2019;111:88-92.
120. Keown K, Abbott S, Kuzeljevic B, Rayment JH, Chilvers MA, Yang CL. An investigation into biomarkers for the diagnosis of ABPA and aspergillus disease in cystic fibrosis. *Pediatr Pulmonol*. 2019;54:1787-1793.
121. de Vries R, Dagelet YWF, Spoor P, et al. Clinical and inflammatory phenotyping by breathomics in chronic airway diseases irrespective of the diagnostic label. *Eur Respir J*. 2018;51:1701817. doi:10.1183/13993003.01817-2017
122. Wickes BL, Wiederhold NP. Molecular diagnostics in medical mycology. *Nat Commun*. 2018;9:5135.
123. Fréalle E, Valot S, Piarroux R, et al. Update on the diagnosis of parasitic and fungal infections. *Ann Biol Clin (Paris)*. 2020;78:299-313.
124. Cassagne C, Normand A-C, L'Ollivier C, Ranque S, Piarroux R. Performance of MALDI-TOF MS platforms for fungal identification. *Mycoses*. 2016;59:678-690.
125. Patterson TF, Donnelly JP. New concepts in diagnostics for invasive mycoses: non-culture-based methodologies. *J Fungi Basel Switz*. 2019;5:E9.
126. Miyabe Y, Tomizawa H, Saito H, et al. Quantification of Aspergillus fumigatus antigen Asp f 1 in airway tissue and allergic inflammation. *Allergy*. 2022;77(10):3154-3156. doi:10.1111/all.15428
127. Figueiredo RT, Neves JS. Eosinophils in fungal diseases: An overview. *J Leukoc Biol*. 2018;104:49-60.
128. Wardlaw AJ, Wharin S, Aung H, Shaffu S, Siddiqui S. The causes of a peripheral blood eosinophilia in a secondary care setting. *Clin Exp Allergy*. 2021;51:902-914.
129. Klion AD, Ackerman SJ, Bochner BS. Contributions of eosinophils to human health and disease. *Annu Rev Pathol*. 2020;15:179-209.
130. Akdis CA, Arkwright PD, Brüggen M-C, et al. Type 2 immunity in the skin and lungs. *Allergy*. 2020;75:1582-1605.
131. McDowell PJ, Heaney LG. Different endotypes and phenotypes drive the heterogeneity in severe asthma. *Allergy*. 2020;75:302-310.
132. Tanimoto H, Fukutomi Y, Yasueda H, et al. Molecular-based allergy diagnosis of allergic bronchopulmonary aspergillosis in aspergillus fumigatus-sensitized Japanese patients. *Clin Exp Allergy*. 2015;45:1790-1800.
133. Vitte J, Romain T, Carsin A, et al. Aspergillus fumigatus components distinguish IgE but not IgG4 profiles between fungal sensitization and allergic broncho-pulmonary aspergillosis. *Allergy*. 2016;71:1640-1643.
134. Piarroux R, Dubus J-C, Reynaud-Gaubert M, Gouitaa M, Ranque S, Vitte J. A new IgE Western blot identifies *Aspergillus fumigatus* sensitization and may discriminate allergic bronchopulmonary aspergillosis. *Allergy*. 2019;74:1808-1810. Published Online First: 20 April. 2019. doi:10.1111/all.13830
135. Michel M, Gomez C, Sereme Y, et al. Evaluation of cellular responses for the diagnosis of allergic bronchopulmonary mycosis: a preliminary study in cystic fibrosis patients. *Front Immunol*. 2020;10:3149.
136. Matricardi PM, Kleine-Tebbe J, Hoffmann HJ, et al. EAACI molecular allergology User's guide. *Pediatr Allergy Immunol*. 2016;27(Suppl 23):1-250.
