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How can Molecular Allergology improve clinical practice?

- What is triggering the reaction?
- Is this true allergy or is the patient sensitised?
- Is the patient at risk to severe allergy?
- How can I rationalise oral food challenges?
Allergy Diagnosis – Historical Overview

- **Provocation testing**
- **Characterisation of IgE**
- **First allergens cloned**
- **Recombinant allergen panels. 1st CRD.**
- **First allergen chip**
- **ImmunoCAP ISAC ® goes global**

**In-vivo testing**

**In-vitro testing**

**Component-resolved diagnosis**

- **1880**
- **1967**
- **1980-91**
- **1995-1999**
- **2000**
- **2007**
Cross Reactivity? What is it?

Pollen

IgE to Grass Pollen

Sensitisation

Grass Profilin, Phl p 12 (Primary Sensitiser)

Allergen protein molecule families are very similar in their macro molecular structure.

Peanut Profilin Ara h 5 (Possible Cross Reactivity)
Pru av 1 (green) and the structure of Bet v 1 (orange)

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
Component Resolved Diagnostics

Allergenic source  Allergenic extract

Specific allergen components  Cross-reactive allergen components
Peanut components

Latin name: Ara h = *Arachis hypogaea*
Risk assessment of peanut components

- Labile protein
  - Profilin
    - Ara h 8
  - PR-10
    - Ara h 9
- Stable protein
  - LTP
  - Storage Proteins
    - Ara h 1
    - Ara h 2
    - Ara h 3

Increasing risk to cause severe symptoms and reactions
Peanut - Ara h2

UK –
MAAS cohort study –
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

France
• Ara h 2 was the most important predictor with a positive cut off of 0.23 ku/l

Australian Infant Cohort Study
• Ara h 2 was the most important predictor with a positive cut off of 1.19 ku/l
• Suggested 0.1 as cut off for home challenge

Denmark
• Ara h 2 was the most important predictor with a positive cut off of 1.63 ku/l
Eller E, Bindslev-Jensen C Clinical value of component-resolved diagnostics in peanut allergic patients Allergy 2013;68(2):190-4
Why do we need to know if allergic or sensitised?

Randomized Trial of Peanut Consumption in Infants at Risk for Peanut Allergy

George Du Toit, M.B., B.Ch., Graham Roberts, D.M., Peter H. Sayre, M.D., Ph.D., Henry T. Bahnson, M.P.H., Suzana Radulovic, M.D., Alexandra F. Santos, M.D., Helen A. Brough, M.B., B.S., Deborah Phippard, Ph.D., Monica Basting, M.A., Mary Feeney, M.Sc., R.D., Victor Turcanu, M.D., Ph.D., Michelle L. Sever, M.S.P.H., Ph.D., Margarita Gomez Lorenzo, M.D., Marshall Plaut, M.D., and Gideon Lack, M.B., B.Ch., for the LEAP Study Team

Early introduction important in high-risk infants.
Nut components

<table>
<thead>
<tr>
<th>Nut</th>
<th>Profilin</th>
<th>PR-10 protein</th>
<th>LTP</th>
<th>Storage Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEANUT</td>
<td>Profilin</td>
<td>Ara h 8</td>
<td>Ara h 9</td>
<td>Ara h 1</td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td>Ara h 2</td>
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<td></td>
<td></td>
<td></td>
<td>Ara h 3</td>
</tr>
<tr>
<td>HAZEL NUT</td>
<td>Profilin</td>
<td>Cor a 1</td>
<td>Cor a 8</td>
<td>Cor a 9</td>
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<td>Cor a 14</td>
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<tr>
<td>WALNUT</td>
<td>Profilin</td>
<td></td>
<td>Jug r 3</td>
<td>Jug r 1</td>
</tr>
<tr>
<td>BRAZIL NUT</td>
<td>Profilin</td>
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<tr>
<td>CASHEW NUT</td>
<td>Profilin</td>
<td></td>
<td></td>
<td>Ana o 3</td>
</tr>
</tbody>
</table>
Food Proteins

**Serum albumins**
- 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 animal species are well known. Cat and dog, cat and pork, Cow’s milk and Cat

**Others**
- **Tropomyosin** - A class of highly conserved
  - Protein, heat stable
- **Egg ovomucoid** - Very heat stable and enzyme resistant
Cow’s Milk

Whey protein

β-lactoglobulin ~35%
α-lactalbumin ~12%
glycomacropeptide ~12%
proteose peptone 3 ~12%
immunoglobulins ~8%
serum albumin ~5%
lactoferrin ~1%
lactoperoxidase ~0.5%
minor proteins ~15%

