Review article

**Alternaria alternata** allergens: Markers of exposure, phylogeny and risk of fungi-induced respiratory allergy

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**Abstract**

*Alternaria alternata* spores are considered a well-known biological contaminant and a very common potent aeroallergen source that is found in environmental samples. The most intense exposure to *A. alternata* allergens is likely to occur outdoors; however, *Alternaria* and other allergenic fungi can colonize in indoor environments and thereby increase the fungal aeroallergen exposure levels. A consequence of human exposure to fungal aeroallergens, sensitization to *A. alternata*, has been unequivocally associated with increased asthma severity. Among allergenic proteins described in this fungal specie, the major allergen, Alt a 1, has been reported as the main elicitor of airborne allergies in patients affected by a mold allergy and considered a marker of primary sensitization to *A. alternata* over, thereby increase the fungal aeroallergen exposure levels. A consequence of human exposure to fungal aeroallergens, sensitization to *A. alternata*, has been unequivocally associated with increased asthma severity. Among allergenic proteins described in this fungal specie, the major allergen, Alt a 1, has been reported as the main elicitor of airborne allergies in patients affected by a mold allergy and considered a marker of primary sensitization to *A. alternata*.

Keywords:
*Alternaria alternata* allergens
Asthma
Cross-reactivity
Diagnosis of allergy
Exposure to fungal allergens
Poly-sensitization

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**Abbreviations:** ABRPA, allergic bronchopulmonary aspergillosis; ABPM, allergic bronchopulmonary mycosis; CRD, component-resolved diagnosis; ELISA, enzyme-linked immunosorbent assays; GA(2)LEN, Global Asthma and Allergy European Network; GST, glutathione-S-transferase; IgE, immunoglobulin E; ITS, internal transcribed spacer; kDa, kilodalton; MnSOD, manganese-dependent superoxide dismutase; MDH, mannoi dehydrogenase; SDAP, Structural Database of Allergenic Proteins; SP, serine protease; WHO/IUIS, World Health Organization and International Union of Immunological Societies.

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1. Introduction

Alternaria alternata is one of the most common saprophytes found worldwide and has been clinically associated with asthma (Bush and Prochnau, 2004; Pulimood et al., 2007), allergic rhinosinusitis (Schell, 2000), hypersensitivity pneumonitis (Ogawa et al., 1997), onychomycosis (Ozbek et al., 2006), onychomycosis (Romano et al., 2001), skin infections (Mayer et al., 2002) and Allergic Bronchopulmonary Mycosis (ABPM) (Singh and Denning, 2012; Chowdhary et al., 2012). Although there is a wide range of clinical manifestations, A. alternata is rarely found to be a cause of invasive infections in humans. This fungal specie is mainly related to the induction of immunoglobulin E (IgE)-mediated respiratory diseases. A. alternata spores are considered one of the most abundant and potent sources of airborne sensitizer allergens. The most intense exposure to A. alternata allergens is likely to occur outdoors; however, Alternaria and other allergenic fungi can colonize in indoor environments and thereby increase exposure levels (Salo et al., 2005).

The true prevalence of sensitization to A. alternata has been difficult to estimate, and several epidemiological and diagnostic studies have reported a highly variable prevalence of human IgE reactivity to Alternaria. The European Community Respiratory Health Survey found that 4.4% of the general adult population (n = 11.355) are sensitized to Alternaria (Bousquet et al., 2007). More recently, in a large skin test study funded by the Global Asthma and Allergy Europe Network (GA(2)LEN) that involved 3034 subjects with a suspected inhalant allergy (median age: 33 years) from 17 collaborating centers located in 14 European countries, Alternaria sensitization prevalence was approximately 9%, with sensitization rates ranging from 2% (Finland) to 23.8% (Greece) (Burbach et al., 2009; Heinzerling et al., 2009). Among a fungal-sensitized population, more than 60% reacted to diagnostic A. alternata extracts (Mari et al., 2003).

There are some population groups in which the A. alternata sensitization rates are much higher. Mold sensitization, particularly to A. alternata, seems to be much more prevalent in the asthmatic population (Neukirch et al., 1999). Younger respiratory allergic patients seem to be more at risk for A. alternata sensitization than older ones (Mari et al., 2003; Katotomichelakis et al., 2012). Farm and sawmill workers have also been reported to be at high risk for occupational airway disease caused by A. alternata because significant levels of A. alternata allergen have been measured in farms (Prester and Macan, 2010).

It is well known that most individuals sensitized to A. alternata present associated allergic manifestations that require medical attention. In particular, in the GA(2)LEN study, clinically relevant symptoms were clearly noticed in 69% of the European patients who were sensitized to Alternaria (Burbach et al., 2009). The severity of typical allergic reactions varies from mild to potentially life-threatening, and it is dependent on several internal and external factors. The most severe IgE-mediated response to A. alternata aeroallergens is severe asthma that is characterized by impaired lung function and frequent exacerbations that may result in patient death. Indeed, sensitization to A. alternata has been reported to be unequivocally associated with increased asthma severity, hospital admissions to intensive care for asthma and deaths related to asthma (Neukirch et al., 1999; Black et al., 2000; Zureik et al., 2002).

Because A. alternata has been increasingly recognized as a powerful respiratory allergic disease inducer (Neukirch et al., 1999), it has been proposed that at least some A. alternata allergens might play a role in the underlying mechanisms of allergic reaction severity. Therefore, the full definition and understanding of the A. alternata allergen repertoire seem to be crucial in finding an explanation for why sensitization to A. alternata is a risk factor for asthma. The study of the allergic protein components of this mold may also provide valuable clues for understanding the interesting recent finding that Alternaria activates the innate immune system and enhances lung inflammation induced by unrelated allergens such as grass pollen (Kim et al., 2014). Although it is well known that there exist irrefutable associations between sensitization to other prevalent aeroallergens, such as house dust mites and pollens, and asthma exacerbations (Baldacci et al., 2015), a cross sectional study from European Community respiratory health survey (Zureik et al., 2002) found that the severity of asthma was more strongly associated with sensitization to molds, namely A. alternata, than with sensitization to other environmental aeroallergens, such as pollens. Nevertheless, compared with other allergenic sources that are commonly found in the environment, fungi, particularly A. alternata, are still a neglected and underestimated source of allergens (Cramer et al., 2014).

