Fungal exposome, human health and unmet needs: a 2022 update with special focus on allergy

Joana Vitte[1], Moïse Michel[1], Andrei Malinovschi[2], Marco Caminati[2], Adeyinka Odebode[2], Isabella Annesi-Maesano[1], Davide Caimmi[1], Carole Cassagne[3], Pascal Demoly[1], Enrico Heffler[2], Estelle Menu[3], Bright Nwaru[2], Youssouf Sereme[3], Stéphane Ranque[3], Monika Raulf[2], Wojciech Feleszko[2], Christer Janson[2], and Carmen Galán[4]

[1]Montpellier Universite d'Excellence
[2]European Academy of Allergy and Clinical Immunology
[3]IHU Mediterranee Infection
[4]Campus de Excelencia Internacional Agroalimentario

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Abstract

Humans inhale, ingest and touch thousands of fungi each day. The ubiquity and diversity of the fungal kingdom are in sharp contrast with their complex and relatively blurred taxonomy and scarce knowledge about their distribution, pathogenic effects, and 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. Improved understanding of the fungal exposome sheds new light on the epidemiology of fungal-related 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.

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Joana Vitte*[1,2], Moïse Michel*[1,2,3], Andrei Malinovschi[4], Marco Caminati*[5], Adeyinka Odebode#[6], Isabella Annesi-Maesano*§[1], Davide Paolo Caimmi[1,7], Carole Cassagne[8], Pascal Demoly[1,7], Enrico Heffler*[9,10], Estelle Menu[8], Bright I. Nwaru*[11,12], Youssouf Sereme[2¶], Stéphane Ranque[8], Monika Raulf*[13], Wojciech Feleszko*[14], Christer Janson[15] , Carmen Galán§[16,17]

EAACI Task Force "Diagnosis and management of Allergic Bronchopulmonary Aspergillosis"; EAACI Working Group Infections; EAACI Working Group Aerobiology and Pollution

* member of the EAACI ABPA Task Force

§ member of the EAACI WG Aerobiology

EAACI Mentorship Programme (Mentor: JV)

1 IDESP, University of Montpellier and INSERM, Montpellier, France

2 Aix-Marseille Univ, IRD, APHM, MEPHI, IHU Méditerranée Infection, Marseille, France

3 Immunology Laboratory, University Hospital Nîmes, Nîmes, France

4 Department of Medical Sciences Clinical Physiology Uppsala University Uppsala Sweden

5 Department of Medicine, Asthma, Allergy and Clinical Immunology Section, University of Verona, Verona, Italy

6 Department of Basic Science, Kampala International University, Uganda

7 Departement of Pneumology, University Hospital of Montpellier, Montpellier, France

8 Aix-Marseille Univ, IRD, APHM, VITROME, IHU Méditerranée Infection, Marseille, France

9 Personalized Medicine, Asthma and Allergy Humanitas Clinical and Research Center IRCCS Rozzano Italy.

10 Department of Biomedical Sciences Humanitas University Pieve Emanuele Italy

11 Krefting Research Centre, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

12 Wallenberg Centre for Molecular and Translational Medicine, University of Gothenburg, Gothenburg, Sweden

13 Department of Allergology and Immunology, Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr-Universität Bochum (IPA), Bochum, Germany

14 Department of Pediatric Pulmonology and Allergy, Medical University of Warsaw, Warsaw, Poland

15 Department of Medical Sciences Respiratory, Allergy and Sleep Research Uppsala University Uppsala Sweden

16 International Campus of Excellence on Agrifood (ceiA3), University of Cordoba, Spain

17 Andalusian Inter-University Institute for Earth System Research (IISTA), University of Cordoba, Spain

¶ Current affiliation: Department of Immunology, Infectiology and Hematology, Institut Necker-Enfants Malades (INEM), INSERM U1151, CNRS UMR 8253, Université Paris Descartes, Paris, France

Corresponding author:

Dr Joana Vitte, IDESP IURC, 641 Avenue du Doyen Gaston Giraud, 34093 Montpellier, France ;*joana.vitte@inserm.fr;*Phone +33620423949

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Abstract

Humans inhale, ingest and touch thousands of fungi each day. The ubiquity and diversity of the fungal kingdom are in sharp contrast with their complex and relatively blurred taxonomy and scarce knowledge about their distribution, pathogenic effects, and 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. Improved understanding of the fungal exposome sheds new light on the epidemiology of fungal-related 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.

