CENTRE DE RECHERCHE PUBLIC

Development of Allergy Markers

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CRP-Santé

- Extract vs purified molecule
- Technical procedures for identification
- Clinical cases:
  - Confirmation of diagnosis and identification of new allergen markers
  - CRD: improvement of diagnosis
- Molecules available at CRP-Santé
- Conclusion

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What is an allergen?

Dermatophagoides pteronyssinus

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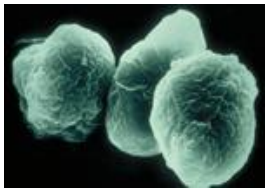
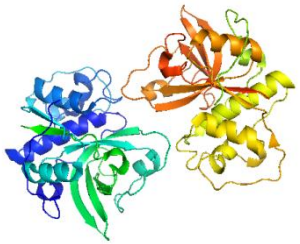
Excrements

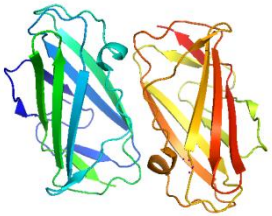


Mix of allergens and non-allergens

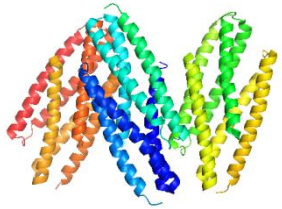
Der p 1



Der p 2



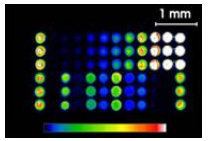
Der p 5



IgE antibodies recognize single molecules

15 different mite allergens have been characterized until now

History of Allergy Diagnosis



Skin-prick-test

Immunotherapy

IgE

RAST

First cloned allergens

Research using recombinant allergens

Microarray

1880 1911 1967 1988-1991 1995-1999 2000

Tests *In vivo*

Tests *In vitro*

Component-resolved diagnosis

Immunotherapy trials with rec allergens

2004

Adapted from C.Harwanegg, CLI Nov 2004

From total extracts to purified molecules

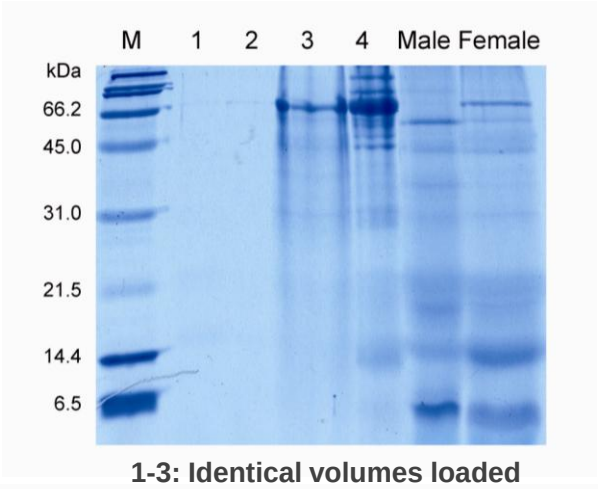
Drawback of extracts

- Extracts are **complex mixtures** of allergenic and non-allergenic molecules
- Allergen **contents vary** depending on the source and the processing of the extract
- Extracts are **not standardized** for quality and quantity of allergen
- Extracts **may be contaminated** by other allergens

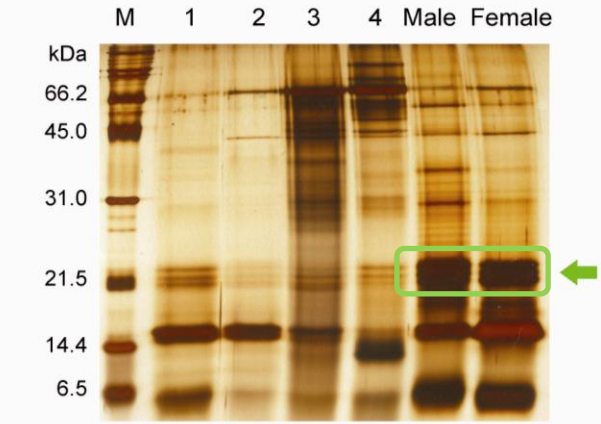
➢ The use of extracts in diagnosis may give false negative or false positive results

