Component-Resolved Diagnosis of Peach and Mugwort Allergy and Cross-Reactivity in China

中国蒿草花粉和桃果实过敏原组分诊断和交叉反应蛋白分析

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Background 研究背景（1－Europe）

- **Pollen-fruit allergy syndrome** 花粉 - 水果交叉过敏（北欧）
  - **Birch-Apple** 桦树花粉 - 苹果、桃、梨、芹菜、胡萝卜等
  - **Mild OAS** 症状轻微，口腔过敏综合症（**OAS** ）
  - **Central and Northern Europe**
  - 与地理位置/环境相关/种群 （欧洲中北部）

- **Mediterranean peach allergy** 地中海区域桃过敏
  - **True food allergy** 桃等核果类为主, 少与花粉症相关
  - **Systematic and OAS symptoms** 口腔综合过敏以及较严重全身性过敏症状
  - **Peach LTP is the primary sensitizer** 桃果实中的脂质转移蛋白是初始激发原

- **Cross-reactivity molecular basis –** 过敏原分子同源性
  - **Bet v 1 --Mal d 1, Pru p 1 etc.** 桦树花粉– 苹果、桃、樱桃
    - **PR-10 protein** 植物病程相关蛋白第**10**家族
  - **LTP, Pru p 3—Mal d 3, Pru av 3,** 水果中的脂质转移蛋白
- **Mugwort pollen allergens (**蒿草花粉过敏原分子鉴定）
  - **South Europe, Austria (Ferreira F group)**