Key words

allergy, allergic broncho-pulmonary aspergillosis, exposome, fungus, hypersensitivity, immunoglobulin, microbiota, mycobiota

Abbreviations

ABPA, allergic broncho-pulmonary aspergillosis; AFRS, allergic fungal rhinosinusitis; AIT, allergen immunotherapy; DGGE, Denaturing Gradient Gel Electrophoresis; ECP, eosinophil cationic protein; FeNO, fractional exhaled nitric oxide; HSP, hypersensitivity pneumonitis; Ig, immunoglobulin; ABPM, allergic broncho-pulmonary mycosis; PAMP, Pathogen-Associated Molecular Pattern; PCR, polymerase chain reaction; PRR, pattern recognition receptors; VOC, volatile organic compounds

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Introduction

The fungal kingdom comprises ubiquitous heterotrophic eukaryotes, specialized in the decomposition of organic material and recycling (1) and with possible symbiosis interactions among fungi or with other organisms, e.g. with algi in lichens or with plants in mycorrhiza. The systematic in fungi is complex, but recent genomic technologies have allowed to improve their phylogenetic tree. Some of them are macroscopic, e.g. edible mushrooms, while others belong to microorganisms (1) and are the focus of this review. Their ubiquity and diversity reflect those of the living world: spores of several fungal species have been detected from samples collected in arctic or desert areas (2), and the number of fungal species has been estimated at 3,8 million (3), being today much of them still unknown. Humans are thus 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. 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 like 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), ligands for 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 associated with immune deficiency, while hypersensitivity occurs mainly in atopic patients (4,5). Some papers have shown that, depending on the ability of a given fungus to grow at human body temperature (thermotolerant fungus able to grow in the airways resulting in fungal colonization) or not (mesophilic), 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 (6).

The third form of the 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 (7).

However, fungal antigens are characterized by intra-kingdom specificity, with a relation between the fungal systematics and IgE sensitization pattern (7). Many fungal proteins have evolved for specific functions associated with heterotroph nutrition. The degree of homology reflects phylogenetic distance (8). 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 immunoglobulins. 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 (7,9). However, other examples of medically relevant cross-reactivity exist, *e.g* . between the skin fungus *Malassezia (M.) sympodialis*and human host proteins (10).

Fungal taxonomy is complex and still evolving, but the main allergenic genera and species belong to three of the ten fungal phyla currently described: *Ascomycota* (comprising *Candida, Alternaria, Aspergillus, Penicillium, Trichophyton* , among other genera),*Basidiomycota* (*e.g* . *Rhodotorula, Ustilago*), and*Mucoromycota* (classified until recently as part of the former phylum *Zygomycota)* (*e.g* . *Mucor*) (11,12) (**Table 1**). Inside each phylum, phylogenetic relationship explains allergen cross-reactivity at the level of genera and species (8). The following sections bring further detail to these topics.

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, and can produce lysine, for some of them, exhibiting a dual state as either yeast or mold (13). Regarding microscopic fungi, yeasts are unicellular forms that reproduce through budding, while molds are multicellular forms displaying hyphae in a mycelium (13). Fungi that present as yeast-like or molds, depending on physicochemical conditions and nutrient availability, are called "dimorphic" (13).

Before 2011, the taxonomy of fungi was blurred by the co-existence of distinct names for the sexual (teleomorph) and asexual (anamorph) states of the same fungus. This dual nomenclature of fungi has hampered research on fungi for decades. 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, the 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 cause a vast variety of diseases, mainly allergic (such as conjunctivitis, asthma, hypersensitivity pneumonitis, rhinitis, and allergic broncho-pulmonary aspergillosis) or infectious (e.g., mucormycoses, invasive aspergillosis, or fusariosis). Fungal hypersensitivity is often found in patients with asthma, chronic obstructive pulmonary disease, cystic fibrosis, and bronchiectasis, ranking among the most prevalent diseases worldwide (4,15). Fungal infections develop predominantly in immunocompromised hosts and may exceed 50% case-fatality rates (4,16). Hypersensitivity pneumonitis (HSP) may occur in subjects without a previous condition (17).

Moreover, fungi release mycotoxins and volatile organic compounds (VOC). Mycotoxin production aims to secure fungal nutrients, while VOC is defined as small molecules containing carbon and able to evaporate under ambient conditions, such as 0.01 kPa and 20°C (18–20). Exposure to mycotoxins may occur through inhalation of airborne mycotoxins or ingestion of contaminated foods, giving rise to mycotoxicosis (19,21).