137. Muthu V, Singh P, Choudhary H, et al. Diagnostic cutoffs and clinical utility of recombinant *Aspergillus fumigatus* antigens in the diagnosis of allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol Pract* Published Online First: 11 September. 2019. doi:10.1016/j.jaip.2019.08.041
138. An J, Lee J-H, Sim JH, et al. Serum eosinophil-derived neurotoxin better reflect asthma control status than blood eosinophil counts. *J Allergy Clin Immunol Pract* Published Online First: 15 April. 2020. doi:10.1016/j.jaip.2020.03.035
139. Sahlberg B, Norbäck D, Wieslander G, Gislason T, Janson C. Onset of mucosal, dermal, and general symptoms in relation to biomarkers and exposures in the dwelling: a cohort study from 1992 to 2002. *Indoor Air*. 2012;22:331-338.
140. Agarwal R, Aggarwal AN, Garg M, Saikia B, Chakrabarti A. Cut-off values of serum IgE (total and *A. fumigatus* -specific) and eosinophil count in differentiating allergic bronchopulmonary aspergillosis from asthma. *Mycoses*. 2014;57:659-663.
141. Lukaszewicz R, Mahay G, Boyer O, Martinet J. Medical algorithm: aspergillus fumigatus components in the diagnosis of allergic bronchopulmonary aspergillosis. *Allergy*. 2022;77:327-330. doi:10.1111/all.15001
142. Caminati M, Feleszko W, Michel M, Annesi-Maesano I, Vitte J. *Aspergillus fumigatus* and personalized medicine: toward a clinically reliable algorithm. *Allergy* Published Online First. 2022. doi:10.1111/all.15299
143. Hoffmann-Sommergruber K, Roesner LM. The clinical impact of cross-reactions between allergens on allergic skin diseases. *Curr Opin Allergy Clin Immunol*. 2020;20:374-380.
144. Hoffmann HJ, Santos AF, Mayorga C, et al. The clinical utility of basophil activation testing in diagnosis and monitoring of allergic disease. *Allergy*. 2015;70:1393-1405.
145. Santos AF, Alpan O, Hoffmann H-J. Basophil activation test: mechanisms and considerations for use in clinical trials and clinical practice. *Allergy*. 2021;76:2420-2432.
146. Katelari A, Tzanoudaki M, Noni M, et al. The role of basophil activation test in allergic bronchopulmonary aspergillosis and aspergillus fumigatus sensitization in cystic fibrosis patients. *J Cyst Fibros*. 2016;15:587-596.
147. Michel M, Sereme Y, Mankouri F, et al. Basophil activation test with aspergillus molecules: the case for ABPA. *Front Allergy*. 2022;3. doi:10.3389/falgy.2022.898731
148. Doron I, Leonardi I, Li XV, et al. Human gut mycobiota tune immunity via CARD9-dependent induction of anti-fungal IgG antibodies. *Cell*. 2021;184:1017-1031.e14.

149. Raulf M, Joest M, Sander I, et al. Update of reference values for IgG antibodies against typical antigens of hypersensitivity pneumonitis. *Allergo J Int*. 2019;28:192-203.
150. Hunter ES, Page ID, Richardson MD, Denning DW. Evaluation of the LDBio aspergillus ICT lateral flow assay for serodiagnosis of allergic bronchopulmonary aspergillosis. *PLoS ONE*. 2020;15:e0238855.
151. Piarroux RP, Romain T, Martin A, et al. Multicenter evaluation of a novel immunochromatographic test for anti-aspergillus IgG detection. *Front Cell Infect Microbiol*. 2019;9:12.
152. Hoffmann-Sommergruber K, de las Vecillas L, Dramburg S, Hilger C, Matricardi P, Santos A. EAACI molecular allergology user's guide 2.0. *Eur Acad Allergy Clin Immunol*. 2022.
153. Borges FM, de Paula TO, Sarmiento MRA, et al. Fungal diversity of human gut microbiota among eutrophic, overweight, and obese individuals based on aerobic culture-dependent approach. *Curr Microbiol*. 2018;75:726-735.
154. Gouba N, Drancourt M. Digestive tract mycobiota: a source of infection. *Med mal Infect*. 2015;45:9-16.
155. Torres PPTES, Rabahi MF, Moreira MAC, Santana PRP, Gomes ACP, Marchiori E. Tomographic assessment of thoracic fungal diseases: a pattern and signs approach. *Radiol Bras*. 2018;51:313-321.

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