Considering the above-mentioned facts, there is a clear need to identify the complete array of allergenic components from A. alternata and elucidate the role of these proteins in the development of respiratory allergies from biology, diagnosis, prognosis and disease management perspectives. At present, a total of 17 allergenic proteins are characterized as allergens of A. alternata and listed in allergen platforms (http://www.allergen.org/ and http://www.allergome.org/). The A. alternata allergens list includes proteins that are restricted to a small number of fungal species that are taxonomically related to A. alternata and ubiquitous proteins that are conserved throughout the evolutionary process; these proteins present their homologs in several fungal families. The existence of multiple homolog allergens across the fungal kingdom that carry cross-reactive epitopes that are structurally indistinguishable from IgE-binding epitopes may raise a considerable problem in the diagnosis and classification of fungal allergies (Bowyer et al., 2006). In agreement with this finding, compelling evidence shows that apparent sensitization to multiple fungi represents a frequent clinical observation in patients sensitized to A. alternata and may be a consequence of the existence of IgE cross-reactivity between fungal proteins (Gupta et al., 2002a,b; Cramer et al., 2009).

Recent phylogenetic studies have increasingly clarified that clinical IgE reactivity data reflect the taxonomical distribution of the fungal allergenic sources in a majority of the cases (Soeria-Atmadja et al., 2010). Thus, individualized fungal allergens should be fully characterized and analyzed with respect to protein family membership, taxonomic distribution and interspecies variability. In this way, establishing relationships between A. alternata allergens and the homologs expressed by other allergologically important fungal species might provide valuable information for defining a taxonomic categorization of fungal allergenic sources and a molecular classification of fungal allergens. The definition of allergens as molecular markers will contribute to an understanding of their potential role in fungal biology, improve diagnostic decisions by prediction of cross-reactivity and develop more robust detection tools to assess exposure allergen levels. Correctly assessing the exposure to aeroallergens in the environment is of major importance for predicting the risk of respiratory symptoms in an atopic population and informing the implementation of appropriate public health measures. The use of specific allergens as tools to accurately detect allergen contaminants and thereby enable the identification of the fungal origin source seems to be a promising strategy.

This review is intended to focus on relevant issues regarding A. alternata sensitization and its allergens, such as the immunological

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characterization and clinical relevance of the allergens identified to date, the phylogenetic relationships based on shared allergen homologs and advances in the assessment of A. alternata exposure and diagnosis of fungal sensitization.

2. Alternaria alternata allergens and their sensitization prevalence

Significant research has been conducted to identify and characterize the proteins that are involved in A. alternata allergy. Alternaria allergens are a diverse group of molecules that possess different chemical and biological properties. To date, 17 IgE-reacting proteins of A. alternata have been identified and isolated. Twelve of them fulfilling the World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee criteria, have been officially listed as A. alternata allergens (Table 1).

The A. alternata allergens recognized to date, as well as their clinical relevance concerning an A. alternata allergy, are discussed extensively here.

2.1. Alt a 1

Alt a 1 is predominantly located in the cell wall of airborne Alternaria spores (Twaroch et al., 2012a,b) that access the respiratory tract and elicit allergic reactions in patients affected by a mold allergy. It is considered to be the marker of primary sensitization to A. alternata and is undoubtedly the more extensively characterized A. alternata allergen. The population that is allergic to A. alternata presents approximately 80% of the sensitization prevalence for Alt a 1. This allergen is released in considerable amounts by A. alternata and related species (Gutiérrez-Rodríguez et al., 2011) and this most likely explains why Alt a 1 displays very high values of sensitization incidence among fungal sensitized population.

Natural Alt a 1 is a 30 kilodalton (kDa) dimer that dissociates into 16.4 and 15.3 subunits under reducing conditions, thus suggesting a disulfide bond linking the monomers (Deards and Montague, 1991). Investigations by means of molecular dynamics suggested that Alt a 1 possesses a greatly stable dimeric structure presenting epitopes with a proper orientation for IgE cross-linking compared with other major allergens such as Bet v 1 and Pru p 3 (Garrido-Arandia et al., 2014). Two linear epitopes (K41-P50 and Y54-K63) showing consistent reactivity with serum IgE from an Alternaria-sensitive patient were identified in the Alt a 1 protein sequence (Kurup et al., 2003). Recently, the recombinant form of Alt a 1 has been analyzed to be capable of three-dimensional structure by X-ray crystallography (Chruszcz et al., 2012). In this work, the Alt a 1 protein presented a dimeric β-barrel structure, a completely novel fold among allergens that potentially define a new fungal protein family with an unknown function (Chruszcz et al., 2012). Although the function of this major allergen remains unclear, evidence has raised some hypotheses for the role played by this protein in fungal biology:

a) Alt a 1 release was detected to be significantly higher in germinated Alternaria spores, which indicates that this allergen might be involved in spore germination (Mitakakis et al., 2001).

b) Its A. brassicicola homolog was found to be highly expressed during the infection process of A. thaliana (Cramer and Lawrence, 2004).

c) Alt a 1 was found to induce the expression of plant defense proteins belonging to the PR5-TLP family and interact with it as a competitive inhibitor (Gómez-Casado et al., 2014).

The last two findings suggest that the role of Alt a 1 can be related to virulence and fungal infection pathogenicity.

Alt a 1 is a conserved protein that is highly specific for Alternaria and its related taxa because homologs of Alt a 1 have been reported in other Pleosporaceae species (Hong et al., 2005). A numerous list of Alt a 1 and Alt a 1-like genes and proteins has been deposited into databases.

2.2. Alt a 3 and Alt a 5

The first report of Alt a 3 and Alt a 5 as minor A. alternata allergens occurred when De Vouge et al. (1998) cloned two sequences encoding IgE-binding fragments of A. alternata allergens by screening the A. alternata cDNA library with A. alternata-sensitive patients’ pooled sera. The IgE-binding fragment that presented high homology in comparison with a region near the C-terminus of a heat shock protein 70 kDa from Cacodorysium herbarum was recognized on immunoblotting by 5% of the sera from A. alternata-sensitized patients, and it was officially named Alt a 3. Members of the HSP 70 protein family are highly conserved across both prokaryotic and eukaryotic organisms and play a major role protecting the cell during thermal and oxidative stress (Kiang and Tsokos, 1998).

The second A. alternata allergenic fragment cloned by De Vouge et al. (Alt a 5) was identified as belonging to the acidic ribosomal protein P2 family, and the recombinant protein had positive IgE-immunoblotting reactivity with 14% of the tested atopic patients’ sera (De Vouge et al., 1998). Biologically, these proteins are known to be ribosomal components that are involved in interactions with elongation factors during the course of protein synthesis (Tchórzewski, 2002).

2.3. Alt a 4, Alt a 7 and Alt a 10

Achatz et al. identified, cloned and characterized Alt a 4, Alt a 7 and Alt a 10 allergens (Achatz et al., 1995, 1996).