➢ **C**omponent - **R**esolved - **D**iagnosis (**CRD**) will help to overcome these draw-backs

Evaluation of skin prick test solutions: guinea pig



1-3: Identical volumes loaded



500 ng of each sample loaded

Major allergens

- Protein concentrations vary by a factor of 20 between SPT samples from 4 different companies
- Major allergens are underrepresented in all 4 samples

Characterization of the allergenic molecules and development of detection tools is important for standardization of extracts

- Extract vs purified molecule
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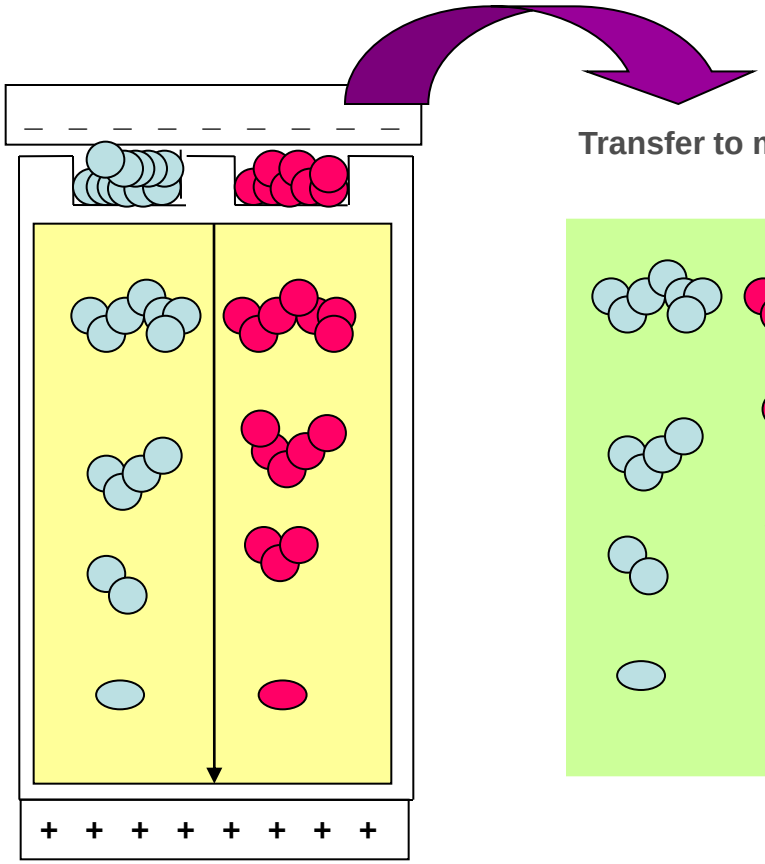
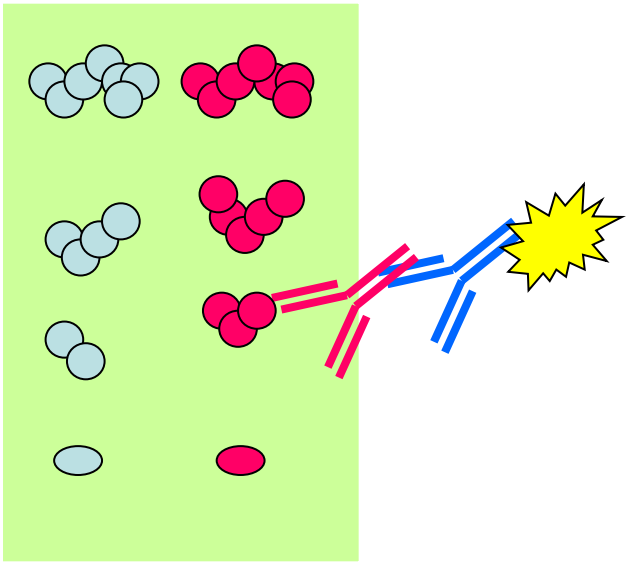
IgE Immunoblot

- The extract is negatively charged.