Fungal VOC are produced by primary and/or secondary metabolism 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 (20,21). Although some fungal VOC have been shown to induce symptoms (fatigue, lethargy, headache, irritation of ocular and upper airway mucosae, wheezing) and upregulate biomarkers of inflammation in healthy volunteers (20,22) their impact on human health is still controversial (18–20). 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.

Fungal exposome

Worldwide, molds are an increasingly acknowledged part of the human exposome, both external (23) and internal as microbiome components collectively named mycobiota (24) **(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 mold exposure shows geographical, seasonal, and urban*versus* rural variability (25–27) and induces immune responses and health effects, often respiratory and allergic, that may start in infancy (28–31). In fact, human-fungus interactions extend from medicine and pharmacy to leisure activities, agriculture, food processing, industry, and even interplanetary travels (13,32,33).

Currently, allergenic molecules from more than 40 fungal genera have been characterized (*www.allergen.org*, accessed March 8th, 2022), but molecular data are still lacking for many environmental fungi **(Table 2)** . Cross-reactivity between fungal allergens may help to identify some, but not all, fungal sensitization outside of the available extracts and molecules (34,35).

III.1. External exposome

Airborne fungal spores and subcellular components originate from different 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, fungal spores, pollen, mites, dead tissues, and pieces of these materials or their metabolic products including endotoxins and mycotoxins) are called bioaerosols (36). 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 before sedimenting in so-called sinks, or settle, e.g. on indoor surfaces or clothing (25,36,37). The wind dispersal fungal spores, ranging from 1 to 30 µm 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 (37–39).

Culture-independent studies have demonstrated a very high diversity of airborne fungal taxa. As an example, each dust sample taken from the outer surface of hundreds of US homes contained more than 1,000 fungal phylotypes, most of which belonging to taxa not been described at the time of the study (25). However, only *Cladosporium* ,*Toxicladosporium* , 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% (25). The results of a questionnaire sent to national and regional networks involved in the European Aeroallergen Network (EAN) and counting fungal spore, 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 other hand, all networks examined *Alternaria* (16), most of them*Cladosporium* (14) and the third more cited spore was*Epicoccum* (10). Other fungal spores where cited (45) in the list, but only by 1 to 6 networks (39). Partly overlapping findings were reported from the European urban areas of Bratislava (Slovakia, temperate continental climate), Thessaloniki (Greece, Mediterranean climate), and Madrid (Spain, warm-temperate subtropical climate). The five top abundant Slovakian fungal spores were *Cladosporium, Leptosphaeria, Coprinus, Alternaria,* and *Ganoderma* (40), while their Greek counterparts were *Cladosporium, Alternaria, Ustilago,*and Ascospores (*Aspergillus/Penicillium*) (41).*Cladosporium, Alternaria, Eurotium, Epicoccum, Penicillium* , and*Sporobolomyces* were detected in more than 90% of samples from Madrid (42). 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 (43) and *Cryptococcus, Candida, Schizophyllum, Fusarium* , and *Gleotinia* in Kuwait (44). 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 (45) and by *Penicillium* and *Aspergillus* in Nigeria using a culture-dependent approach (46).

Under temperate climates, seasonal variations usually increase fungal abundance with higher temperatures and rainfall, such as during summer and fall (42). However, very high temperature values may negatively affect the abundance of airborne fungal spores, as observed in Karachi and Lagos (43,46). Significant interannual variations in rainfall are common and associated with variations in airborne fungal abundance (42,45).

Depending on the considered fungi, spore release may occur preferentially during the daytime, as observed for *Alternaria,Cladosporium* , *Epicoccum,* and *Exosporium* or at night, e.g. *Coprinus* and *Leptosphaeria* (40). Particulate air pollution such as PM10 is positively correlated with the fungal spore load (40).

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 airborne fungi, such as *Fusarium, Curvularia, Cryptococcus,* and *Ustilago* .

Indoor exposure is paramount, as most people now spend most of their time indoors (47). 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” (48), is an essential contributor to indoor mold growth, often related to climatic events (*e.g* ., floods, storms, rising ocean levels) or poor housing standards, including older homes (23,47,49). 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 (50–52).

