Abstract:
Birch and hazel pollen are the most common causes of allergic diseases that occur during the spring season. For decades, the method of quantitative determination of specific IgE (sIgE) against allergen extracts has been used in the laboratory diagnosis of allergies. In recent years, there has been the possibility of determining sIgE by individual allergenic molecules, either native or recombinant. This method, known as Component Resolved Diagnosis (CRD), has made it possible to identify major and cross-reactive allergenic molecules.

Genuine allergenic molecules in birch (Bet v 1), hazel (Cor a 1) and hazelnut (Cor a 8, 9, 11, 14) are responsible for allergic reactions in most patients. Moreover, they cause a severe clinical picture in patients with hypersensitivity, in contrast to cross-reactive molecules, eg. Bet v 1 homologues in vegetables (celery, soy) and fruits (apple, cherry, pear), or Bet v 2 homologues in vegetables (celery, carrot) and fruits (musk melon, peach, banana), that cause milder symptoms.

In the diagnostic context, future research will make it possible to determine the diagnostic significance of the detection of individual allergen molecules responsible for allergy symptoms in each individual patient.

The application of bioinformatics will certainly help in the interpretation of the findings, especially in cases when multiplex assays are applied. Particular progress is also expected in the application of allergen molecules to allergen-specific immunotherapy.

Keywords: allergy; birch pollen; hazel pollen; hazelnut, component resolved diagnosis

Genuine and cross-reactive allergenic molecules of birch and hazel

Slavica Dodig1, Marta Navratil1, Ivan Pavić3, Ivana Čepelak1

1 Department of Medical Biochemistry and Hematology, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia
2 Department of Pulmonology, Allergology, Immunology and Rheumatology, University Children’s Hospital Zagreb; Faculty of Medicine, University of Osijek, Croatia
3 Department of Pulmonology, Allergology, Immunology and Rheumatology, University Children’s Hospital Zagreb; School of Medicine, University of Split, Croatia

SAŽETAK:
Prave i križno-reaktivne alergenske molekule breze i lijeske
Pelud breze i lijeske najsječići su uzročnici alergijskih bolesti koje se pojavitljju tijekom proljeća. U laboratorijskoj dijagnostici alergija već se desetljećima koristi metoda kvantitativnog određivanja specifičnog IgE (sIgE) prema alergenskim ekstraktima. Posljednjih godina postoji mogućnost određivanja sIgE prema pojedinačnim alergenskim molekulama, bilo nativnim ili rekombinantnim. Ova metoda, poznata kao komponentna dijagnostika (CRD), omogućila je identifikaciju glavnih i križno-reaktivnih alergenskih molekula.

Prave alergenske molekule breze (Bet v 1), lijeske (Cor a 1) i liješnjaku (Cor a 8, 9, 11, 14) su prave molekule koje uzimaju poseban pozornik kod pacijenata. Kao i kod križno-reaktivnih molekula, nekoliko postoji mogućnost određivanja sIgE prema pojedinačnim alergenskim molekulama, bilo nativnim ili rekombinantnim. Ova metoda, poznata kao komponentna dijagnostika (CRD), omogućila je identifikaciju glavnih i križno-reaktivnih alergenskih molekula.

Prave alergenske molekule breze (Bet v 1), lijeske (Cor a 1) i liješnjaku (Cor a 8, 9, 11, 14) odgovorne su za alergijske reakcije kod većine pacijenata. Stoviše, kod pacijenata s preosjetljivostu izazivaju tešku kliničku sliku, za razliku od križno-reaktivnih molekula, npr. homologa Bet v 1 iz povrća (celer, soja) i voća (jabuka, trešnja, kruška) odnosno homologa Bet v 2 iz povrća (celer, mrkva) i voća (dinja, breška, banana), koji uzrokuju blaže simptome.
U dijagnostičkom kontekstu, buduća će istraživanja omogućiti utvrđivanje dijagnostičke značajnosti otkrivanja pojedinačnih alergenskih molekula odgovornih za simptome alergije kod svakog pojedinog pacijenta.

Primjena bioinformatike zasigurno će pomoći u tumačenju nalaza, osobito u slučajevima kada se primjenjuju multipleks-testovi. Poseban napredak očekuje se i u primjeni alergenskih molekula u alergen-specifičnoj imunoterapiji.

**Kljучне риеčи:** alergija; pelud breze; pelud lijeske; lješnjak; komponentna dijagnostika

**Introduction**

Pollens play a major role in the onset of seasonal allergic disorders, mediated by IgE (eg, conjunctivitis, rhinitis and/or asthma) all over the world (1,2). The most clinically relevant allergenic plants in Europe are grasses, trees and weeds (3). Springtime allergies are caused by tree pollen that mostly belong to the orders Fagales (birch, hazelnut, alder, beech, oak), Proteales (plane tree, sycamore), Pinales (cypress), and Lamiales (ash, olive, lilac) (4). The order Fagales encompasses two main families - Betulaceae and Fagaceae; Betulaceae includes the genera Betula (birch), Corylus (hazel), Alnus (alder), Carpinus (hornbeam), and Ostrya (hop-hornbeam). The family Fagaceae comprise the genera Quercus (oak), Castanea (chestnut), and Fagus (beech) (5,6). Among order Fagales, birch, alder and hazel are the most potent cause of tree pollen allergy. During pollination, the onset of symptoms in hypersensitive patients depends on the number of allergenic grains in the air. The birch pollen concentrations greater than 30 grains/m³/24 h trigger severe symptoms, and values greater than 80 grains/m³/24 h produce allergy symptoms in 90% of patients. The hazel pollen causes allergic reaction at concentrations between 20 and 30 grains/m³/24 h (7). The threshold value for the occurrence of allergy symptoms in subjects with hypersensitivity to alder pollen is 45 pollen grains in m³/24 h) (8).