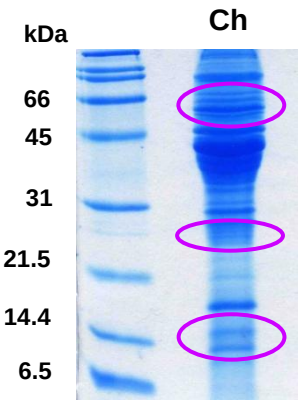
-Big molecules migrate much more slowly than small molecules.

- After electrophoresis, the gel is colored with Coomassie Blue

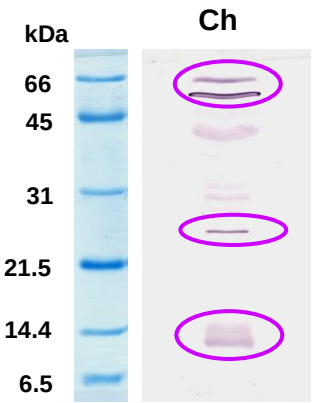




Coomassie blue

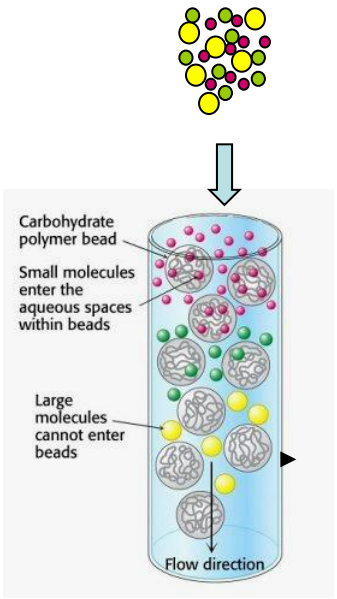


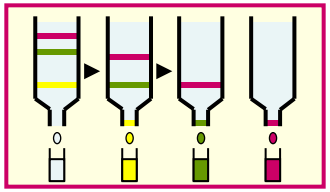
IgE immunoblot



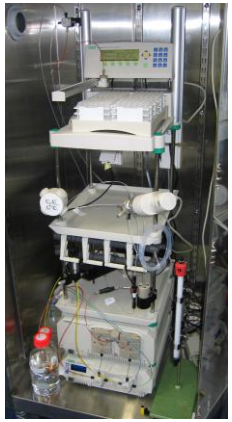
- first antibody e.g. patient serum
- detection by anti-IgE antibody
- bound antibodies are visualized by color