Alt a 4 is a 57 kDa A. alternata protein that bound IgE from 42% of an A. alternata sensitized population. In turn, Alt a 7 was identified as a clear, evidence has raised some hypotheses for the role played by this protein in fungal biology:

Table 1

<table>
<thead>
<tr>
<th>Allergen</th>
<th>Biochemical name</th>
<th>MW (SDS-PAGE) kDa</th>
<th>Clinical relevance</th>
<th>Isoallergen and variants</th>
<th>Nucleotide (NCBI)</th>
<th>Protein (UniProt)</th>
<th>PDB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alt a 1</td>
<td></td>
<td>16.4 and 15.3 band</td>
<td>Major allergen</td>
<td>Alt a 1.0101</td>
<td>U82633/U86752</td>
<td>AAB40400/AAB47552</td>
<td>P79085</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Alt a 1.0102</td>
<td>A568627</td>
<td>A575297</td>
<td>Q6K323</td>
</tr>
<tr>
<td>Alt a 3</td>
<td></td>
<td></td>
<td>Minor allergen</td>
<td>Alt a 3.0101</td>
<td>U87807/U87808</td>
<td>AAB48402/AAB48041</td>
<td>P79893</td>
</tr>
<tr>
<td>Alt a 4</td>
<td></td>
<td></td>
<td>Minor allergen</td>
<td>Alt a 4.0101</td>
<td>X84217</td>
<td>CAAS8999</td>
<td>Q00002</td>
</tr>
<tr>
<td>Alt a 5</td>
<td></td>
<td></td>
<td>Minor allergen</td>
<td>Alt a 5.0101</td>
<td>X89222/U87806</td>
<td>CAAS5066/AAB48041</td>
<td>P42037</td>
</tr>
<tr>
<td>Alt a 6</td>
<td></td>
<td></td>
<td>Minor allergen</td>
<td>Alt a 6.0101</td>
<td>U82437</td>
<td>AAG42022</td>
<td>Q5HTD3</td>
</tr>
<tr>
<td>Alt a 7</td>
<td></td>
<td></td>
<td>Minor allergen</td>
<td>Alt a 7.0101</td>
<td>X78225</td>
<td>CAAS5069</td>
<td>P42058</td>
</tr>
<tr>
<td>Alt a 8</td>
<td></td>
<td></td>
<td>Minor allergen</td>
<td>Alt a 8.0101</td>
<td>AY191815</td>
<td>AA091800</td>
<td>POCOY4</td>
</tr>
<tr>
<td>Alt a 10</td>
<td></td>
<td></td>
<td>Minor allergen</td>
<td>Alt a 10.0101</td>
<td>X78227</td>
<td>CAAS5071</td>
<td>P42041</td>
</tr>
<tr>
<td>Alt a 12</td>
<td></td>
<td></td>
<td>Minor allergen</td>
<td>Alt a 12.0101</td>
<td>X4216</td>
<td>CAAS8998</td>
<td>P49148</td>
</tr>
<tr>
<td>Alt a 13</td>
<td></td>
<td></td>
<td>Minor allergen</td>
<td>Alt a 13.0101</td>
<td>A514673</td>
<td>AAR88813</td>
<td>Q04854</td>
</tr>
<tr>
<td>Alt a 14</td>
<td></td>
<td></td>
<td>Minor allergen</td>
<td>Alt a 14.0101</td>
<td>KCR32979</td>
<td>AG580276</td>
<td>P86254</td>
</tr>
<tr>
<td>Alt a 15</td>
<td></td>
<td></td>
<td>Minor allergen</td>
<td>Alt a 15.0101</td>
<td>KJS58435</td>
<td>AH297469</td>
<td>–</td>
</tr>
</tbody>
</table>


MW, molecular weight; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.
22 kDa Alternaria protein highly homologous to YCP4 yeast, that bound IgE from 7% of the Alternaria-sensitized subjects. An 11 kDa aldehyde dehydrogenase (Alt a 10) reactive with 2% of the same Alternaria-sensitized population was also identified. (Achatz et al., 1995, 1996).

2.4. Alt a 6

cDNA coding for A. alternata enolase was cloned by two research groups using a similar approach that consisted of screening the cDNA library using a C. herbarum DNA fragment as a probe (Unger et al., 1999, Simon-Nobbe et al., 2000). In one of the studies, a skin test with recombinant protein showed a positive response in two of the seven allergics to Alternaria (Unger et al., 1999). In the second study, blotted purified recombinant Alt a 6, was recognized by 22% of A. alternata sensitized patients’ sera (n = 23) (Simon-Nobbe et al., 2000). Recently, a similar Alt a 6 sensitization prevalence among an A. alternata allergic population (n = 30) was found by the application of resolved-component diagnostics (Postigo et al., 2011). Native enolase purified from an A. alternata extract possesses high thermostability and presents a molecular weight of 47 kDa and an optimal pH of 6.8 (Kustrzeba-Wójcicka and Kmiecik, 2001). Enolases (also called 2-phospho-d-glycerate hydrolyses or phosphopyruvate hydratase) are highly expressed key enzymes of glycolysis and gluconeogenesis catalyzing the interconversion of 2-phospho-d-glycerate and phosphoenolpyruvate (Wagner et al., 2000). Because these enzymes are highly conserved, investigations of IgE reactivities of various fungal enolases by cross-inhibition experiments revealed that enolase is a highly cross-reactive fungal allergen (Simon-Nobbe et al., 2000), as described in detail below.

2.5. Alt a 8

The recombinant A. alternata NADP+-dependent mannitol dehydrogenase (MtDH), 28.6 kDa was recognized by 41% of A. alternata allergic patients (n = 22), and its cross-reactivity with MtDH of C. herbarum was confirmed by inhibition-ELISA experiments (Schneider et al., 2006). These enzymes mediate the reversible conversion of mannitol to fructose in the metabolism cycle of mannitol, which serves as a storage or translocated carbohydrate that seems to be needed for fungal phytopathogenesis (Véllez et al., 2007, 2008).

2.6. Alt a 13

Alt a 13 is a 26 kDa protein that belongs to the glutathione-S-transferase (GST) protein family. The biochemical function of these enzymes is that they take part in the detoxification of endogenous and xenobiotic compounds or their metabolites by catalyzing their biotransformation in glutathione conjugates that present lower toxicity and are more easily excreted outside the cell than their parental compounds (McGoldrick et al., 2005).

Shankar et al. produced the recombinant protein and referred to Alt a 13 as a major A. alternata allergen demonstrating that 14 of the 17 patients who presented a skin reaction to an A. alternata extract recognized rGST and nGST (Shankar et al., 2006). In a recent report, the same authors bioinformatically identified epitopes and residues of the Alt a 13 molecule that seemed to be involved in the IgE-reactivity (Shankar et al., 2009).

2.7. Alt a 14

Manganese-dependent superoxide dismutases (MnSODs) are phylogenetically conserved enzymes that are involved in defense against oxidative stress by preventing oxidative damage (Cramer et al., 1996). Our research group identified the natural A. alternata MnSOD, Alt a 14, as an IgE-reactive protein of 24 kDa (Postigo et al., 2011). More recently, the recombinant molecule was produced, and it was demonstrated that 11.5% of the sixty-one A. alternata-sensitized patients’ sera reacted to the rAlt a 14 by IgE immunoblotting (Gabriel et al., 2015b). In the same work, we found that rAlt a 14 was also able to bind IgE from patients who were clinically diagnosed as suffering of Allergic Bronchopulmonary Aspergillosis (ABPA), which suggested that the sensitization to this A. alternata allergen may be related to the pathogenesis of ABPM.