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 (23). The degree of exposure to indoor molds was estimated at 5-10% under cold-temperate climates, and up to 30% in warmer climates (27). An Environmental Relative Moldiness Index (ERMI) may be used as a quantitative marker of indoor mold exposure (49). The original ERMI, developed in the United States of America, is computed using PCR quantification of 36 common indoor molds, of which 26 are related to water damage and 10 are not (53). In order to acknowledge local fungal variability at the levels of species and abundance, the need for an adapted ERMI was demonstrated (54).

Exposure to mold also occurs at various workplaces. A distinction has to be made between the intentional use of molds and unintentional exposure to moldy materials. The intentional use of molds in workplaces is found in food production, pharmaceutical production and microbiological laboratories. Representatives of the fungal genus *Penicillium* are found in ripen cheese and salamis and produce antibiotics.*Aspergillus niger* produces citric acid from residues of the sugar industry. In microbiology laboratories, workers come into contact with molds when growing and multiplying microorganisms. Many more workers come into contact with mold 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.

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 (55,56), there are only four genera of

significant importance: *Aspergillus, Penicillium, Alternaria* , and *Cladosporium* (47).

III.2. Internal exposome

Animal and plant microbiota contain a fungal component, the mycobiota; conversely, fungi possess their own microbiota (57). Crosstalk between fungal and bacterial components of the microbiota and between each of them and the host are essential for sustained commensalism (24,57). 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 (24). The most prevalent fungal genera in the healthy gut are*Saccharomyces* , *Malassezia* , *Candida* , and*Cyberlindnera* (24). 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 (58). Strikingly, frequent fungal taxa associated with oral, pulmonary, intestinal, or cutaneous locations, such as *Aspergillus, Cladosporium, Alternaria* , or *Penicillium (24)* overlap with environmental counterparts described in the previous section. On the other hand, 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 (59). A special case could be represented by breast milk mycobiota, which comprises*Malassezia, Penicillium, Davidiella,* and *Sistotrema*genera, possibly explaining the abundance of *Malassezia* in the neonatal and young infant gut mycobiota (24). 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, and that a higher abundance of gut fungi in infants was predictive of later development of allergic diseases (31,60).

Among fungi associated with human skin, *Malassezia* , mainly*M. sympodialis* , is 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 (10,24).

III.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, feces), to the environment (temperature, wind, relative humidity, building material) or personal conditions (adult vs child, professional vs home exposure), and to the purpose of the study (e.g. epidemiological study vs examination of a patient's case) (61–63).

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 molecular methods. For most environmental fungal taxa, culture cannot be achieved (64). On the other hand, for those growing in vitro, their growth rate will depend on the type of fungal culture, the nutrient media in use (57). 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 (65). Alternatively, DNA-based approaches such as polymerase chain reaction (PCR) targeting taxonomic marker sequences, or DNA metabarcoding, allow the identification of considerably higher taxonomic biodiversity within the collected samples (64,66). However, this new technology also has some shortcomings, including primer bias which can heavily alter sequencing results (67,68), 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 (64). 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 (69,70).

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 (71).

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 (24,72).

Overview of major mold-related hypersensitivity diseases

Fungus-human host interactions involve a combination of hypersensitivity, toxicity, and opportunistic infections (26–28,73–76)**(Figure 2)** . Indoor and outdoor exposure to fungi is ubiquitous (33,47) and altered by climate change (29,41,77).

IV.1. Upper airways: Allergic fungal rhinosinusitis (AFRS)

AFRS is a unique form of immune-mediated non-invasive fungal rhinosinusitis exhibiting prominent Th2 responses, eosinophilic inflammation, and IgE responses (78). Its prevalence is definitely higher in arid and tropical climates, such as in the Asia-Pacific region, Australia, Thailand, Malaysia, India, the Middle East, Saudi Arabia, North Africa, and Southeastern and Southwestern parts of the US, especially the Mississippi basin (79,80). Climate influence is prominent, as demonstrated by a prevalence of 0.4% in Northern US states compared to over 10% in Southern ones. *A. fumigatus* is the most frequent fungus involved in AFRS (4,79,80).