Isolation of purified allergen

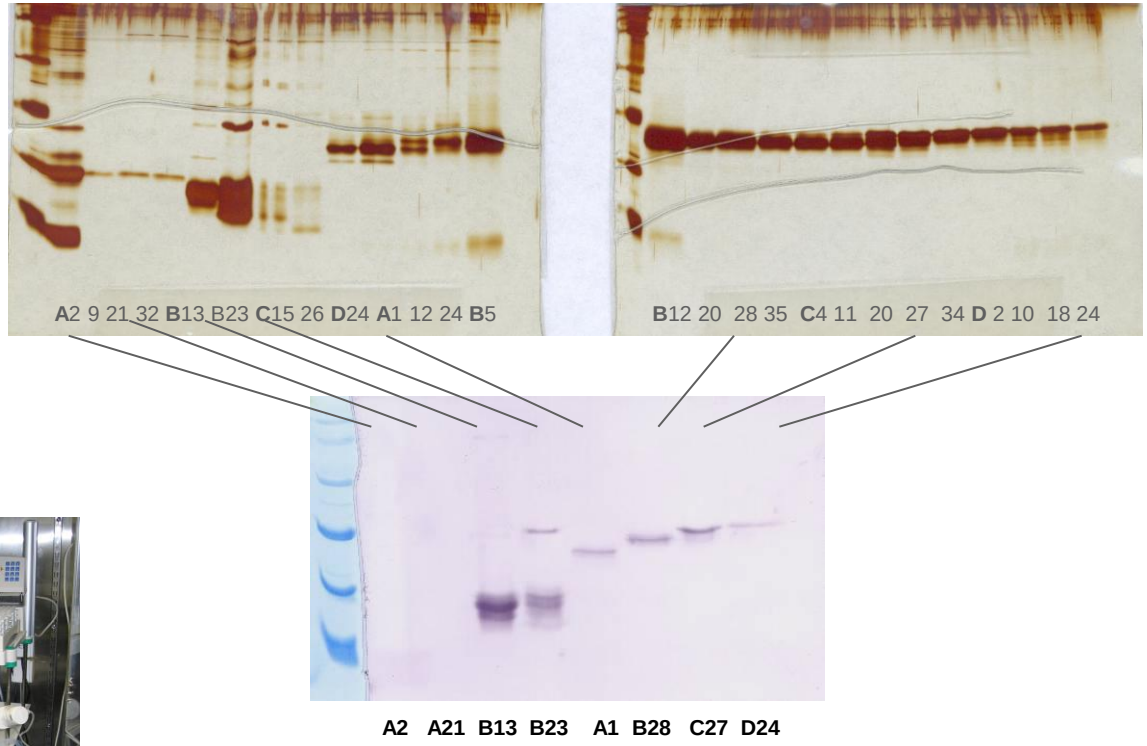




Up to 180 fractions are collected and analysed

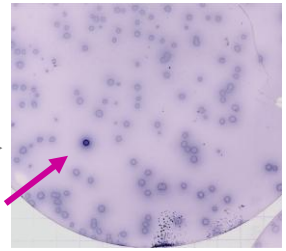


Isolation of an allergen

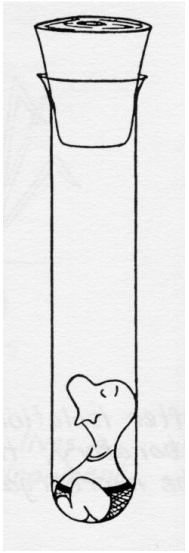


Identification of the isolated molecules can be done by
N-terminal sequencing and / or
Mass spectrometry

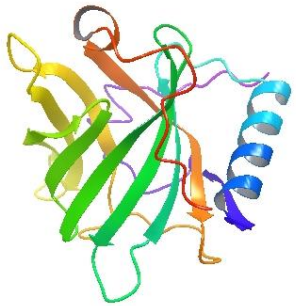
Production of recombinant allergens



cDNA library



Bacteria *Escherichia coli* produce the recombinant allergen



Isolated allergen

Use of recombinant allergens allows production of high quantities of pure protein, independent from the original source

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Clinical case: Identification of a new allergen marker (1)