In Croatia, birch pollination was found to be most pronounced in the period from February to May, with peak grain count in April (7). The highest pollination of alder and hazel was recorded during February and March. About 70% of patients with rhinitis / asthma caused by hypersensitivity to birch pollen have an associated hypersensitivity to cross-reacting food allergens (9).

The basic laboratory diagnosis of allergies is based on 1. the detection of the type of allergic reaction (IgE-mediated allergies or non-IgE-mediated allergies) and 2. the detection of the triggers that led to allergic reaction (determination of IgE against allergen extracts and allergenic components) (10). This is followed by 3. the detection of cross-reactivity between individual allergens or allergenic components or molecules, respectively, Figure 1 (11).

**Figure 1.** Simplified allergy diagnosis algorithm. In laboratory diagnostics, only procedures related to IgE-mediated allergies are shown; CRD – component resolved diagnosis.
Persons with atopy have an increased concentration of total IgE (tIgE) and specific IgE (sIgE) against causative allergens. When determining the concentration of either tIgE or sIgE, it is important to keep in mind the time elapsed since exposure to the causative allergen. (12,13). Everyday practice has shown that IgE concentration was always significantly higher 2 to 6 months after exposure to seasonal allergens. After that time, its concentration begins to decrease.

Component Resolved Diagnosis, CRD, or Molecular Allergy Diagnosis, MAD, respectively, for determination of serum sIgE concentration against purified natural and recombinant allergenic molecules is used recently (14 - 16). Molecules of natural (n) allergens are obtained by purification of allergen extracts using chemical, chromatographic, electrophoretic and / or immunoaffinity methods. The production of recombinant (r) allergen molecules is a complex process that includes 1. extraction and isolation of messenger RNA (mRNA) from allergen sources, 2. synthesis of complementary DNA (cDNA), 3. electrophoretic separation of individual components of allergen sources, 4. preparation of primers for polymerase chain reaction (PCR), 5. amplification of cDNA of individual allergenic components, and 6. expression of (r) allergens (eg. rBet v1, rBet v2, rBet v4, etc.). CRD thus allows detection of sIgE to potential causative allergenic molecules that trigger an allergic reaction in a person with atopic disease.

**Allergen Nomenclature**

Allergen name consists of three letters from the genus, one letter from the species epithet and an Arabic numeral, eg. Bet v 1 - *Betula verrucosa* (the major allergen from birch pollen), Cor a 1 - *Corylus avellana* (the major allergen from hazel pollen). The number is given in order of their discovery, so these mentioned allergens were the first discovered allergens from birch and hazel pollen, respectively (17,18).

<table>
<thead>
<tr>
<th>Allergen family</th>
<th>Genuine molecules</th>
<th>Cross reactive molecules</th>
</tr>
</thead>
</table>
| **Profilins**   | Birch pollen (Bet v 2)  
Bet v 2 homologues:  
Tree pollen: olive (Ole e 2), goosefoot (Che a 2) | Bet v 2 homologues:  
Vegetables:* celery (Api g 4), carrot (Dau c 4)  
Fruits:* muskmelon (Cuc m 2), peach (Pru p 4), banana (Mus xp 1)  
Seeds:* mustard (Sin a 1) |
| **PR-10-P**     | Birch pollen (Bet v 1) #  
Bet v 1 homologues:  
Tree pollen: alder (Aln g 1), hazel (Cor a 1), hornbeam (Car b 1), chestnut (Cas s 1), beech (Fag s 1), oak (Que a1) cypress (Cup a 1), | Bet v 1 homologues:  
Fruit (apple (Mal d 1), cherry (Pru av 1), pear (Pyr c 1), stone fruits, eg. peanut (Ara h 8));  
Vegetables:* celery (Api g 1), soy (Gly m 4) |
| **nsLTPs**      | Stone fruit:  
Hazelnut (Cor a 8) | Stone fruit  
Hazelnut (Cor a 8) cross-reactive with peach (Pru p 3), both ## |
| **Storage proteins** | Stone fruit  
Hazelnut (Cor a 9, Cor a 11, Cor a 14) ## | |

# – Marker of genuine (species-specific) sensitization; ## – risk- or severity-associated molecules; * – sensitive to heat/digestion

**Allergen Definition**

Allergens are proteins, glycoproteins, lipoproteins or conjugates of proteins and haptons, with a known nucleotide and / or amino acid sequence, carbohydrate composition and relative molecular mass (Mr 5 - 150 kDa) (16,19).