Casein

αS1-, αS2-, β-, κ-casein

Composition of Milk Protein

Whey protein 20%
Casein 80%
Casein

• Heat stable protein

• Proteins remain stable even after 120mins boiling at 100°C

• α-lactalbumin disappeared after 30mins
• β-lactoglobulin after 15mins
• Lactoferrin after 10mins

Casein

The usefulness of casein-specific IgE and IgG4 antibodies in cow’s milk allergic children

Komei Ito1*, Masaki Futa1ura1,2, Robert Mové1are3,4, Akira Tanaka5, Tsutomu Kawabe6, Tatsuo Sakamoto7 and Magnus P. Borres5,8

- High levels of casein-sIgE antibodies are strongly associated with milk allergy in children and might be associated with prolonged allergy

- The casein test showed the best discriminating performance with a clinical decision point of 6.6 kUA/L corresponding to 100% specificity for milk allergy

Children with Specific IgE to Casein above 1.8kUA had six fold increase in risk of anaphylaxis

1. Ito et al. Clinical and Molecular Allergy 2012, 10:1 The usefulness of casein-specific IgE and IgG4 antibodies in cow’s milk allergic children
Casein

- Low levels of Casein can indicate tolerance to baked milk products
- 75% of children with a recent diagnosis of CMPA tolerated baked milk

(25% high risk of Anaphylaxis)

Why baked milk?

Dietary baked milk accelerates the resolution of cow’s milk allergy in children

Jennifer S. Kim, MD,* Anna Nowak-Węgrzyn, MD,* Scott H. Sicherer, MD, Sally Noone, RN, Erin L. Moshier, MS, and Hugh A. Sampson, MD New York, NY

• Tolerance of baked milk is a good indicator for outgrowing allergy

• The addition of baked milk into the diet appears to accelerate tolerance
Egg – Ovomucoid

- Allergy to egg is generally agreed to be one of the most common causes of food allergy in infants and young children.
- IgE antibodies to egg white in infancy are a good indicator of atopy and predict the development of disease later in life.
- Common clinical decision - reintroducing cooked egg back into the diet.

Major Egg Allergen Components

Gal d 1  **Ovomucoid**
Gal d 2  Ovalbumin
Gal d 3  Conalbumin
Gal d 4  Lysozyme

**DIETARY BAKED EGG ACCELERATES RESOLUTION OF EGG ALLERGY IN CHILDREN**

Stephanie A. Leonard, MD, Hugh A. Sampson, MD, Scott H. Sicherer, MD, Sally Noone, RN, Erin L. Moshier, MS*, James Godbold, PhD*, and Anna Nowak-Wegrzyn, MD
Jaffe Food Allergy Institute, Division of Pediatric Allergy, Mount Sinai School of Medicine, New York, NY

*Department of Preventative Medicine, Mount Sinai School of Medicine, New York, NY

It is useful to investigate recombinant specific IgE to better diagnose food-dependent exercise-induced anaphylaxis

**Soya – Gly m 5 & 6**

- Raised specific IgE to Gly m 5 and Gly m 6 indicates true soya allergy and risk of severe reactions.

Caution: Sensitisation to Gly m 4, a PR-10 protein, is common in patients allergic to birch-related tree pollen. Local reactions with large quantities of raw protein.

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2. Ito K et al. IgE to Gly m 5 and Gly m 6 is associated with severe allergic reactions to soybean in Japanese children. J Allergy Clin Immunol. 2011
<table>
<thead>
<tr>
<th>Allergen Components as severity markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut</td>
</tr>
<tr>
<td>Soy</td>
</tr>
<tr>
<td>Wheat</td>
</tr>
<tr>
<td>Milk</td>
</tr>
<tr>
<td>Egg</td>
</tr>
</tbody>
</table>
Microarray Testing

- ImmunoCAP ISAC includes common allergens, plus many more
- Overall 112 allergen components from 51 sources
- Components represented are:
  - Species-specific
  - Cross-reactive
Which patients?

- Complex history patients – What is their current atopic status?
- Eczema patients – multiple allergens
- Idiopathic anaphylaxis - ImmunoCAP ISAC identified further useful clinical information in 20% of this group of patients from a UK study\(^1\)
- Multi-sensitised patients – possible cross reactions? Or genuine primary allergens?
- Immunotherapy patients
  - Has the primary allergen been identified?