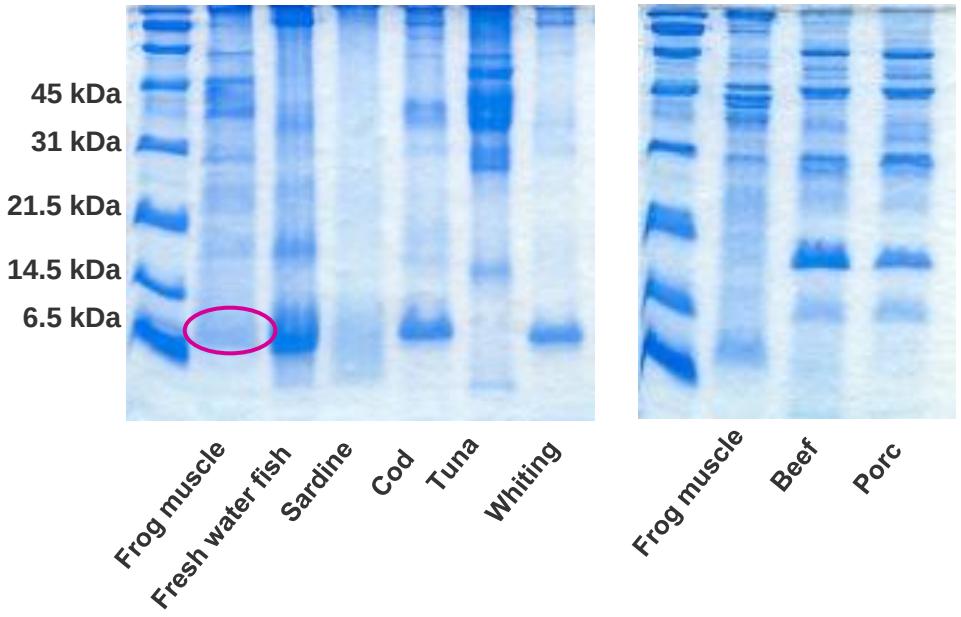
Clinical case of a patient who experienced an anaphylactic shock after ingestion of fried frog legs

- **Clinical history** including prick test with food
- Collection of **serum**
- **Sample** of incriminated substance

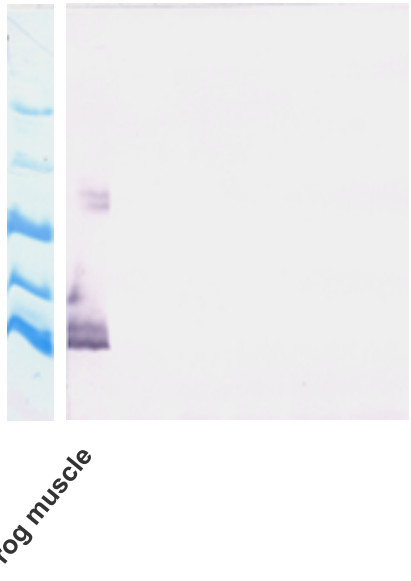
For this case, there was no commercial diagnostic available

Objective: confirmation of allergenic source by detection in immunoblot

Analysis of different protein extracts



Analysis in IgE immunoblot

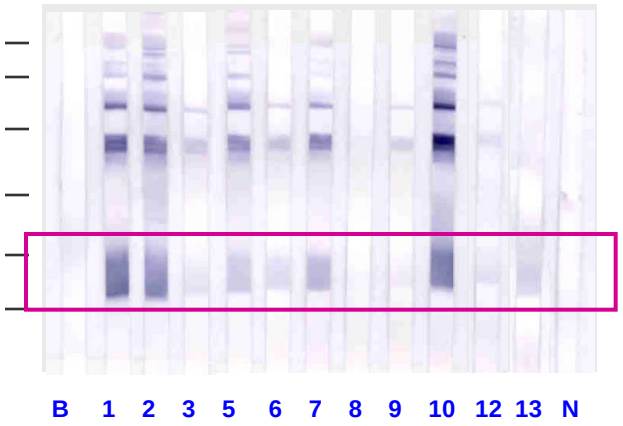


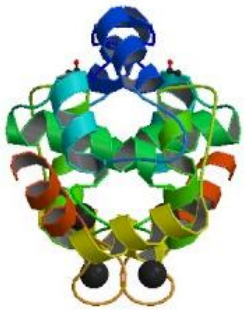
IgE reactivity was demonstrated against frog meat