According to the chemical structure, major allergens can be classified into a list of different structural families. Some of them are, for example: pathogenesis-related class 10 proteins (PR-10-P), protease inhibitor/storage lipid transfer proteins (LTP), cupin superfamily (11S and 7S plant seed storage globulins), lipocalin/cytosolic fatty-acid binding protein family, EF hand family, papain-like cysteine protease, profilins, thiamatin-like proteins, group 1/2/3 grass pollen allergen i.e. expansin family, expansin with N-terminal domain, group 5/6 grass pollen allergen, glycosyl hydrolases family 17 (endo-beta-1,3-glucosidase), hyaluronidase, cyclophilin type peptidyl-prolyl cis–trans isomerase, and FAD linked oxidase/berberine bridge enyzme-like family, etc. (19).

In clinical practice, it is important to distinguish genuine (primary) sensitization and cross reactivity, particularly in presumed polysensitizations. Today, the allergist has the possibility to refer a patient who has symptoms during the spring season to a laboratory diagnosis i.e. determination of sIgE against genuine allergen molecules and cross-reactive molecules of birch and hazel pollen, but also allergen molecules present in plant-food, especially in patients who have plant-food syndrome. Table 1. shows genuine and cross-reactive allergenic molecules from different allergen families with associated data on the stability of individual molecules according to heat and digestion, as well as the risks and severity of symptoms that can trigger in patients with hypersensitivity.
According to the chemical structure, allergic plant molecules are classified into several families, e.g. profilins, pathogenesis-related proteins (PR-10-P), non-specific lipid transfer proteins (nsLTPs), storage globulins (eg. 7S seed storage globulin, 11S seed storage globulin), binding proteins (eg. polcalcins) (Table 2). Some plant allergens are enzymes, eg. phenyl coumaran benzyl ether reductase, cyclphilin (peptidyl-prolyl isomerase), glutathione-S-transferase (Table 3.). Allergens are characterized by acidic isoelectric point, pl = 5.7±0.15 (with exception nsLTPs) and negative electrostatic potential (20). These molecules are responsible, for example, for plant growth, differentiation, defense of the plant from bacteria, viruses and fungi.

**Genuine and Cross-Reactive Allergen Molecules**

The allergenicity of an allergenic molecule can be defined as the potential of that molecule to trigger an allergic reaction. Allergenicity depends on the structure of the allergenic molecule, i.e. size (Mr), acidity (pl), charge, solubility, 3D protein-folding, and stability (20,25).

Until the introduction CRD (MAD) of allergies, allergens were classified as major and minor allergens, taking into account two criteria: the IgE-binding frequency and the induction of immediate skin prick test response. According to the first criterion, “major” allergens can sensitize more than half of individuals with atopy (> 50% IgE-binding), in contrast to “minor” allergens that can sensitize less than half of allergic persons (<50% IgE-binding), and according to another criterion, “major allergens” can induce immediate skin test responses in > 90% of allergic individuals, and “minor allergens” in <20% of patients (26). Certainly, the major epitope of allergenic molecule should be located at the molecular surface, so that it could be accessible to its slgE.

CRD has advanced the accurately define the genuine sensitization to allergens, and has made it possible to distinguish between genuine (primary or marker molecules) and cross-reactive allergenic molecules. Genuine pollen allergen molecules are those allergenic molecules that are responsible for family- or species-specific sensitization (6). They induce sensitization and the production of slgE, and triggers a reaction between the allergenic molecule and its slgE, which ultimately leads to the appearance of allergy symptoms.

Cross-reactive allergenic molecules are similar (homologues) to genuine molecules, belong to the same genus, and can trigger an allergic reaction when the similarity with the genuine molecule is greater than 70%. If their similarity is less than 50% cross-reactive molecules will not trigger an allergic reaction (25). Cross-reactive allergenic molecules can cause an allergic reaction after previous contact of patient with the main sensitizer (6). Cross-reactive molecules are found in plant fruits, and are similar to the genuine molecule in pollen. Because of their similarity, the IgE molecule recognizes both, the genuine allergen molecule and its sIgE, which ultimately leads to the appearance of allergy symptoms.

Cross-reactive allergenic molecules are similar (homologues) to genuine molecules, belong to the same genus, and can trigger an allergic reaction when the similarity with the genuine molecule is greater than 70%. If their similarity is less than 50% cross-reactive molecules will not trigger an allergic reaction (25). Cross-reactive allergenic molecules can cause an allergic reaction after previous contact of patient with the main sensitizer (6). Cross-reactive molecules are found in plant fruits, and are similar to the genuine molecule in pollen. Because of their similarity, the IgE molecule recognizes both, the genuine allergen molecule and its sIgE, which ultimately leads to the appearance of allergy symptoms.

Cross-reactive allergenic molecules are similar (homologues) to genuine molecules, belong to the same genus, and can trigger an allergic reaction when the similarity with the genuine molecule is greater than 70%. If their similarity is less than 50% cross-reactive molecules will not trigger an allergic reaction (25). Cross-reactive allergenic molecules can cause an allergic reaction after previous contact of patient with the main sensitizer (6). Cross-reactive molecules are found in plant fruits, and are similar to the genuine molecule in pollen. Because of their similarity, the IgE molecule recognizes both, the genuine allergen molecule and its sIgE, which ultimately leads to the appearance of allergy symptoms.

