Molecular Allergology: The Clinical Utility of Allergen Components

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Phadia
Overview

- What are allergen components?
- What type of diagnostics are there?
- Back ground – allergen components
- Study Work
- Clinical Cases
- ImmunoCAP ISAC
How can molecular allergology improve my clinical practice?

- What is triggering the reaction?
- Is this true allergy or is the patient sensitised?
- How severe is the reaction going to be?
- How long will the allergy persist?
- How can I rationalise oral food challenges?
- Can I make immunotherapy more effective in this patient?
Helping Patients Improve QoL
Allergy diagnosis - Historical overview

1880
Provocation testing

1967
Characterisation of IgE

1988-91
First allergens cloned

1995-1999
Diagnostic recombinant allergen panels

2000
First allergen chip

2007
ImmunoCAP ISAC ® goes global

In-vivo testing

1907
RAST*

2007
Diagnostic recombinant allergen panels

2007
First allergen chip

Component-resolved diagnosis
Limitations of Current Testing

- Current tests define allergen-containing sources, not specific allergenic molecules
  - Up to 50% of patients asymptomatic sensitisation\(^1\)
  - Up to 30% patients with false positive results in “open” challenges due to bias\(^2\)
- The major challenge to clinicians is distinguishing sensitisation versus true allergy\(^3\)

Two Platforms

• Allergen Components – ImmunoCAP Specific IgE
  – Over 85 different specific IgE component diagnostics
• ImmunoCAP ISAC
  – 21st Century allergy diagnostics
  – Protein array biochip
  – 103 individual components
What is an allergen?
More than one protein!

Proteins of Peanut
Whole allergen extracts vs components

Allergen raw material → Extraction → Recombinant Technology → Purification

Extract ImmunoCAP → Component ImmunoCAP

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Component Nomenclature

**Can f 1**

*Canis familiaris*, allergen # 1

Prefix “r” for recombinant or “n” for native

rCan f 1 - nCan f 1
Protein groups

Plant Foods

PR-10 proteins, Bet v 1 homologue
- Heat labile protein

Profilins
- Highly cross-reactive, present in most plants

Storage proteins
- Proteins found in seeds
- Often stable and heat resistant
- Often associated with systemic and severe reactions

LTP, lipid transfer protein
- Stable to digestion and heat
- Often associated with systemic and severe reactions in addition to OAS

CCD, cross-reactive carbohydrates
- Highly cross-reactive, present in most plants
- Seldom associated with clinical symptoms

Important:
Some species have very similar DNA and therefore share the same or similar allergen proteins. Plant Foods demonstrate high levels of cross reactivity between species e.g. wheat and grass

Others

Serum albumin’s
- A common protein present in different biological fluids and solids e.g. cow’s milk and beef, egg and chicken
- Cross-reaction between albumin from different anima species are well known. Cat and dog, cat and pork.

Enzymes
- Including Bromelin and Pepsin

Moulds
- Limited cross reactivity
- Useful for specific diagnosis

Others
- Tropomyosin- A class of highly conserved Protein, heat stable
- Egg ovomucoid - Very heat stable and enzyme resistant

Venoms
- Determine venom allergy in relation to specific species for immunotherapy purposes
PR-10 proteins
Bet v 1-homologous allergens

**PR-10 proteins, Bet v 1 homologue**

- Heat labile protein
- Often associated with local symptoms
- Often associated with allergic reactions to fruits and vegetables in northern Europe

- Tree pollen
- Fruits
- Vegetables
- Nuts

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Bet v1</td>
<td>Pr u p 1</td>
</tr>
<tr>
<td>Cor a 1</td>
<td>Ara h 8</td>
</tr>
<tr>
<td>Mal d 1</td>
<td>Gly m 4</td>
</tr>
</tbody>
</table>
Profilin’s
Bet v 2-homologous allergens

Profilins

- Highly cross-reactive, present in most plants
- Seldom associated with clinical symptoms but may cause demonstrable or even severe reactions in a small minority of patients

• Tree pollen
• Fruits
• Vegetables
• Nuts
• Grass Pollen
• Weed Pollen

<table>
<thead>
<tr>
<th>Bet v 2</th>
<th>Phl p 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pru p 4</td>
<td>Hev b 8</td>
</tr>
</tbody>
</table>
LTPs
non-specific Lipid Transfer Proteins

LTP, lipid transfer protein

• Stable to digestion and heat
• Often associated with allergic reactions to fruits and vegetables in southern Europe
• Often associated with systemic and severe reactions in addition to OAS