Hilger et al., Allergy 2002;57:1053-8

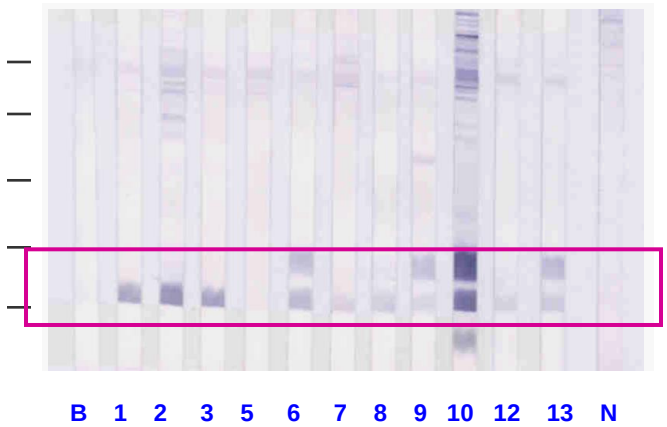
Clinical case: Impact on patient advice after molecular diagnosis (3)

A) *Gadus morhua*
Cod





B) *Rana esculenta*
Frog



Cod and frog parvalbumin share 60 % of aa identity

Fish allergic patients are at risk when consuming frog legs

Hilger et al., Allergy 2004;59:653

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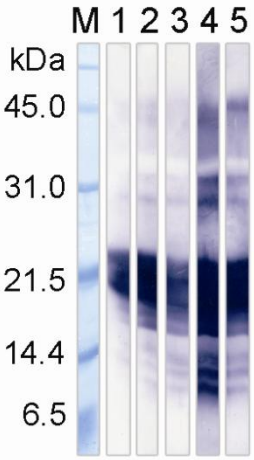
CRD: Isolation of guinea pig allergens