Cross-reactive allergenic molecules are similar (homologues) to genuine molecules, belong to the same genus, and can trigger an allergic reaction when the similarity with the genuine molecule is greater than 70%. If their similarity is less than 50% cross-reactive molecules will not trigger an allergic reaction (25). Cross-reactive allergenic molecules can cause an allergic reaction after previous contact of patient with the main sensitizer (6). Cross-reactive molecules are found in plant fruits, and are similar to the genuine molecule in pollen. Because of their similarity, the IgE molecule recognizes both, the genuine allergen molecule and its sIgE, which ultimately leads to the appearance of allergy symptoms.

**Genuine and Cross-Reactive Allergen Molecules**

The allergenicity of an allergenic molecule can be defined as the potential of that molecule to trigger an allergic reaction. Allergenicity depends on the structure of the allergenic molecule, i.e. size (Mr), acidity (pl), charge, solubility, 3D protein-folding, and stability (20,25).

Until the introduction CRD (MAD) of allergies, allergens were classified as major and minor allergens, taking into account two criteria: the IgE-binding frequency and the induction of immediate skin prick test response. According to the first criterion, “major” allergens can sensitize more than half of individuals with atopy (> 50% IgE-binding), in contrast to “minor” allergens that can sensitize less than half of allergic persons (<50% IgE-binding), and according to another criterion, “major allergens” can induce immediate skin test responses in > 90% of allergic individuals, and “minor allergens” in <20% of patients (26). Certainly, the major epitope of allergenic molecule should be located at the molecular surface, so that it could be accessible to its slgE.

CRD has advanced the accurately define the genuine sensitization to allergens, and has made it possible to distinguish between genuine (primary or marker molecules) and cross-reactive allergenic molecules. Genuine pollen allergen molecules are those allergenic molecules that are responsible for family- or species-specific sensitization (6). They induce sensitization and the production of slgE, and triggers a reaction between the allergenic molecule and its slgE, which ultimately leads to the appearance of allergy symptoms.

Cross-reactive allergenic molecules are similar (homologues) to genuine molecules, belong to the same genus, and can trigger an allergic reaction when the similarity with the genuine molecule is greater than 70%. If their similarity is less than 50% cross-reactive molecules will not trigger an allergic reaction (25). Cross-reactive allergenic molecules can cause an allergic reaction after previous contact of patient with the main sensitizer (6). Cross-reactive molecules are found in plant fruits, and are similar to the genuine molecule in pollen. Because of their similarity, the IgE molecule recognizes both, the genuine allergen molecule and its sIgE, which ultimately leads to the appearance of allergy symptoms.

Cross-reactive allergenic molecules are similar (homologues) to genuine molecules, belong to the same genus, and can trigger an allergic reaction when the similarity with the genuine molecule is greater than 70%. If their similarity is less than 50% cross-reactive molecules will not trigger an allergic reaction (25). Cross-reactive allergenic molecules can cause an allergic reaction after previous contact of patient with the main sensitizer (6). Cross-reactive molecules are found in plant fruits, and are similar to the genuine molecule in pollen. Because of their similarity, the IgE molecule recognizes both, the genuine allergen molecule and its sIgE, which ultimately leads to the appearance of allergy symptoms.

Cross-reactive allergenic molecules are similar (homologues) to genuine molecules, belong to the same genus, and can trigger an allergic reaction when the similarity with the genuine molecule is greater than 70%. If their similarity is less than 50% cross-reactive molecules will not trigger an allergic reaction (25). Cross-reactive allergenic molecules can cause an allergic reaction after previous contact of patient with the main sensitizer (6). Cross-reactive molecules are found in plant fruits, and are similar to the genuine molecule in pollen. Because of their similarity, the IgE molecule recognizes both, the genuine allergen molecule and its sIgE, which ultimately leads to the appearance of allergy symptoms.

Cross-reactive allergenic molecules are similar (homologues) to genuine molecules, belong to the same genus, and can trigger an allergic reaction when the similarity with the genuine molecule is greater than 70%. If their similarity is less than 50% cross-reactive molecules will not trigger an allergic reaction (25). Cross-reactive allergenic molecules can cause an allergic reaction after previous contact of patient with the main sensitizer (6). Cross-reactive molecules are found in plant fruits, and are similar to the genuine molecule in pollen. Because of their similarity, the IgE molecule recognizes both, the genuine allergen molecule and its sIgE, which ultimately leads to the appearance of allergy symptoms.

Cross-reactive allergenic molecules are similar (homologues) to genuine molecules, belong to the same genus, and can trigger an allergic reaction when the similarity with the genuine molecule is greater than 70%. If their similarity is less than 50% cross-reactive molecules will not trigger an allergic reaction (25). Cross-reactive allergenic molecules can cause an allergic reaction after previous contact of patient with the main sensitizer (6). Cross-reactive molecules are found in plant fruits, and are similar to the genuine molecule in pollen. Because of their similarity, the IgE molecule recognizes both, the genuine allergen molecule and its sIgE, which ultimately leads to the appearance of allergy symptoms.