2.8. Alta 15

Serine proteases (SPs), particularly subtilisin SP, are recognized as one of the most important families of allergenic proteins (Radauer et al., 2008), and they have been extensively designed as pan-allergens (Lee et al., 2007; Shen et al., 2007). Recently, a subtilisin-like serine protease was cloned and characterized as an official allergen of A. alternata, named Alt a 15 (Gabriel et al., 2016). Immunoblotting analyses revealed that IgE antibodies from a population sensitized to A. alternata (n = 59) bound to rAlt a 15 with a prevalence of 10.2%. It was also showed that IgE-mediated sensitization to this allergen is highly specific for patients who are poly-sensitized to molds, namely to Curvularia lunata (Gabriel et al., 2016).

It is well known that the proteases produced by various allergenic sources may directly impact respiratory epithelial biology, thus playing an important role in initiating an allergic response in the lung (Yike, 2011; Wills-Karp et al., 2010). Proteases, which act at the same time as allergen, may enhance bystander-type reactions, thereby facilitating antigen access to the subepithelium and potentially modifying other allergens in such a way that they become more potent antigens.

Furthermore, it has been demonstrated that intrinsic SP-specific activity of Alternaria alternata extracts plays an important role in the physiopathogenesis of asthma via the elicitation of an increase in the permeability of bronchial epithelial cells (Leino et al., 2013) and the promotion of a rapid and robust release of early innate mediators and prolonged Th2 inflammation (Kouzaki et al., 2009; Boitano et al., 2011; Snegrove et al., 2014). The above-mentioned data support the need to further investigate the allergic properties of A. alternata SPs.

2.9. Non-IUIS A. alternata allergenic proteins

Five other A. alternata proteins (Alt a TCTP, Alt a NTF2, Alt a 2, Alt a 9 and Alt a 70 kDa) that were reported to be able to bind IgE from sensitized patients are not encompassed in the referred official allergen list, but they were already comprised in the Allergome database (http://www.allergome.org/). The translationally controlled tumor protein from A. alternata (Alt a TCTP) was characterized as an allergenic protein with the demonstrated ability to react with 4% (n = 16) of A. alternata patients’ sera (Rid et al., 2009). A. alternata nuclear transport factor 2, Alt a NTF2, was described as a 13.7 kDa IgE-reactive protein that reacts with a low prevalence (4 of n = 480) with sera proceeding from mold-sensitized individuals (Weichel et al., 2003). However, the sensitization prevalence in a population of patients sensitized to Alternaria was not studied. Reports respective to Alt a 2 allergen characterization are controversial. First, it was reported to be a major allergen with a sensitization rate of 61% among Alternaria-sensitive patients (Bush et al., 1999). In a study performed by Asturias et al. (Asturias et al., 2005), it was surprisingly found that none of the 42 A. alternata-sensitized patients’ sera reacted with Alt a 2. Alt a 9 was identified as a 42 kDa protein that bound IgE in 5% of patients (Achatz et al., 1995). For Alt a 2, Alt a 9 and Alt a 70 kDa proteins, the structural and biochemical functions are unknown. The last one was reported to induce skin reactivity in 87% (n = 16) of the A. alternata-sensitized patients (Portnoy et al., 1990).

3. A. alternata allergens and cross-reactivity

The allergen cross-reactivity phenomenon typically occurs when IgE antibodies that are originally raised against one allergen bind or recognize a similar protein from another allergenic source. Such recognition
can trigger an allergic reaction or can be completely irrelevant for the patient. The clinical relevance of cross-reactivity depends on factors such as the host, exposure and implied allergen (Ferreira et al., 2004). Cross-reacting allergens are evolutionary conserved proteins that play vital functions and are therefore ubiquitously distributed in several biological sources (Hauser et al., 2010).

The cross-reactivity of A. alternata with other airborne fungal species has been extensively described (Gupta et al., 2002a,b), and there is evidence that a significantly high percentage of patients sensitized to A. alternata are poly-sensitized to more than one other fungal species and might also be sensitized to other environmental aeroallergen sources such as pollens, mites or even to food allergens (Mari et al., 2003). It is well known that the existence of cross-reactivity, particularly among different molds, may have implications for the diagnosis and treatment of fungal allergies (Cramer et al., 2009). In terms of diagnosis, it is common to observe that if a patient is primarily sensitized to highly conserved allergenic proteins, the patient's serum could also give a positive result for a cross-reactive component from different allergic mold species. This occurrence often complicates the identification of the primary sensitizer specie and results in defective management of the allergic disease. From both diagnostic and treatment perspectives, it is important that poly-sensitization resulting from allergen cross-reactivity be distinguished from co-sensitization to multiple allergenic sources. Immunologically, co-sensitization occurs when genuine sensivity be distinguished from co-sensitization to multiple allergenic the allergic disease. From both diagnostic and treatment perspectives, it is important that poly-sensitization resulting from allergen cross-reactivity be distinguished from co-sensitization to multiple allergenic sources. Immunologically, co-sensitization occurs when genuine sensitivity to more than one allergen source is not due to cross-reactivity because it is not mediated by shared epitope-specific antibodies (Canonica et al., 2013). Therefore, for all allergological relevant allergen source, clarifying the cross-reactive structures and genuine markers among the whole allergen array is necessary.

Most A. alternata allergenic proteins are at least potentially cross-reactive across one or more species. As can be observed in Table 2, A. alternata possesses allergens that have their homologs in the 3 other genera that, together with Alternaria, are most commonly associated with the development of fungal allergy: Cladosporium, Penicillium and Aspergillus.

The high identity scores of the alignments are strong evidence of the potential for the existence of IgE cross-reactions (Bowyer et al., 2006). A sequence identity of at least 50% has been accepted as required for allergenic cross-reactivity (Aalberse, 2005); however, it can also rarely be detected when lowest sequence homologies are noticed. Differences in the amino acid sequence or structure, localization and/or amount of allergenic proteins reflect differences of allergenic properties of homologs and are determinant factors of cross-reactivity.