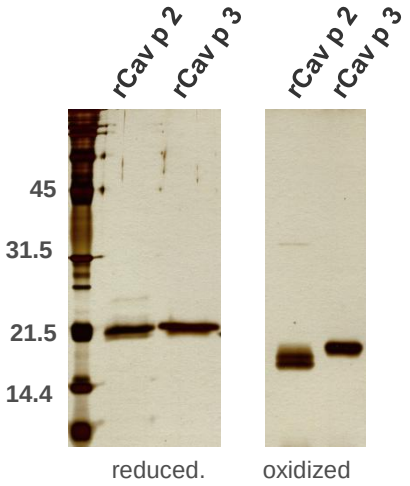


IgE immunoblot

Major IgE reactive bands are detected in hair extract by different patient sera

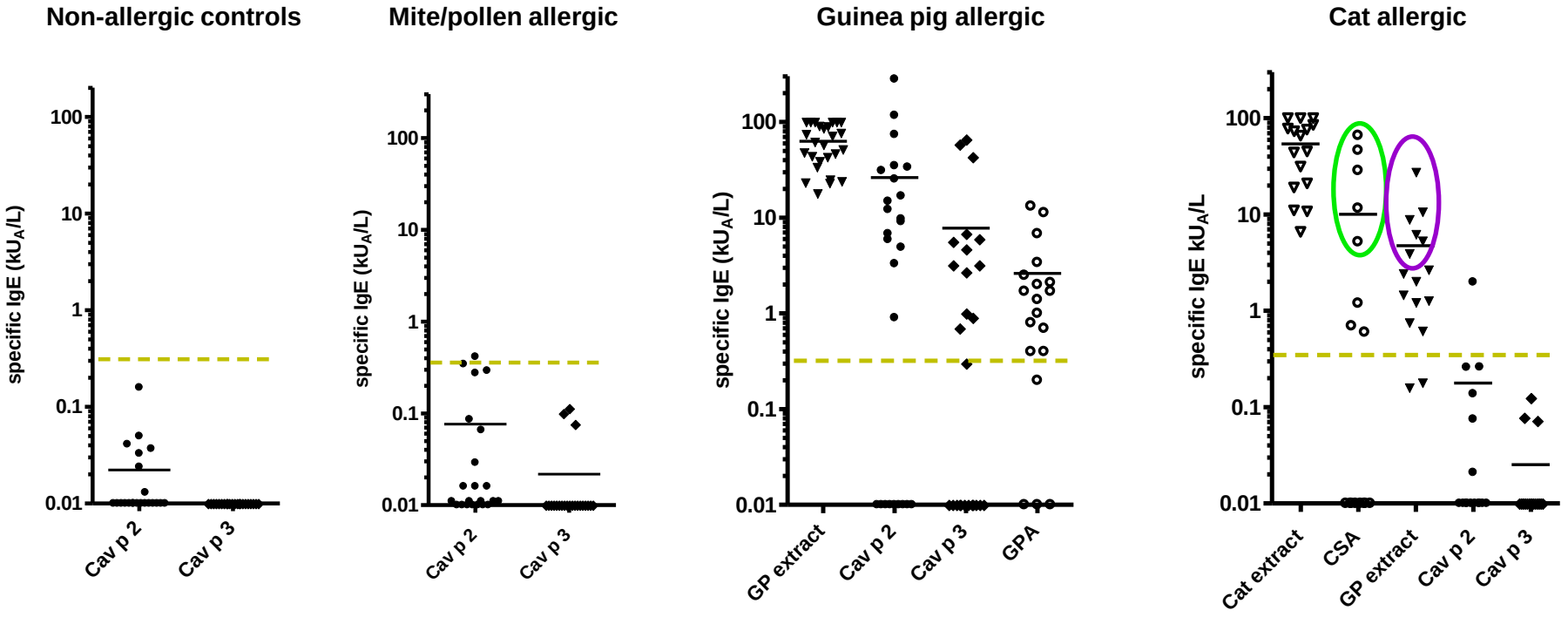
Screening of different tissues to detect source of allergen expression (e.g. salivary glands)

Isolation & cloning



Hilger et al., Clin Exp Allergy 2011; 41:899

CRD: Cav p 2 and Cav p 3 as diagnostic tools



- Cav p 2 and/or Cav p 3 are recognized by 75% of guinea pig allergic patients.
- False positive results in cat allergic patients by using GP extract

Hilger et al., Clin Exp Allergy 2011; 41:899

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Markers available for diagnosis at CRP-Santé

Animal	Commercially available	CRP-Santé
Dog	Can f 1, Can f 2, Can f 3	Can f 6 
Guinea pig 	Extract	Cav p 1, 2, 3, GPA, 1 new molecule
Rabbit	Extract 	3 new molecules
Pigeon Tick	No diagnosis available	Arg r 1 
Fish 	Extracts Cod and Carp parvalbumin	Parvalbumins from 11 fish species

Dog allergy: a patient cohort of 44 cat and dog allergic patients from Luxembourg was analysed. 61% had antibodies to the new allergen

Guinea pig allergy: 26 guinea pig allergic patients from Luxembourg were characterised for 5 allergens

Pigeon tick: 41 patients from France, Italy, Germany and Czech Republic were diagnosed for this allergy

Fish: IgE profile to different fish allergens was done for 62 patients from Luxembourg and France

Allergen source	Published in:
Dog	• J Allergy Clin Immunol 2011
Guinea pig	• Clin Exp Allergy 2011 • patent application for 4 guinea pig allergens (PCT/EP2009/067604)
Fish	• Allergy 2011 • Int Arch Allergy Immunol 2010 • J Investig Allergol Clin Immunol 2010 •J Allergy Clin Immunol 2009 • Allergy 2004
Pigeon tick	• Eur J Dermatol 2011 • Eur J Dermatol 2010 • J Allergy Clin Immunol 2005
Meat	• Int Arch Allergy Immunol 2010 • Allergy 2009 • Revue française d'allergologie et d'immunologie clinique 2006 • Allergy 2002

Conclusion

Characterization of allergens is mandatory to

- generate **individual patient IgE profiles** for optimisation of patient diagnosis and advice
- develop **tools for standardisation** of extracts (skin prick test, IgE tests)
- study the **mechanism of allergy**
- develop **personalised immunotherapy**

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