Cross-reactive allergenic molecules are similar (homologues) to genuine molecules, belong to the same genus, and can trigger an allergic reaction when the similarity with the genuine molecule is greater than 70%. If their similarity is less than 50% cross-reactive molecules will not trigger an allergic reaction (25). Cross-reactive allergenic molecules can cause an allergic reaction after previous contact of patient with the main sensitizer (6). Cross-reactive molecules are found in plant fruits, and are similar to the genuine molecule in pollen. Because of their similarity, the IgE molecule recognizes both, the genuine allergen molecule and its sIgE, which ultimately leads to the appearance of allergy symptoms.

Cross-reactive allergenic molecules are similar (homologues) to genuine molecules, belong to the same genus, and can trigger an allergic reaction when the similarity with the genuine molecule is greater than 70%. If their similarity is less than 50% cross-reactive molecules will not trigger an allergic reaction (25). Cross-reactive allergenic molecules can cause an allergic reaction after previous contact of patient with the main sensitizer (6). Cross-reactive molecules are found in plant fruits, and are similar to the genuine molecule in pollen. Because of their similarity, the IgE molecule recognizes both, the genuine allergen molecule and its sIgE, which ultimately leads to the appearance of allergy symptoms.
Table 2. Basic characteristics of plant allergenic proteins

<table>
<thead>
<tr>
<th></th>
<th>Profilins</th>
<th>PR-10 proteins#</th>
<th>nsLTPs#</th>
<th>Polcalcins</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Allergen source</strong></td>
<td>Timothy grass pollen</td>
<td>Birch pollen</td>
<td>pomegranate; Peach: seeds, fruits, leaves, roots, pollens</td>
<td>PolLens of trees, grasses and weeds</td>
</tr>
<tr>
<td><strong>Mr (kDa)</strong></td>
<td>14 - 17</td>
<td>17</td>
<td>LTP1s: 9-10; LTP2s: 7</td>
<td>8 - 9</td>
</tr>
<tr>
<td><strong>Length (amino acids)</strong></td>
<td>100 - 131</td>
<td>154-163</td>
<td>LTP1s: 90-95; LTP2s: 70</td>
<td>78 (monomer)</td>
</tr>
<tr>
<td><strong>Isoelectric point</strong></td>
<td>5.07</td>
<td>5.39</td>
<td>9.25 (8.8 to 12)</td>
<td>4.39</td>
</tr>
<tr>
<td><strong>3D structure</strong></td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Molecular structure</strong></td>
<td>Central 6-stranded β sheet and two α-helices</td>
<td>Seven-stranded anti-parallel β-sheet flanked by three α-helices</td>
<td>C-terminal alpha-helices, 6 - 8 cysteine residues</td>
<td>α-helices</td>
</tr>
<tr>
<td><strong>Disulfide bonds</strong></td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>Glycosylation</strong></td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><strong>Heat and digestion resistant</strong></td>
<td>Thermally labile</td>
<td>Thermolabile/ resistant to proteases</td>
<td>High resistance to gastrointestinal proteolysis, to pH and high temperatures</td>
<td>Stable to thermal denaturation</td>
</tr>
<tr>
<td><strong>Function</strong></td>
<td>Cell growth, differentiation, proliferation, motility, and cytokinesis</td>
<td>Protective role against pathogens bacteria, fungi and viruses</td>
<td>Transfer membrane lipids; defending plants from bacteria, fungi and viruses</td>
<td>Control of intracellular calcium levels during pollen germination</td>
</tr>
<tr>
<td><strong>Cross-reactivity</strong></td>
<td>Between different pollen sources and some plant foods, and latex</td>
<td>Some pollens (within order Fagales), plant food (mostly fruits and some vegetables)</td>
<td>Within nsLTP family; in plant food and vegetables, but also in pollen and latex</td>
<td>Within pollens, only</td>
</tr>
<tr>
<td><strong>Allergy symptoms</strong></td>
<td>Mostly mild (rhinitis, conjunctivitis)</td>
<td>Mild to severe (conjunctivitis, rhinitis, asthma)</td>
<td>High risk of severe systemic reactions (angioedema, anaphylaxis), following the intake of foods (raw, cooked or preserved foods)</td>
<td>Mild to severe respiratory symptoms; marker of polysensitisation; no connection with food allergy</td>
</tr>
</tbody>
</table>

# – major allergens; 3D – three dimensional structure available.
Allergenic Birch Pollen Molecules

Currently, seven allergen molecules have been defined in birch pollen (18,28-33). They belong to different protein families, i.e. PR-10-P profilins, polcalcin-like proteins, phenyl coumaran benzyl ether reductase, cyclophilin and glutathione-S-transferase, as shown in Table 3. Among them, Bet v 1 belongs to genuine allergenic molecules, and other molecules are cross-reactive. Bet v 1 homologous molecules can be found in pollen of related Fagales trees (hazel - Cor a 1, alder Aln g 1, beech - Fag s 1, oak - Que a 1, hornbeam - Car b 1, chestnut - Cas s 1).