In relation to the major A. alternata allergen, the Allergome database (http://www.allergome.org/) includes a list of 171 Ait a 1-related proteins from several species of Alternaria and related taxa; however, to date, no evidence of IgE reactivity has been assessed for most of these molecules. In fact, most A. alternata-related taxa have been described to be associated mainly with the pathogenicity of plants and spoilage and the biodeterioration of fruits, feedstuffs and foods (Thomma, 2003) instead of environmental allergenic sources. Analysis of the molecular diversity of indoor Alternaria isolates in the USA revealed that species of Alternaria section Alternaria represented 98% (153 isolates), of which 137 isolates were assigned to A. alternata, 15 to the A. arborescens specie and a single isolate to A. burnsii (Woudenberg et al., 2015). Gutiérrez-Rodríguez et al. demonstrated that fungal species from the Pleosporaceae family, such as Stemphylium and Ulocladium, have a common allergen that shows a high level of cross-reactivity with Ait a 1 from A. alternata (Gutiérrez-Rodríguez et al., 2011). Given the scarce but existing evidence of Ait a 1 homolog allergenicity and even with their expected low frequencies as environmental contaminants, evaluating the presence of other species that liberate Ait a 1-like proteins could contribute to the total allergenic Ait a 1 load to which atopic patients are exposed and may be an interesting task to consider further.

The remaining A. alternata allergens are mostly phylogenetically conserved proteins that are recognized as cross-reactive fungal allergen classes that may cross-react with homologous protein from other fungal species but also from organisms from different phyla. Numerous reports present the mediation of the cross-reaction between minor A. alternata allergens and other fungal allergenic species (Breitenbach et al., 1997; Rid et al., 2009; Shankar et al., 2005; Simon-Nobbe et al., 2000, 2008; Weichel et al., 2003). Among these, enolase (Ait a 6), for which cross-reactivity with Cladosporium, Saccharomyces, Candida, Aspergillus and Hevea brasiliensis was demonstrated (Breitenbach et al., 1997; Simon-Nobbe et al., 2000), is considered to be the A. alternata cross-reactive allergen with high allergological importance fundamentally in terms of diagnosis (Postigo et al., 2011). Recently, we undertook efforts to identify and characterize two new cross-reacting allergens: Ait a 14, a MnSOD, and Ait a 15, a vacuolar SP. In our experiments, these two allergens seemed to play a role in the cross-reactivity phenomena between A. alternata and other important allergenic molds, namely A. fumigatus and C. lunata. Specifically, it was found that Ait a 14 showed cross-reactivity with Asp f 6 (Gabriel et al., 2015b), an A. fumigatus allergen that, together with Asp f 4, is regarded as a specific marker for ABPA (Fricke-Hidalgo et al., 2010; Hemmann et al., 1999). Some studies have referred to A. alternata as one of the potential etiologic agents of this hypersensitivity-mediated disease of the lower airways (Chowdhry et al., 2012; Singh and Denning, 2012; Chowdhry et al., 2014). Related to this, Jubit et al. observed a significant association of ABPA with a prior or concomitant sensitization to Alternaria and proposed that it may be the result of an allergenic cross-reactivity between products from Alternaria and Aspergillus classes that may cross-react with homologous protein from other fungal species but also from organisms from different phyla. Numerous reports present the mediation of the cross-reaction between minor A. alternata allergens and other fungal allergenic species (Breitenbach et al., 1997; Rid et al., 2009; Shankar et al., 2005; Simon-Nobbe et al., 2000, 2008; Weichel et al., 2003). Among these, enolase (Ait a 6), for which cross-reactivity with Cladosporium, Saccharomyces, Candida, Aspergillus and Hevea brasiliensis was demonstrated (Breitenbach et al., 1997; Simon-Nobbe et al., 2000), is considered to be the A. alternata cross-reactive allergen with high allergological importance fundamentally in terms of diagnosis (Postigo et al., 2011). Recently, we undertook efforts to identify and characterize two new cross-reacting allergens: Ait a 14, a MnSOD, and Ait a 15, a vacuolar SP. In our experiments, these two allergens seemed to play a role in the cross-reactivity phenomena between A. alternata and other important allergenic molds, namely A. fumigatus and C. lunata. Specifically, it was found that Ait a 14 showed cross-reactivity with Asp f 6 (Gabriel et al., 2015b), an A. fumigatus allergen that, together with Asp f 4, is regarded as a specific marker for ABPA (Fricke-Hidalgo et al., 2010; Hemmann et al., 1999). Some studies have referred to A. alternata as one of the potential etiologic agents of this hypersensitivity-mediated disease of the lower airways (Chowdhry et al., 2012; Singh and Denning, 2012; Chowdhry et al., 2014). Related to this, Jubit et al. observed a significant association of ABPA with a prior or concomitant sensitization to Alternaria and proposed that it may be the result of an allergenic cross-reactivity between products from Alternaria and Aspergillus

### Table 2

<table>
<thead>
<tr>
<th>Biochemical function</th>
<th>A. alternata allergen</th>
<th>Fungal Homologs</th>
<th>Species</th>
<th>IUIS allergens</th>
<th>SDAP E score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat shock proteins</td>
<td>Alt a 3</td>
<td>Pen c 19</td>
<td>Penicillium citrinum</td>
<td>1.9e – 27</td>
<td></td>
</tr>
<tr>
<td>Ribosomal protein P2</td>
<td>Alt a 5</td>
<td>Clh h 5</td>
<td>Cladosporium herbarum</td>
<td>8.4e – 25</td>
<td></td>
</tr>
<tr>
<td>Enolase</td>
<td>Alt a 6</td>
<td>Cla h 6</td>
<td>Aspergillus fumigatus</td>
<td>2.0e – 22</td>
<td></td>
</tr>
<tr>
<td>Acid ribosomal protein</td>
<td>Alt a 12</td>
<td>Pen c 22</td>
<td>Aspergillus fumigatus</td>
<td>1.9e – 154</td>
<td></td>
</tr>
<tr>
<td>Manganese superoxide</td>
<td>Alt a 14</td>
<td>Mala s 11</td>
<td>Malassezia sympodialis</td>
<td>4.9e – 36</td>
<td></td>
</tr>
<tr>
<td>Serine protease</td>
<td>Alt a 15</td>
<td>Curvularia lunata</td>
<td>Cladosporium herbarum</td>
<td>5.0e – 91</td>
<td></td>
</tr>
</tbody>
</table>

The link to Structural Database of Allergenic Proteins (SDAP) is http://fermi.utmb.edu/. IUIS, International Union of Immunological Societies.
species (Jubin et al., 2010). Thus, because rAlt a 14 was also able to bind IgE from ABPA patient sera, it was suggested that this MsnSOD could have important implications in A. alternata sensitization as a risk factor for the development of ABPA (Gabriel et al., 2015b). Moreover, it has been described that allergens belonging to the MsnSOD protein family may cross-react also to homologous human MnSOD, which could contribute to the perpetuation of the inflammatory response in asthma (Cramer et al., 1996).

Associations between sensitization to airborne molds (A. alternata, C. herbarum and A. fumigatus) and food allergies, namely to mushrooms and spinach, have also been reported (Herrera-Mozo et al., 2006). In a recent case report, a prior sensitization to A. alternata was associated with a severe food reaction to cross-reacting homolog mushroom proteins. A MtDH and a MnSOD from Agaricus bisporus mushroom with significant homology to Alt a 8 and Alt a 14, respectively, were identified as patient-specific IgE-binding proteins (Gabriel et al., 2015a).