Rachel

Re-referral to Allergy Clinic (Age 12)

• Had been under local paediatrician
• Family request 2\textsuperscript{nd} Opinion
• Struggling with allergies
Rachel

- Multiple Food Allergy
  (Cow’s Milk, Egg, Peanut, Tree Nuts, Shellfish, Sesame, Tomato, Onion, Peach)
- Eczema (now improving)
- Perennial Allergic Rhinitis with Seasonal Exacerbation
- Latex Allergy
- Chronic Urticaria
Rachel

- Allergic Rhinitis –
  Maximal pharmacotherapy
  ?immunotherapy

At worst April - June
Food Allergy

Cow’s Milk – Reaction to formula as infant
Egg – Reaction to scrambled egg (when weaning)
Peanut & Tree Nuts – Never exposed
Shellfish – never exposed
Sesame - Reaction to sesame biscuit (age 4)
Tomato, Onion, Peach – Oral tingle, eczema flare

Latex - ?flare of eczema as baby / avoided since
## Skin Prick Test Results

<table>
<thead>
<tr>
<th>Positive control</th>
<th>5mm</th>
</tr>
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<tbody>
<tr>
<td>Grass pollen</td>
<td>14mm</td>
</tr>
<tr>
<td>Tree pollen</td>
<td>16mm</td>
</tr>
<tr>
<td>Silver birch pollen</td>
<td>9mm</td>
</tr>
<tr>
<td>House dust mite</td>
<td>8mm</td>
</tr>
<tr>
<td>Alternaria</td>
<td>Negative</td>
</tr>
<tr>
<td>Cat dander</td>
<td>3mm</td>
</tr>
<tr>
<td>Latex</td>
<td>Negative</td>
</tr>
<tr>
<td>Egg</td>
<td>7mm</td>
</tr>
<tr>
<td>Cow’s Milk</td>
<td>8mm</td>
</tr>
<tr>
<td>Sesame</td>
<td>4mm</td>
</tr>
<tr>
<td>Prawn</td>
<td>5mm</td>
</tr>
<tr>
<td>Tomato (fresh)</td>
<td>Negative</td>
</tr>
<tr>
<td>Peach (fresh)</td>
<td>Negative</td>
</tr>
<tr>
<td>Onion (fresh)</td>
<td>Negative</td>
</tr>
<tr>
<td>Peanut</td>
<td>8mm</td>
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<tr>
<td>Hazelnut</td>
<td>6mm</td>
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<tr>
<td>Almond</td>
<td>3mm</td>
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<tr>
<td>Walnut</td>
<td>9mm</td>
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<tr>
<td>Brazil nut</td>
<td>6mms</td>
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<tr>
<td>Cashew nut</td>
<td>6mms</td>
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<tr>
<td>Pistachio</td>
<td>7mms</td>
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## Specific IgE Results

<table>
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<tr>
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<th>Value</th>
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<th>Value</th>
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<tbody>
<tr>
<td>Egg</td>
<td>32.00</td>
<td>Cow’s Milk</td>
<td>22.40</td>
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<tr>
<td>Ovomucoid</td>
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<td>Milk BOS D8</td>
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<td>RAST Peanut</td>
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<td>Sesame</td>
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<tr>
<td>Peanut ARA H 2</td>
<td>&lt;0.35</td>
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<tr>
<td>Peanut ARA H 8</td>
<td>84.64</td>
<td>Shrimp</td>
<td>5.23</td>
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<tr>
<td>Cashew</td>
<td>1.40</td>
<td>Shrimp PEN A1</td>
<td>12.8</td>
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<tr>
<td>Cashew ANA O3</td>
<td>&lt;0.35</td>
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<tr>
<td>Walnut</td>
<td>3.96</td>
<td>Peach Pru P3</td>
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<td>Walnut JUG R1</td>
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<tr>
<td>Walnut JUG R3</td>
<td>0.37</td>
<td>Latex</td>
<td>&lt;0.35</td>
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<td>Hazelnut</td>
<td>23.4</td>
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<tr>
<td>Hazelnut COR A1</td>
<td>68.2</td>
<td>Grass Pollen</td>
<td>&gt;100</td>
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<td>Hazelnut COR A8</td>
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<td>Birch Pollen</td>
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<td>Hazelnut COR A9</td>
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<td>Bet v1</td>
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<td>Hazelnut COR A14</td>
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<tr>
<td>Brazil Nut</td>
<td>4.2</td>
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<tr>
<td>Brazil Nut BER E1</td>
<td>&lt;0.35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Food Challenges

• Peanut
• Hazelnut
• Cashew
• Brazil Nut
• Baked Milk
• Baked Egg
• Shrimp
• Latex
Immunotherapy

• Grass & Birch Pollen
Conclusions

• Component testing gives us the ability to diagnose more accurately and perform a risk assessment
• Microarray testing is useful and more cost effective in the multiply sensitised patient / eczema patient
• Increased QOL for patients
• Health economic benefit
Thank you...

Any Questions...

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