IV.2. Lower airways

IV.2.1. Asthma

Thermotolerant mold-sensitized individuals are at high risk for developing chronic severe lung disease, including life-threatening asthma, bronchiectasis, and lung fibrosis (6). Conversely, the severity of mesophilic-mold related lung diseases resides mostly in acute allergenic stimulation (81). Examples are *Aspergillus fumigatus* -related fungal asthma, as opposed to *A. alternata* -related asthma attacks in the aftermath of summer storms. With respect to *A. 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 (6). In adults from the European Community Respiratory Health Survey (ECRHS), sensitization to *Alternaria*was associated with severe asthma (82) and a decrease in lung function, especially in women (83); also, sensitization to molds (*Cladosporium* and *Alternaria*) was more prevalent in individuals living in damp dwellings and related to current asthma (84). In pediatric asthma, mold sensitization related to impaired pulmonary function and increased airway hyperresponsiveness (85). In adults with severe asthma, multiple fungal sensitizations are related to poorer asthma control (86). Conversely, *A. fumigatus* specific IgG has not been associated with modified clinical outcomes in asthma, in the absence of allergic broncho-pulmonary aspergillosis (ABPA) or hypersensitivity pneumonitis (HSP).

IV.2.2. Allergic broncho-pulmonary mycosis

Allergic broncho-pulmonary mycosis (ABPM) is probably the most severe allergic fungal diseases (73). It is typically induced by *A. fumigatus* (ABPA), often presents as an asthmatic exacerbation in asthma or cystic fibrosis patients, and involves an exuberant anti-fungal allergic response with 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 (87–89).

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

IV.2.3. Hypersensitivity pneumonitis (HSP)

Hypersensitivity pneumonitis is defined as "an interstitial lung disease resulting from an immune-mediated response in susceptible and sensitized individuals to a large variety of inhaled antigens found in the environment", with fungal antigens among the most frequent. Fungal-related HSP usually occurs because of prolonged exposure to fungal material, often in an occupational setting, and manifests as lung functional deterioration accompanied by exaggerated antigen-specific IgG and cellular responses (17,19).

IV.2.4. Skin disease: Atopic dermatitis

The most notable connection between atopic dermatitis and fungi is*Malassezia sympodialis* , a frequent skin colonizer in healthy and affected individuals (90). Some *Malassezia* allergens share structural epitopes and IgE reactivity with human proteins, e.g. thioredoxins (Mala s 13) (10). *M. sympodialis* induces a T-lymphocyte response only in susceptible, atopic individuals (90).

Overview of diagnostic tools

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.

V.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 (91).

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 (92) while in HSP the most common finding is a patched ground glass pattern (91). The limited studies on magnetic resonance imaging (MRI) did not provide evidence for any added diagnostic value of these investigations (93).

Exhaled nitric oxide, a marker of type 2 inflammation, appears to be elevated in cystic fibrosis subjects suffering from ABPA compared with only *Aspergillus* -sensitized subjects. Therefore FeNO (fractional exhaled nitric oxide) might have a role as a diagnostic test in the context of cystic fibrosis (94).

V.2. Laboratory tools

Evidence of IgE responses and eosinophil involvement support a hypersensitivity mechanism, while mycological evidence of fungal persistence is more challenging to obtain (4,6,83,85). Particularly in the case of HSP and ABPA, the determination of mold-specific IgG (*A. fumigatus*- specific IgG in the case of ABPA) is an additional useful diagnostic tool (95).

V.2.1. Mycology

The detection of fungi at various human body site, 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.

Conventional mycological diagnosis relies on the macroscopic and microscopic assessment of fresh and culture samples (96,97). Direct examination of fresh samples aims at recognizing characteristic features, such as fungal hyphae. Inoculation on fungal growth media, followed by a 5 to 7 days incubation yields colonies further identified at the species level using MALDI-TOF Mass Spectrometry and a specialized fungal reference spectra database (98). 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 (99).

V.2.2. Cytology & 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 (89). Eosinophils, eosinophilic inflammation markers such as Charcot-Leyden crystals, and fungal hyphae were recently proposed as pathognomonic for ABPA/ABPM (88).

V.2.3. Hematology

Systemic or local eosinophilia is a hallmark of fungal allergic diseases (100). Eosinophils are readily counted and interpreted through a basic white blood cell count. Blood eosinophilia, often defined as $>$ 500 elements/mm3 but sometimes at lower levels, e.g. 150 elements/mm3 (101), is an inconsistent marker of a predominantly type 2 response and atopic orientation, without specificity for ongoing fungal-related atopic disease. It is subject to variations related to comorbidities and ongoing treatments, especially corticosteroids (102).

V.2.4. Immunology

Immunoglobulin responses and inflammatory mediators are markers of the fungal-host interaction (103–108). Such biomarkers are valuable endpoints of individual susceptibility because fungal exposure is not a direct predictor of health effects at the individual level (77). In the ECRHS cohort of general adult population, indoor mold exposure was associated with a higher risk for adult-onset asthma, oculo-respiratory and general symptoms, and increased asthma severity scores, predicted by higher levels of IgE and eosinophil cationic protein (ECP) (74,75,77,109). Pre-existing sensitization to airborne molds was associated with a higher risk of mold-related disease (74).