In addition, it should be mentioned that allergenic molecules homologous to the Bet v 1 molecule can be found in fruits and vegetables, such as Rosaceae fruits (e.g. apple - Mal d 1, pear - Pyr c 1, cherry - Pru av 1, apricot - Pru ar 1), Apiaceae vegetables (e.g. celery- Api g 1, carrot - Dau c 1), as well as in nuts, seeds, legumes (eg. hazelnut - Cor a 1, peanut - Ara h 8, soybean - Gly m 4 (6,24). Amino acid identities between Bet v1 and its homologous molecules from the PR-10-P family (Bet v 1, Mal d 1, Pru av 1, Act d 8, Api g 1, Ara h 8, Gly m 4 and Cor a 1) are between 39 and 67%.

Table 3. Genuine and cross-reactive allergenic molecules in birch pollen (adapted according to the references 18,28-33.

<table>
<thead>
<tr>
<th>Allergenic molecule</th>
<th>Protein</th>
<th>Mr (kDa)</th>
<th>Allergenicity / cross-reactivity **</th>
<th>Prevalence among birch pollen allergic patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bet v 1</td>
<td>PR-10-P (Bet v 1 family member)</td>
<td>17</td>
<td>95 - 100 % of patients have sIgE to Bet v 1</td>
<td></td>
</tr>
<tr>
<td>Bet v 2</td>
<td>Profilin</td>
<td>15</td>
<td>22% of patients have sIgE to rBet v 2. Bet v 2 induces histamine release from blood basophils of profilin-allergic individuals, but not of individuals sensitized to other plant allergens.</td>
<td></td>
</tr>
<tr>
<td>Bet v 3</td>
<td>Polcalcin-like protein</td>
<td>24</td>
<td>Approximately 10% of patients show IgE binding to rBet v 3</td>
<td></td>
</tr>
<tr>
<td>Bet v 4</td>
<td>Polcalcin</td>
<td>7-8</td>
<td>5% of patients have sIgE to rBet v 4</td>
<td></td>
</tr>
<tr>
<td>Bet v 6 #</td>
<td>Phenyl coumaran benzyl ether reductase</td>
<td>33</td>
<td>32% of patients have sIgE ≥3.5 kUa/L (≥ class 3) to rBet v 6</td>
<td></td>
</tr>
<tr>
<td>Bet v 7</td>
<td>Cyclophilin (peptidyl-prolyl isomerase)</td>
<td>18</td>
<td>20.8% of subjects recognize Bet v 7. Patients demonstrate positive SPT with birch pollen extract.</td>
<td></td>
</tr>
<tr>
<td>Bet v 8</td>
<td>Glutathione-S-transferase</td>
<td>27</td>
<td>13% birch pollen allergic subjects recognise rBet v 8</td>
<td></td>
</tr>
</tbody>
</table>

S IgE – specific IgE; # – previously known as Bet v 5; ** – tested in vitro; r – recombinant molecule.
Allergenic Hazel Pollen and Hazelnut Molecules

According to the Allergen Nomenclature Sub-Committee three allergenic molecule have been defined in hazel pollen and eight in hazelnut (18). They all belong to different protein families, i.e. PR-10-P (Cor a 1), iso flavone reductase homologue (Cor a 6), ns-LTPs (Cor a 10), profilins (Cor a 2), luminal binding protein (Cor a 8), seed storage globulins (Cor a 9, Cor a 11), oleosins (Cor a 12, Cor a 13, Cor a 15), albumins (Cor a 14) (Table 4.). Hazel pollen allergenic molecule Cor a 1 belongs to major and genuine allergenic molecule, respectively. It should be noted that Cor a 1 belongs to the group Bet v 1- homologous allergenic molecules. Amino acid identities between Cor a1 and its homologues from the PR-10-P family (Bet v 1, Mal d 1, Pru av 1, Act d 8, Api g 1, Ara h 8 and Gly m 4) are between 43 and 67%. (EAACI). Among plant fruit allergens, Cor a 9 is also a major allergen. Genuine allergenic molecule Cor a 1 has two isoforms, with a closely related sequence identity; one is found in hazel pollen, and the other in hazelnut (6,34 - 36). Cor a 1, is heat labile, and also unstable to gastric digestion.

### Table 4. Genuine and cross-reactive allergenic molecules in hazel and hazelnut allergens (adapted according to the references 6,18,34 - 36.)