<table>
<thead>
<tr>
<th>Pru p 3</th>
<th>Ole e 7</th>
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<tbody>
<tr>
<td>Cor a 8</td>
<td>Ara h 9</td>
</tr>
<tr>
<td>Par j 2</td>
<td>Art v 3</td>
</tr>
</tbody>
</table>

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Storage proteins

- Legumes
- Nuts
- Grains and seeds

Storage proteins

- Proteins found in seeds
- Often stable and heat resistant
- Often associated with systemic and severe reactions

<table>
<thead>
<tr>
<th>Protein Type</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 S albumin, Ara h 2</td>
<td>Gliadins</td>
</tr>
<tr>
<td>7 S globulin, Ses i 3</td>
<td>Alpha amylase inhibitors</td>
</tr>
<tr>
<td>11 S globulin, Gly m 6</td>
<td>Vicilin, Jug r 2</td>
</tr>
</tbody>
</table>
Rule of Thumb

- Profilin and PR10 proteins
  - Highly cross reactive (PR 10 especially to Birch)
  - Often associated with less severe reactions e.g. OAS
- nsLTP’s and Storage Proteins
  - Associated with more severe reactions
  - More heat/digestive enzyme resistant and therefore can be more often associated with OAS and well as digestive problems
Tropomyosin

- A class of highly conserved protein
- Physically associated with actin and myosin in muscle fibres
- Heat stable
- Found in most edible parts of crustaceans

- Dust mite
- Cockroach
- Crustacean

Der p 10 | Pen a 1
CCD (Cross Reactive Carbohydrate Determinants)

- Many allergen are glycpcroteins
- And therefore share similar glyco-epitopes
- CCD’s therefore be highly cross reactive
- these determinants are treated as foreign epitopes in humans they are considered highly immunogenic

- Plants
- Venom
- Dust mite
Peanut Allergen Components

- Ara h 1: Storage proteins
- Ara h 2: Storage proteins
- Ara h 3: Storage proteins
- Ara h 5: Profilin
- Ara h 8: PR-10
- Ara h 9: LTP

- Ara h 1-3 are the major peanut allergens.

Peanut allergy in 3-5 year old children in UK

Hourihane, 2007

Hourihane, 2007

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MAAS Peanut Study

MAAS
1085 born into unselected population based cohort

1029 attended 8-year Follow-up

Inclusion criteria
- SPT ≥ 3mm
- sIgE > 0.2 kUa/l
- Suggestive history

110 children were peanut sensitized

81 Oral Food Challenges

11.8% were peanut sensitised

The prevalence of clinical peanut allergy among sensitised subjects 22.4%

Nicolaou, Woodcock, Custovic et al. JACI 2010
Pattern of sensitisation to allergen components among peanut allergic and tolerant individuals

Conclusion MAAS Peanut study

- The majority of children considered peanut-sensitised on the basis of standard tests do not have peanut allergy
- Ara h 2 was the most important predictor of clinical peanut allergy

Clinical implications
Measurement of IgE response to major peanut allergen Ara h 2 is more useful in predicting clinical allergy than currently used skin or blood tests
Assessment of peanut allergy

Peanut (SPT/specific IgE) + Ara h 2

- Peanut: neg
  - Ara h 2: neg
    - Low risk for severe reactions to peanut
    - Further testing: In geographical areas where birch is common consider testing for Ara h 8

- Peanut: pos
  - Ara h 2: neg
    - Risk for severe reactions to peanut
    - Further testing:
      - Risk grading:
        - Ara h 1: ●●●●
        - Ara h 3: ●●●●
        - Ara h 9: ●●●●
        - Ara h 8: ●●
        - CCD: ●

- Peanut: pos
  - Ara h 2: pos
    - High risk for severe reactions to peanut

Peanut: pos
Ara h 2: neg
Low risk for severe reactions to peanut
Case: 7 year-old girl
Consultation for regular control of eczema, rhinitis and food allergy

Personal history
• Eczema since childhood and uses steroid ointments
• Allergic rhinitis against birch
• Oral itching when eating peanuts and tree nuts
• The parents want to know if it is ok if their daughter eats peanuts & tree nuts because recently no reactions to small amounts.