Although a high number of fungal species shares IgE-binding homolog molecules, not all of them possess the same clinical importance. In fact, as an example, the well-recognized fungal pan-allergens belonging to the serine protease protein family are major allergens in several fungi, namely Aspergillus, Cladosporium, Curvularia, Penicillium, Rhodotorula and Trichophyton species; however, the A. alternata homolog (Alt a 15) presents a relatively low prevalence of recognition among A. alternata-sensitive patients. Quantitative differences rather than qualitative ones, e.g., the amount of allergen released, most likely explain why some of these proteins are minor or major allergens depending on the producer’s fungal species.

Because a significantly high number of A. alternata-sensitized patients are poly-sensitized to other aeroallergens that are simultaneously present in high frequencies such as grass, olive, cat epithelia, Dermatophagoides and cypress (Katotomichelakis et al., 2012), the existence of cross-reactive or adjuvant phenomena must be considered.

4. Alternaria allergens and their phylogenetic information

Traditionally, fungal species are systematically categorized on the basis of their morphologic characteristics. However, in recent years, promissory phylogenetic approaches mainly based on sequence comparison of ribosomal RNA genes have emerged and have allowed a more advanced classification of fungi (Schoch et al., 2012). Simultaneously, it has been increasingly proposed that the knowledge of fungal biology and how it is evolving may explain the antigenic nature of allergologically important fungal species.

The evolutionary process seems to be responsible for the observation that a limited number of fungal species produce a specific allergen that is highly recognized by the human immune system. In fact, the immune system of each atopic individual will recognize the foreign proteins existing in the surrounding environment, and the degree of the foreign nature of the recognized allergens is highly dependent on their evolutionary distance from human proteins. Accordingly, recently, Soeria-Atmadja et al. demonstrated that IgE sensitization patterns of individuals sensitized to fungi strikingly mirrors fungal phylogenetic relationships (Soeria-Atmadja et al., 2010). These observations reflect the premise that more closely phylogenetically related fungi will have greater shared antigens.

The existence of shared allergens across fungal species causing IgE cross-reactivity suggests that some genes coding for allergens recognized by IgE antibodies may be used as powerful phylogenetic markers that are useful to categorize fungi systematically and to identify proteins causing IgE cross-reactivity through the fungal kingdom. In this regard because A. alternata has been considered the main producer of fungal allergens, it is proposed that information on the evolution process of the gene coding for the allergen may be valuable in understanding the allergen importance in fungal biology and the stimulation of the allergic response. Generally, the molecular studies founded on fungal allergen proteins are limited but results are quite promising (Davolos and Pietrangeli, 2007). In one of their studies, Hong et al. found gene homologs of the major A. alternata allergen, Alt a 1, exclusively in Alternaria and taxonomically related species. In the same study, the authors used Alt a 1 as a molecular marker to produce phylogenetic trees of Alternaria and related taxa with high resolution and bootstrap supports (Hong et al., 2005). Indeed, the evolutionary process seems to have led to the development of a species-specific allergenic protein, Alt a 1, which is restricted to several allergenic fungal species phylogenetically related to A. alternata belonging to Pleosporaceae family. This process probably explains the reason why Alt a 1 is the main primary sensitizer in a fungi-sensitized population and is widely considered to be a marker of sensitization to Pleosporaceae members (Postigo et al., 2011). Given its high phylogenetic content, the Alt a 1 gene and protein may be useful to unequivocally identify and detect a restricted group of fungal species (Gabriel et al., 2015c); this issue will be more extensively addressed below. In contrast, as described above, most A. alternata allergenic proteins are minor allergens that possess homologs across diverse fungal species.

The taxonomic distribution of Alternaria allergens homologs may be highly correlated with sensitization and cross-reactivity data. Species phylogenetically related to A. alternata are likely to produce a similar allergen array and thus produce similar sensitization patterns whereas phylogenetically distant ones will only share ubiquitous cross-reactive molecules, thus explaining why the induced IgE sensitization data are less correlative. The IgE-based hierarchical tree proposed by Soeria-Atmadja et al. positioned A. alternata in the Pleosporales species cluster with the least similarity to other species of the same order (Soeria-Atmadja et al., 2010). Because this study included only a few Pleosporales members (Curvularia lunata, Stemphylium herbarum, Epicoccum purpurascens, Phoma betae and Setomelanomro rostrato) in which an Alt a 1-like protein is not described, the lower similarity of sensitization pattern reflects that, as expected, the patients are sensitized to A. alternata mainly through Alt a 1. Future similar studies including a higher number of fungal species, namely those that are phylogenetically close to A. alternata, should be performed. This will allow obtaining the complete pattern of association between fungal molecular systematic and IgE sensitization data and ascertaining the value of Alt a 1 and other cross-reactive fungal components in the delineation of a hierarchical organization among allergy-related fungi.

To establish complete phylogenetic analyses conducted on gene coding for allergens recognized by IgE antibodies and fully correlate the data with the immune system’s response displayed in fungi-sensitized individuals, research on allergenic protein homologs existing across fungal species is critical.

5. Exposure to A. alternata allergens and its assessment

Airborne measurement of mold exposure showed that increased atmospheric levels of fungal spores are linked to severe manifestations of respiratory allergic responses, occurrence of hospital admissions and deaths related to asthma (Targonski et al., 1995).

The threshold Alternaria count in the air necessary to elicit allergic symptoms has been estimated to be 100 spores/m³ (Ricci et al., 1995). Compared with the concentration associated with the onset of allergic symptoms for Cladosporium spores (3000 spores/m³) (Gravesen, 1981), that with Alternaria are the most widespread mold spores found in aerobiological surveys, the potent allergenicity of Alternaria is clear. This is in agreement with a previous study, where the comparison between sensitization to fungi with corresponding fungal spore counts found in the same environment showed that Alternaria presented a significantly higher score for sensitization (Beaumont et al., 1985).

Knowledge of the aerodynamic characteristics and distribution of allergens and how and when exposure is increased is essential to try to establish relationships between allergen exposure and specific immune responses. Alternaria distribution is reported to be related to the geographic area, season, atmospheric condition and time of day (Rotem,
1994). Given that _Alternaria_ species are prevalent phytopathogens, the concentration of allergenic airborne spores can also be linked to the release of spores from infected plants; thus, areas where agricultural activities are prevalent may be conducive to the environmental dissemination of allergenic _A. alternata_ spores (Corden et al., 2003).

Fungal exposures differ from prevalent environmental allergens, such as pollens, both in their quantity and in the duration of exposure to the allergenic source. In fact, airborne spore counts are often 1000-fold higher than pollen counts (Salvaggio et al., 1971), and a prolonged intense exposure to _Alternaria_ spores normally occurs for months. Typically, exposure to pollens, for example ragweed pollen, occurs for weeks (Dang and Lawrence, 2014). However, because the pattern of pollen exposure is also described to be quite variable dependent on plant species, geographical location and climate conditions pollen season can last for months (Burge, 2002).