<table>
<thead>
<tr>
<th>Allergenic molecule</th>
<th>Protein</th>
<th>Mr (kDa)</th>
<th>Allergenicity / cross-reactivity **</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pollen molecules</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cor a1</td>
<td>PR-10-P (Bet v 1 family member)</td>
<td>17</td>
<td>100% patients allergic to hazelnut show IgE binding to rCor a 1</td>
</tr>
<tr>
<td>Cor a6</td>
<td>Isoflavone reductase homologue</td>
<td>35</td>
<td>No data available</td>
</tr>
<tr>
<td>Cor a10</td>
<td>nsLTP type 1</td>
<td>70</td>
<td>Between proteins from different pollens and plant foods</td>
</tr>
<tr>
<td><strong>Food molecules</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cor a2</td>
<td>Profilin</td>
<td>14</td>
<td>15.4% of hazelnut allergic patients had IgE binding to rCor a 2</td>
</tr>
<tr>
<td>Cor a8</td>
<td>nsLTP</td>
<td>9</td>
<td>Some patients with severe anaphylactic reactions to hazelnut showed sIgE binding to Cor a 8</td>
</tr>
<tr>
<td>Cor a9</td>
<td>11S seed storage globulin (legumin)</td>
<td>40</td>
<td>86% of hazelnut allergic patients with systemic reactions had IgE biding to rCor a 9</td>
</tr>
<tr>
<td>Cor a11</td>
<td>7S seed storage globulin (vicilin)</td>
<td>48</td>
<td>Cross-reactivity with Cor a 1 in about half of patients</td>
</tr>
<tr>
<td>Cor a12</td>
<td>17 kDa oleosin</td>
<td>17</td>
<td>63% of patients with hazelnut and/or peanut ingestion related symptoms.</td>
</tr>
<tr>
<td>Cor a13</td>
<td>14-16 kDa oleosin</td>
<td>14-16</td>
<td>63% of patients with hazelnut and/or peanut ingestion related symptoms.</td>
</tr>
<tr>
<td>Cor a14</td>
<td>2S albumin</td>
<td>10</td>
<td>1/3 of hazelnut allergic individuals had IgE that reacted with natural 2S albumin from hazelnut.</td>
</tr>
<tr>
<td>Cor a15</td>
<td>Oleosin</td>
<td>17</td>
<td>Found in all hazelnut SPT positive children</td>
</tr>
</tbody>
</table>

Cor a – *Corylus avellana*; PR-10-P – Pathogenesis-related protein; nsLTP – non-specific lipid transport protein; ** – tested in vitro; SPT – skin prick test.
Identification of Allergenic Molecules

Identification of particular genuine and cross-reactive allergenic molecules became possible after individual allergen molecules were isolated from allergen sources and after production of some recombinant allergenic molecules. Subsequently, methods have been developed for the determination of sIgE precisely according to these particular molecules (i.e. CDR). CRD is based on a sandwich fluoro-immunochemical or lumino-immunochemical method on a three-dimensional carrier. It can be performed as a singleplex (one sample - one allergenic molecule) and multiplex assay. The latter assay involves the simultaneous determination of the concentration of sIgE against a great number (up to 120) of allergenic molecules (37).

Recently, CRD has been increasingly used in laboratory diagnosis of allergies. The choice of allergen for singleplex assay is made based on the anamnesis, clinical findings of a patient and on the results of a skin prick test (SPT). The results are expressed in semi-quantitative ISAC Standardized Units for sIgE (ISU-E), based on the World Health Organization IgE standard (WHO 75/502). The detection range is 0 - 100 ISU-E. In the future, computer programs could help in interpretation of multiplex assays results, which would significantly help physicians in their daily practice.

Allergenic Molecules in Clinical Practice

Indirect detection of allergenic molecules by determining the concentration of sIgE has brought significant advances in understanding the immune mechanisms involved in allergic diseases. It is important for clinical practice to know whether a patient is hypersensitive to genuine allergenic molecules, cross-reactive molecules. In addition, it should be determined whether the patient has simultaneous hypersensitivity to allergenic molecules from different allergenic sources, eg. weeds and birch, i.e. cross-sensitization (38,39). It is now possible to detect hypersensitivity to genuine or cross-reactive allergenic components, which is not possible either with SPT or with the determination of sIgE against allergenic extracts. CRD allows physicians to more accurately interpret severe, moderate, or mild allergy symptoms in each individual patient, complete a previous diagnosis based on allergen extract diagnostics (using both SPT and sIgE), and adjust individual symptom prevention (advising the patient to avoid contact with causative allergens) and treatment of diseases, including allergen specific immunotherapy (ASIT).

Identification of genuine and cross-reactive molecules may be useful in subjects with: i) anaphylaxis caused by non-steroidal anti-inflammatory drugs, effort, or with idiopathic anaphylaxis; ii) delayed anaphylaxis (e.g. 3 - 6h after consumption of red meat); iii) multiple hypersensitivity, i.e. sensitivity to pollen and plant food allergens; iv) latex allergy; v) food allergy (40,41). In addition, CRD should be applied when selecting allergens for ASIT. The severity of the symptoms depends on the family to which the causative allergen molecules belong. It is known that storage proteins (genuine allergens) are generally responsible for the severe and long-lasting symptoms. Marker of genuine (species-specific) sensitization is Bet v 1. Milder and shorter-lasting symptoms are caused by cross-reactive allergens. The severity of symptoms in hypersensitivity to the allergenic molecules of birch and hazel increases from profilins to storage proteins, i.e. profilins < PR-10-P < nsLTPs < storage proteins (Figure 2.) (6,36).