IgE
Hazelnut (f17): 78 kUₐ/l
Peanut (f13): 0.40 kUₐ/l

→ Can we answer the parent’s question?
Component testing

Markers of primary sensitisation

<table>
<thead>
<tr>
<th>Allergen</th>
<th>Component</th>
<th>Marker</th>
<th>Value (kU A/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut</td>
<td>f13</td>
<td>Ara h 2</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ara h 8</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>21.5</td>
</tr>
<tr>
<td>Hazelnut</td>
<td>f17</td>
<td>Cor a 1</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cor a 8</td>
<td>69.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>Birch</td>
<td>t3</td>
<td></td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

Markers of cross reactions

PR-10

Interpretation

Primarily cross reaction with birch
If eating peanuts – mild reaction may occur
”May contain peanuts/nuts” label of less concern

Treatment
Epipen likely not needed
Wheat

Wheat allergy

• Among the 6 foods responsible for most food allergic reactions in children
• Extensive cross-reactions to grasses and fruits

• What components are important?
• Omega-5 gliadin
• Linked Clinically to Exercised Induced Anaphylaxis

1 Palosuo et al J Allergy Clinical Immunol 1999;103:912-7
2 Ito et al Allergy 2008 63:1536 - 1542
sIgE Tri a 19 (omega-5 gliadin)

88 wheat sensitised japanese children, 44 had wheat allergy and 44 had no wheat allergy

Ito et al., Allergy 2008
# Wheat cases

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Country</th>
<th>sIgE (kU_A/L)</th>
<th>Protein</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65</td>
<td>Sweden</td>
<td>3.06</td>
<td>wheat (f4)</td>
<td>8.0</td>
</tr>
<tr>
<td>2</td>
<td>32</td>
<td>Spain</td>
<td>6.14</td>
<td>omega-5 gliadin (f416)</td>
<td>0.08</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>Spain</td>
<td>24.2</td>
<td>wheat LTP (f214)</td>
<td>0.01</td>
</tr>
<tr>
<td>4</td>
<td>29</td>
<td>Spain</td>
<td>2.32</td>
<td>profilin (g212)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

* Experimental ImmunoCAP

**Tri a 14 (LTP) and Tri a 19 (ω5-gliadin) can help to discriminate wheat allergy from clinically irrelevant sensitisation**

C. Eriksson, unpublished data
Egg

- One of the most common allergies in infants and young children
- Common clinical decision - reintroducing cooked egg back into the diet

Major Egg Allergen Components\(^1\)

<table>
<thead>
<tr>
<th>Gal d 1</th>
<th>Ovomucoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gal d 2</td>
<td>Ovalbumin</td>
</tr>
<tr>
<td>Gal d 3</td>
<td>Conalbumin</td>
</tr>
<tr>
<td>Gal d 4</td>
<td>Lysozyme</td>
</tr>
</tbody>
</table>

Ovomucoid

- Consists of 3 domains containing 3 disulfide bridges each (total 9 SS-bridges)
- Very heat stable and resistance to proteases
- Relative resistant to enzymatic degradation
- High specific IgE concentrations to ovomucoid is associated with persistent egg allergy

1, Bernhisel-Broadbent et al. JACI 1994, 2, Urisu et al. IAAI 1999
3, Takagi et al. IAAI 2005 4, Järvinen et al. Allergy 2007
Ovomucoid - Ando et al 2008

- 180 Patients
- DBPFCs - Raw Egg White, Cooked Egg
- Measured patients IgE for Egg White, Ovomucoid and Oval Albumin
- Investigate the Clinical predictability of the three tests
Ovomucoid - Ando et al 2008

- Ovomucoid test demonstrated superior predicative values for cooked egg (than the other tests)
- Decision points- positive 10.8 kUA/l (PPV 95%) and negative at 1.2 PV kUA/l (PPV 95%)

Conclusions – Quantitative decision points for both egg white and ovomucoid will be useful in the diagnosis of egg allergy
Assessment of egg allergy

Egg white (SPT/specific IgE) + Ovomucoid

- Egg white: neg
  - Ovomucoid: neg
  - Low risk for clinical reactions to egg

- Egg white: pos
  - Ovomucoid: neg
  - Risk for clinical reactions to egg

- Egg white: pos
  - Ovomucoid: pos
  - High risk for clinical reactions to egg

Absence of IgE antibodies to ovomucoid indicates tolerance to ingestion to baked egg

Increased risk for persistent egg allergy
3 years old boy

Eczema since 4 months old,
eczema improved after avoidance of egg.

ImmunoCAP results (kU/L):
Egg white: 7

Is there a risk of reaction after occasional intake of small amounts of egg?

ImmunoCAP results (kU/L)
egg: 3,2
Ovomucoid: < 0,1
Egg Case
Patient Management

- The decrease of sIgE to Ovomucoid indicates the child has a good prognosis of outgrow his egg allergy.
- Ovomucoid < 0.1 indicates a low risk of reaction after intake of egg in baked or cooked food.
Case: 2 year-old boy
Consultation for regular control of food allergy

History
• Has eczema and milk / egg allergy
• Pasta with egg gave general urticaria which led to an ER visit
• Licking of ice cream gave rash around mouth.