It has been documented that asthmatic patients sensitized to _Alternaria_ tend to suffer a more severe outcome during the late summer and early autumn (Chakrabarti et al., 2012; Canova et al., 2013), when the highest _Alternaria_ spore counts are recorded (Rodriguez-Rajo et al., 2005; Segvic Klarić and Peljepjak, 2006). Pulimood et al. found that epidemic thunderstorm-related asthma is strongly associated with _Alternaria_ species sensitization and fragmentation of _Alternaria_ spores results in easily breathable allergenic fragments (Pulimood et al., 2007). A high concentration of fungal spores, their transportation over large distances and other environmental factors that might contribute to bronchial hyper-responsiveness are associated with thunderstorms, such as ozone and a sudden reduction of temperature, and may explain the association. Because _Alternaria_ species grow on cereals, it has been proposed that the farming practice contributes to increasing fungal spore levels (Pulimood et al., 2007). Global climatic change and CO₂ concentration also appear to stimulate _A. alternata_ sporulation and total antigen production (Wolf et al., 2010), also consistent with the increase in the prevalence of pollen allergies and asthma severity (Beggs and Bambrick, 2005).

The presence of _A. alternata_ allergen indoors has also been detected, namely colonizing in textile home stuffs such as carpets and bedding (Peters et al., 2008). In a US Indoors survey, the prevalence of symptomatric asthma was strongly correlated with _A. alternata_ counts in the dust samples of patients’ homes (Salo et al., 2006).

Because of the significant negative effects of _A. alternata_ on human health and plants, the correct detection of _A. alternata_ and its allergens is of great importance not only in the clinical setting but also in environmental, epidemiological and plant pathology studies. Several strategies have evolved to sample, identify and interpret exposure to fungi (Tovey and Green, 2005). The traditional methods of fungal identification and exposure evaluation are based on readily observable morphological features and spore counts (Guarro et al., 1999). The delineation of the _Alternaria_ species is complicated by methods that have several associated limitations; these methods are time consuming, laborious, and they are not always reliable because they require the skills of a trained person.

Recently, the epidemiological information obtained by traditional methods has been actively used to protect and enhance the quality of life in the sensitized/allergic subjects, though much work is needed in this field. Several protein- and DNA-based methods have merged and are considered useful tools for monitoring fungal exposure levels in a given indoor or outdoor environment. They can be performed on a large number of samples, often quickly and without the need of technical skills in mycological techniques.

Protein-based methods include immunoassays such as enzyme-linked immunosorbert assays (ELISA) that use polyclonal or monoclonal antibodies to quantify _A. alternata_ antigens. Currently, some ELISA kits are commercially available for the specific detection and quantification of the _A. alternata_ major allergen, Alt a 1. ELISA techniques are based on the interaction between antibodies and the specific allergens, and the possibility of cross-reactivity with other nontarget proteins, namely homologs proteins from other sources, can lead to false-positive results. The main advantages of immunochemical assays such as ELISA are that they provide quantitative results and allow direct assessment of the allergen with a low set-up cost, moderated running time, and no special requirements for expert knowledge.

Recently, Meng et al. found no significant difference in the detection rate of _Alternaria_ between asthmatic and non-asthmatic homes by either spore counting or cultivable airborne detection (Meng et al., 2012). However, in a previous cross-sectional study, a polyclonal anti- _Alternaria_ antibody assay was successfully employed to detect _Alternaria_ allergens in US homes; exposure to _A. alternata_ allergens in US homes has been observed to be associated with active asthma symptoms (Salo et al., 2006). The same assay allowed ascertaining that _Alternaria_ antigen levels are influenced not only by regional and housing characteristics but also by residents’ behavior (Salo et al., 2005). The use of a monoclonal antibody-based ELISA confirmed that risk of respiratory symptoms in patients sensitized to _A. alternata_ was significantly correlated with atmospheric Alt a 1 levels (Feo Brito et al., 2012). Capture ELISA was also described as a very sensitive, specific and reproducible assay for Alt a 1 detection in dust samples collected in poultry farms (Prester and Macan, 2010).

Alternatively, development of DNA-based methods has enabled more reliably quantifying fungal species. Several detection and quantification PCR-based methods with acceptable set-up costs and running times have emerged. Given its highly variable content, the large number of copies per cell and deposited sequences in international databases, Internal Transcribed Spacer (ITS) regions of the rRNA gene have been utilized as a target for assessing the fungal diversity in environmental samples (Rittenour et al., 2012). The main advantages of such molecular techniques are their specificity and minimal susceptibility to cross-reactivity phenomena because the chosen target sequence can be adapted from target allergen-encoding sequences to species-specific DNA markers. Despite the advantages of DNA-based methods, PCR is still much contested because when detecting a gene encoding for an allergen, it does not necessarily imply its expression. Consequently, the results obtained by DNA detection do not account for the actual allergenic potential. However, the same happens with some, if not most, ELISA tests that do not necessarily detect the allergenic proteins but rather detect species-specific protein markers. In fact, the detection of a molecular marker gives indirect information regarding the allergenic potential, but provides evidence of the presence of the allergenic ingredient.

A recent molecular study demonstrated that Alt a 1 can be used as a marker to successfully detect allergenic and pathogenic _Alternaria_ and related taxa by PCR (Gabriel et al., 2015c). The PCR system using a primer set, previously used by other authors for the production of recombinant Alt a 1, allowed the detection of the closely taxonomically related species _A. alternata_ and _A. tenuissima_.

6. Allergen based-component diagnosis: a new era of _Alternaria_ allergy diagnosis

It is now generally accepted that the diagnosis of an allergy must be based on the clinical history of symptoms and on confirmative assays that may include the determination of allergen specific IgE antibodies by skin prick tests, laboratory-based in vitro analyses and/or provocation tests. According to a large Pan-European GA(2)LEN skin prick test study (Bousquet et al., 2009), an _Alternaria_ extract should be included in the minimum standard battery of test inhalant allergens that are used to appropriately assess sensitization across Europe (Table 3).

The combination of skin prick tests and determination of allergen-specific IgE levels in the serum is currently recommended for a reliable assessment of _Alternaria_ sensitization. In diagnosis investigations, complex crude extracts are widely used. However, there are several...
limitations associated with the use of whole complex extracts that include the presence of irrelevant molecules and potentially highly cross-reactive allergens, the limited representation of some allergens and the well-documented variability between preparations of crude extracts (Shreffler, 2011). This could be the origin of the significant low concordance between SPT and serum IgE test results for Alternaria (O’Driscoll et al., 2009).