V.2.4.1. Determination of fungal-specific antibody responses: IgE, IgG

a. 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 (87) (110) (103) (104).

Quantitative laboratory IgE methods allow dynamic monitoring and cross-reactivity assessment. Given the variations in fungal extract preparation and inter-method 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 relies on the availability of molecular allergens for in vitro diagnostics, which is currently limited to *Aspergillus fumigatus, A. alternata, C. herbarum* , and *Malassezia sympodialis* .

b. Fungal serology: anti-fungal specific IgG

Since fungi are ubiquitous, detecting anti-fungal specific IgG in the blood of individuals free of fungal disease is common. 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 (111). High concentrations of anti-fungal specific IgG may support ABPA/ABPM, HSP, infectious aspergillosis.

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.

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, e.g. general population, specific allergic populations, and severe asthma.

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.

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.

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 allergic and hypersensitivity reactions to fungi in the context of the current climate change and global need for sustainable housing.

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Table 1. Overview of the current classification of fungi at the phylum level. Phyla comprising established allergenic species are shown in bold font. References: 8, 11, 12, 19, 25, 75, 80, and www.allergen.org.

Fungal phyla	**Examples of known allergenic genera and species**
Ascomycota	*Alternaria, Aspergillus, Aureobasidium, Candida, Chaetomium, Chrysonilla, Cladosporium, Coccidioid*
Basidiomycota	*Coprinus, Malassezia, Psilocybe, Rhodotorula, Trichosporon, Ustilago*
Blastocladiomycota	None
Chytridiomycota	None
Cryptomycota	None
Microsporidia	None
Mucoromycota	*Mucor, Rhizopus*
Olpidiomycota	*None*
Sanchytriomycota	None
Zoopagomycota	None

Table 2. Fungal molecular allergens currently included in the WHO/IUIS nomenclature.

Data are presented as the allergen number per fungal genus, and biochemical groups in each phylum, with the distribution of the most frequent protein families among the three phyla. The table is not exhaustive: other fungal allergen families have been identified, such as heat shock proteins (HSP70), e.g. Alt a 3 and Pen c 19, while important allergens, such as Alt a 1, the major allergen from *Alternaria alternata* , belong to allergen families with as yet unknown biological functions.

Source: *www.allergen.org* accessed March 8th, 2022 (the phylum for *Rhizopus* was updated from *Zygomycota* , now obsolete, to *Mucoromycota*).

Phylum	**Genus**	**Genus**	**Protein families (total number at the phylum**
	Name	**Number of validated allergens**	**Serine-proteases**
Ascomycota	*Alternaria*	12	19
	Aspergillus	31	
	Candida	3	
	Cladosporium	10	
	Curvularia	4	
	Epicoccum	1	
	Fusarium	4	
	Penicillium	17	
	Stachybotrys	1	
	Trichophyton	4	
	Ulocladium	1	
Basidiomycota	*Coprinus*	5	0
	Malassezia	13	

Phylum	Genus	Genus	Protein families (total number at the phylum
	Psilocybe	2	
	Rhodotorula	2	
	Schizophyllum	1	
Mucoromycota	*Rhizopus*	2	1
Total		**113**	**20**

Figure legends

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 and anthropic sources, and are collectively designated 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. Fungi interact with other eukaryota, 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. Upper left box: ± denotes inconstantly demonstrated airborne fungal genera (as opposed to ubiquitous ones).

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 various diseases.

Figure 3. Determination of *Aspergillus fumigatus*-specific IgE in serum. a, quantitative assays; b, semi-quantitative assays.a, The x axis denotes *A. fumigatus* -specific IgE levels (kUA/L); **b,** the x axis denotes the number of responses for each intensity class of *A. fumigatus* -specific IgE, shown as a discrete 0 to 6 scale on the y axis (0: no detectable *A. fumigatus* -specific IgE; 6: very high *A. fumigatus* -specific IgE result).

The lack of intermethod standardization results in high variability of the results obtained with the same sample during an external quality assessment program. The scattering of the results is seen with quantitative as well as semi-quantitative assays. CV, coefficient of variation, n, number of participating laboratories, SD, standard deviation.

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