Figure 2. Relationship between the severity of symptoms and the family to which individual allergen molecules belong. PR-10-Ps - Pathogenesis-related proteins; (Adapted according to the ref.)
Risk- or severity associated allergenic molecules families are 2S albumins (Cor a 14 (hazelnut), Gly m 8 (soy), other seed storage proteins (Cor a 9, Cor a 11, Gly m 5, Gly m 6), nsLTP (eg. Pru p 3, peach), Cor a 8 (hazelnut). Pollen-food allergy syndrome occurs in patients with panallergen allergy, especially in hypersensitivity to allergen molecules from the profilins, PR-10s, and nsLTPs families (42). It should be noted that stone fruits, seeds and legumes contain stable seed storage proteins (2S albumins, 7S and 11 S globulins), which are involved in direct sensitization of subjects with atopy, and can therefore cause more severe symptoms. Although hazelnut allergenic molecules Cor a 14 and Cor a 9 belong to minor allergens, they can cause clinically significant allergy symptoms due to their chemical structure (2S albumin and 11 S globulin, respectively) (6). Moderate symptoms are manifested on the conjunctiva (itch, tearing, redness), nose (itch, sneezing, runny nose, stuffy nose), ears, eg. internally, Eustachian tubes (itch), eye lids, lips, cheeks, earlobe, face (tissue swelling / angioedema). Extremely rarely, an allergic reaction can be systemic, which means the appearance of symptoms such as localized, multifocal or generalized angioedema, nausea, vomiting, diarrhea, abdominal pain, breathing difficulties, wheezing, cough, general weakness. Mild symptoms are itch of lips mucosa, oral mucosa, palate, burning, stinging of palate and throat or mild mucosal swelling at all mentioned localizations.

nsLTPs can cause allergy symptoms of varying severity, from mild pollen food allergy syndrome (PFAS) to anaphylaxis (36,43). Due to cross-reactivity between homologous allergenic molecules, patients hypersensitive to tree pollen (birch, hazel and alder) may experience transient oropharyngeal symptoms (mostly PFAS) after consumption of particular Bet v 1-related plant fruits (eg apple, pear, apricot, cherry, plum, hazelnut, walnut, almond, mango, avocado, kiwi) and vegetables (tomato, carrot, fennel, celery, soybean), those containing PR-10-P. Generally, symptoms appear often within a few minutes, (sometimes immediately) after consumption of raw plant food, not after being heat-treated or acidified (6). Symptoms of PFAS can be mild and transient, oropharyngeal symptoms, testing for sensitization and prophylactic avoidance of potentially cross-reactive foods is generally not recommended. Patients are instructed to avoid the specific raw fruits or vegetables or the nuts (raw and thermally processed) that have caused symptoms in the past (51,52) Patients who have previously eaten thermally processed (cooked, microwaved, pasteurized, or baked) fruits and vegetables without side effects may continue to eat these forms of the food (53). According to the literature, in patients with PFAS risk for systemic reactions is estimated to be between 2 and 10 percent. In case of patient with PFAS who have experienced systemic reaction, it is suggested a strict avoidance of the offending foods in all formula (42) Tolerance to cross-reactive foods should be carefully evaluated and clear instruction given of what to avoid. EPI is recommended in the following situations i) in patients with past systemic reaction, ii) allergy to peant, tree nuts and mustard, iii) reactions to cooked plant food and iv) sensitization to peach and apple in Western Mediterranean areas. Prevention of allergy symptoms implies avoiding the consumption only those Bet v 1 homologues of raw plant foods, which have caused allergic signs and symptoms. Allergen specific immunotherapy is the only etiological therapy of allergic diseases with long-term and post-treatment benefits. Several trials have demonstrated that subcutaneous immunotherapy (SCIT) or sublingual Immunotherapy (SLIT) with birch alone or birch, alder and hazel mixture is effective treatment for respiratory allergy. One trial with recombinat Bet v 1 have demonstrated similar clinical efficacy supporting the thesis that major allergen is the main cause of disease. Recently, it is suggested that ASIT
with birch pollen extract is also effective treatment of Fagales tree pollen allergy due to high degree of IgE cross-reactivity of the tree species (54). However it is not clear that ASIT with tree pollen extracts has a beneficial effect on cross-reacting plant food allergies. (55). Therefore, PFAS caused by Bet v 1 homologous allergenic molecules from plant foods without the presence of pollen induced respiratory symptoms is not an indication for ASIT in patients with tree pollen allergy (56,57).

Although, there are some evidence that sublingual ASIT to pollen allergens has some positive effects in terms of T-cell tolerance, immune deviation, and regulatory T cells, as well as allergen-specific IgG4 further prospective studies will be needed to obtain definitive confirmation (42).

**Conclusion**

Identification of genuine and cross-reactive allergens makes it possible to predict or assess the risk for the severity of allergy symptoms and their duration. Today it is known that milder and shorter symptoms are initiated by cross-reactive allergens, and that severe and long symptoms are initiated by genuine allergenic molecules. The severity of symptoms in hypersensitivity to the allergenic molecules of birch and hazel increases from profilins to storage proteins, i.e. profilins < PR-10-P < nsLTPs < storage proteins). Storage proteins are generally responsible for the severe symptoms. It is not yet clear that ASIT with pollen allergen extracts has a beneficial effect on cross-reacting plant food allergies.

**References:**

18. Allergen nomenclature, WHO/IUIS Allergen nomenclature Sub-Committee. Available at: http://www.allergen.org


38. Versluis A, van Os-Medendorp H, Kruizinga AG, Blom WM, Houwen GF, Knulst AC. Cofactors in allergic reactions to food: physical exercise and alcohol are the most important. *Immunity Inflamm Dis.* 2016;4:392-400.


42. Luengo O, Cardona V. Component resolved diagnosis: when should it be used? *Clin Translat Allergy.* 2014;4:28.