Some studies investigating the biochemical and immunological content of commercially available Alternaria skin prick test solutions found high variability with respect to their protein, antigen and allergen contents (Kespohl et al., 2013; Esch, 2004; Vailes et al., 2001). Many factors attributed to the different steps of fungal extract production could be responsible for an inconsistent skin test preparation, which can result in an erroneous diagnosis. The use a specific mold strain and growth conditions preferred by the manufacturers is considered to be the first source of variation. Then, different extraction methods, with or without additives and final allergen quantification systems are also likely to be potential sources of variability (Kespohl et al., 2013; Saenz-de-Santamaria et al., 2006; Portnoy et al., 1993). Recently, Twaroch et al. observed that Alternaria strains, media nutritional components and growth periods have an enormous impact on the presence of IgE-binding proteins in the final extract (Twaroch et al., 2015). With regard to the most clinically relevant Alternaria allergen, it was demonstrated that Alt a 1 expression is highly dependent on fungal growing time, cultivation media (Ibarrola et al., 2004) and strain (Martinez et al., 2006). In cases of poly-sensitized patients, the application of diagnosis methods based on extracts obtained from related and non-related allergenic sources where the existence of cross-reactive allergens is well known results in difficulty in identifying the primary sensitizer (Crameri, 2011); therefore, no discrimination is allowed between cross-reactivity and co-sensitization.

To overcome crude extract-based diagnosis limitations, molecular or component-resolved diagnosis (CRD) using individual recombinant or native allergenic molecules tested on a fluorescence enzyme immunoassay (ImmunoCAP) or a microarray-based assay platform are now widely available in Europe (Shreffler, 2011). The availability of high-quality recombinant allergens by the recent advances in recombinant DNA technology for allergen research has allowed definition of individual sensitization profiles and an understanding of the nature of sensitization as well as noted makers of severity, persistence or cross-reactivity. Thus, more informed choices can be made regarding strategies for allergen-specific immunotherapy (Cromwell et al., 2011).

To date, only two allergens (rAlt a 1, rAlt a 6) are commercially available for the molecular diagnosis of an Alternaria allergy. It has been currently accepted that the use of the species-specific Alternaria allergen, Alt a 1, in molecular diagnosis, indicates a genuine sensitization to A. alternata, even if close homolog allergens might occur across the fungal kingdom (Twaroch et al., 2012a,b). However, in the absence of sensitization to Alt a 1, the application of an individual A. alternata allergen with known cross-reactivity can improve diagnostic sensitivity, thereby permitting the identification of cross-sensitization and allowing the unequivocal definition of primary sensitizers in poly-sensitization cases.

In a preliminary study, the use of two recombinant molecules, Alt a 1 and Alt a 6, by skin prick test seemed to correctly diagnose Alternaria sensitization in 7 tested subjects (Unger et al., 1999). Asturias et al. observed that replacing A. alternata extracts with either natural or recombinant Alt a 1 forms seemed to be sufficient for a reliable diagnosis of Alternaria sensitization (Asturias et al., 2005). Recently, Postigo et al. revealed that 2 of the 30 A. alternata allergic-patients did not react to Alt a 1 or Alt a 6 but did react to a potentially cross-reactive MnSOD, now officially named Alt a 14; according to the authors, Alt a 14 should be included in the molecular diagnosis allergen array (Postigo et al., 2011).

It is hoped that the identification and characterization of the whole array of Alternaria allergens and new techniques based on allergenic recombinant proteins will allow for the preparation of better quality test solutions, improve Alternaria allergy diagnoses and guide to a more effective specific immunotherapy using a single or a few allergenic molecules. In fact, from a clinical point of view, the achievement of good quality biological materials for diagnosis and immunotherapy of allergic patients is one of critical aspects to consider in the IgE-mediated allergy field. As previously mentioned, the absence of a standardized extract could undoubtedly have clinical relevance and the replacement of crude extracts with purified allergens can contribute to solve this issue. However there is still a long way to go until immunotherapy of mold allergy will be safe and successful (Simon-Nobbe et al., 2008).

The most important steps to achieve an effective therapy for a fungal allergy are, indeed, the accurate diagnosis and exposure assessment to A. alternata and its individual components. Because A. alternata is mainly an outdoor aeroallergen, its avoidance as a preventive therapeutic measure is difficult to achieve; however, advances in aerobiological research aiming to define clinical thresholds for fungal allergen loads and to determine the extent and timing of exposure may provide information on the implementation of appropriate protective health measures at both individual and population levels. Thus, ideally aeroallergen-counting centers should allow prediction of future epidemics and warn patients with fungal sensitization to avoid places or activities in areas with high allergen/spores loads, use personal protective equipment (e.g. masks) and/or programmed prophylactic therapy. In addition, some precautions, especially at home, may reduce and/or prevent indoor exposure to allergenic fungal species (Salo et al., 2005; Sanchez and Bush, 2001). Regular cleaning to avoid the accumulation of debris and dust, reducing dampness in buildings to prevent moisture-related problems and avoiding indoor smoking are some of the reported protective measures that may significantly abate indoor fungal growth (Salo et al., 2005; Khan and Karuppayil, 2012).

### 7. Conclusion

Compared to other common environmental allergen sources, fungi are reported to be neglected and underestimated. As A. alternata allergens continue to rise, enabling research on the role of this fungal species and its allergenic components, particularly in asthma, makes sense.

Establishing correlations of A. alternata exposure and allergic symptoms remains problematic. It is critically important to adopt a multidisciplinary approach using both epidemiologic and molecular tools to accurately evaluate the exposure trends to A. alternata and its individual allergens and to determine the potential health effects.

As presented in this review, A. alternata allergens may be considered potent markers of phylogeny, sensitization and exposure. Complete characterization of fungal allergens and the analysis of a distinct pattern of allergenic species distribution will aid in understanding and predicting cross-reactivity and improving allergy diagnostic methodologies. Moreover, an understanding of the phylogenetic content of unique and shared allergens will provide insights into fungal biology, allergenicity and fungi-induced immune responses.

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**Table 3**

Skin prick test standard allergen battery to assess sensitization across Europe suggested by Global Allergy and Asthma European Network (Bousquet et al., 2005; Bousquet et al., 2012).

<table>
<thead>
<tr>
<th>Pollen</th>
<th>Grass</th>
<th>Birch</th>
<th>Artemisia</th>
<th>Olive</th>
<th>Parietaria</th>
<th>Ambrosia</th>
<th>Cypress</th>
<th>Plane</th>
</tr>
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<tbody>
<tr>
<td>Mites</td>
<td>Dermatophagoides pteronyssinus (Dermatophagoides farinae)</td>
<td></td>
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<tr>
<td>Animals</td>
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<td>Dog dander</td>
<td>Alternaria</td>
<td>Cladosporium</td>
<td>Blatella</td>
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<tr>
<td>Molds</td>
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<td>Insects</td>
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