How severe is this boy’s allergy?

<table>
<thead>
<tr>
<th>Protein</th>
<th>f</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg white</td>
<td>f1</td>
<td>13 kU_A/l</td>
</tr>
<tr>
<td>Ovomucoid</td>
<td>f233</td>
<td>10.8 kU_A/l</td>
</tr>
</tbody>
</table>
Case: 2.5 year-old girl
Consultation for regular control of food allergy + asthma?

History
- Has egg allergy, no eczema and suspicion of asthma.
- Is sensitised to cat and gets rashes when close to cat.
- Egg allergy diagnosed for the first time at 11 m of age. Urticaria and edema.
- Avoiding egg without mistakes

Can egg be introduced now into the diet?

<table>
<thead>
<tr>
<th>Egg white f1</th>
<th>0.50 kU_A/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovomucoid</td>
<td>0.47 kU_A/l</td>
</tr>
</tbody>
</table>
• Next generation allergy test for 21st Century
• Micro Protein Array
• 47 Food Types in Panel + Others
• 103 Component Tests
• Marker allergens (specific and cross reactive)
• Only 20 µl of serum needed
• Detection of IgE, IgG, IgG4 antibodies possible
Where does ISAC fit In?

• Remove subjectivity
• Patients with serve symptoms
  – Anaphylaxis
  – Rule out as many allergens as possible
• Patients with difficult to diagnose allergy
  – What is triggering the symptoms?
  – Idiopathic anaphylaxis
  – Hyper IgE syndrome
• Cost effective method if having to access multiple components
1. Summary of positive allergen-specific IgE results

Species specific components

<table>
<thead>
<tr>
<th>Allergen</th>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grass pollen</td>
<td>rCyn d 1</td>
<td>1 ISU</td>
</tr>
<tr>
<td>Grass pollen</td>
<td>rPhl p 1</td>
<td>1.6 ISU</td>
</tr>
<tr>
<td>Grass pollen</td>
<td>rPhl p 5</td>
<td>1.1 ISU</td>
</tr>
<tr>
<td>Grass pollen</td>
<td>rPhl p 6</td>
<td>0.9 ISU</td>
</tr>
<tr>
<td>Tree pollen</td>
<td>rBet v 1</td>
<td>27 ISU</td>
</tr>
<tr>
<td>Animal</td>
<td>rFel d 1</td>
<td>5.5 ISU</td>
</tr>
<tr>
<td>Animal</td>
<td>rFel d 4</td>
<td>1.5 ISU</td>
</tr>
<tr>
<td>Animal</td>
<td>rCan f 1</td>
<td>2 ISU</td>
</tr>
<tr>
<td>Mould</td>
<td>rAll e 1</td>
<td>9.4 ISU</td>
</tr>
<tr>
<td>Mite</td>
<td>rDer p 2</td>
<td>11 ISU</td>
</tr>
<tr>
<td>Mite</td>
<td>rDer f 1</td>
<td>14 ISU</td>
</tr>
<tr>
<td>Components with limited cross-reactivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR-10 protein</td>
<td>rBet v 1</td>
<td>27 ISU</td>
</tr>
<tr>
<td>Apple</td>
<td>rMa l d 1</td>
<td>2 ISU</td>
</tr>
<tr>
<td>Peach</td>
<td>rPru p 1</td>
<td>1.4 ISU</td>
</tr>
<tr>
<td>Cross-reactive components</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protholin</td>
<td>rBet v 2</td>
<td>1.6 ISU</td>
</tr>
</tbody>
</table>
Sensitisation ≠ Allergy

Traditional tests
- Detection of sensitisation to natural allergens using extract based tests (protein soup)

Component resolved Diagnostics (CRD)
- Detection of sensitisation to allergen components
  - Higher specificity
  - Higher sensitivity (quantitative performance)
  - Understanding cross-reactivity
  - Identifying severity markers

Exposure → Sensitization → Allergy
Impact on clinical use

• Identify patients at risk of severe reactions
  – Peanut – Ara h 2
  – Wheat – Tri a 14 and Tri a 19

• Identify patients at risk of persistent symptoms
  – Egg – Gal d 1 = ovomucoid

• Predict cross-reactivity
  – Hazel Nut and Birch – Gly m 4 and Bet v 1
  – Latex and fruit – Hev b 6 och Hev b 8

• Identity the correct components in use with Immunotherapy
Thank you!