Peach allergen identification
桃过敏原分子鉴定

⊃ **Allergen components 桃过敏原组分**

➢ **Pru p 1: PR-10,** 与桦树花粉中 **Bet v 1**交叉

➢ **Pru p 2: PR-5,** 类甜蛋白, 较少,但可以引起严重休克
(西班牙,意大利**)**

➢ **Pru p 3: PR-14,** 脂质转移蛋白**(LTP,** 欧洲地中海地
区,初始致敏原**)**

➢ **Pru p 4: profilin** 肌动蛋白结合**(**少,一般与其它混合
出现**)**

Mugwort pollen allergens
已鉴定蒿花粉过敏原分子

- **Art v 1: defensin-like (类防护蛋白)**

- **Art v 2: PR-1 (病程相关1蛋白）**

- **Art v 3: PR-14(LTP，脂质转移蛋白，9 kda)**

- **Art v 4: Profilin, actin-binding (肌动蛋白结合蛋白, 抑制蛋白）**

- **Art v 5：EF-hand calcium-binding protein(钙结合蛋白）**

- **Art v 6：Pectate lyase (果胶酸裂解酶）**

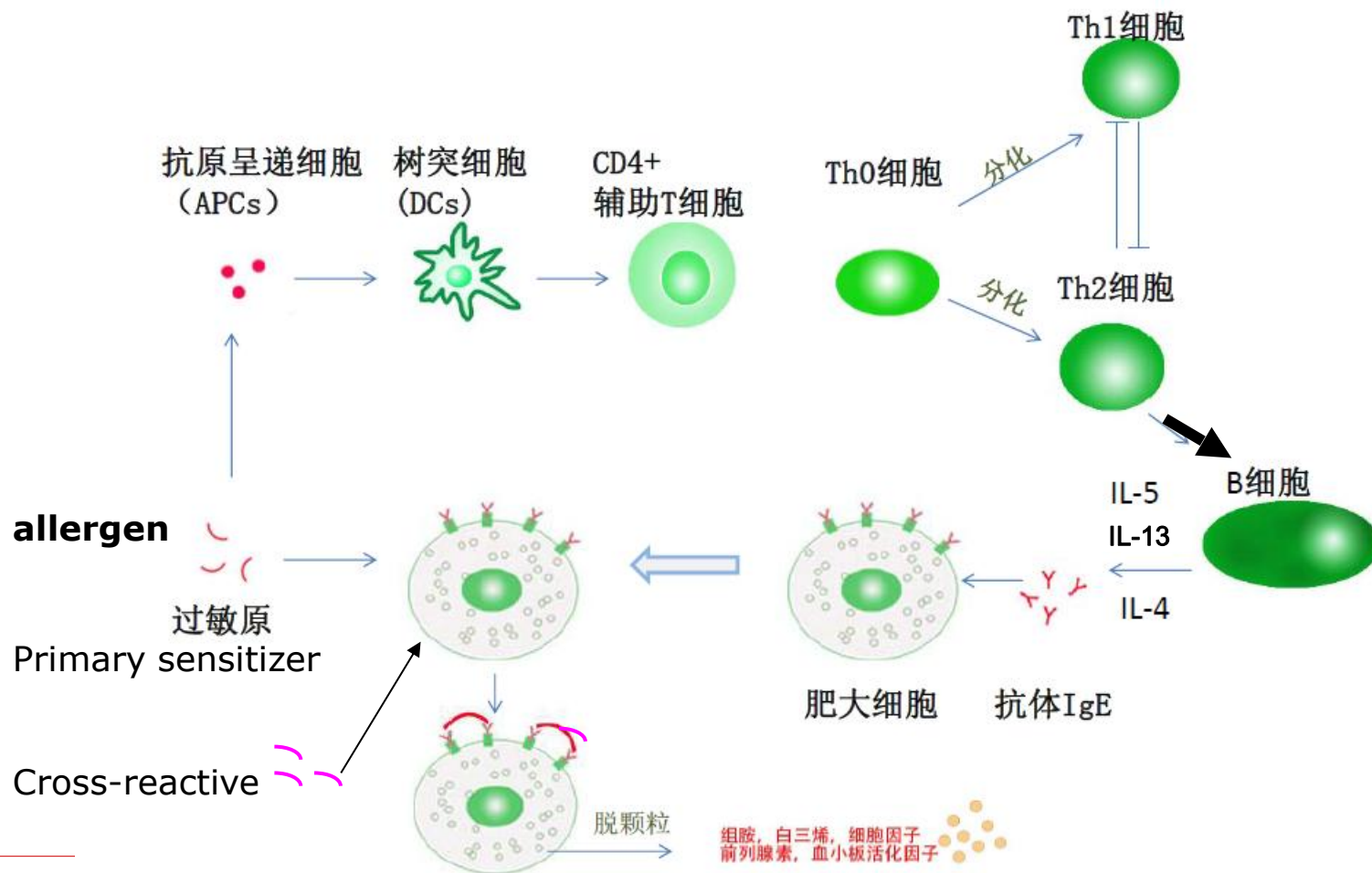# Background 研究背景(2－China)

- **Fruit allergy in China**（中国水果过敏问题）
  - **Subtropical fruits, like mango, lychee, pineapple, Chinese bayberry**（热带和亚热带水果）
  - **Stone fruits, peach, plum, some severe cases report**（核果类，桃等, 严重休克报道）
  - **Pome fruits: apple, pear, loquat**（仁果类）
  - **Grapes**（葡萄）
  - **Vegetable fruits (tomato, cucumber,番茄，黄瓜）**
  - **Due to consumption increase, diversity and availability (** 水果种类多样、充足性和消费量

- **Food allergy associated mugwort allergy** 花粉与食物过敏交叉
  - First report by Wen and Ye, 2002 (文昭明,叶世泰)
    - **Beans, peach, sunflower seeds, peanut** 豆科蔬菜植物、桃、葵花籽、花生
    - **70% Food allergy occurred later or at the same time** 食物过敏迟于或者同于花粉过敏
    - **Systematic accompanying OAS symptoms** 以全身性伴随口腔综合反应

  - Allergic rhinitis associated food allergy in Beijing过敏性鼻炎伴发食物过敏（Zhang et al.2011）
    - **70% are mugwort pollen allergy** 蒿草花粉过敏
    - **Food allergy source curry, shrimp, peach, pineapple, peanut, spinach.** 食物过敏：咖喱(茴香？），虾，桃，凤梨，花生，菠菜

Mechanism of Allergy（过敏机理）

Hypothesis on molecules involved in Mugwort-Peach allergy in China

□ **Peach –mugwort 桃–蒿草花粉共同的过敏原组分**

➤ **LTP 脂质转移蛋白家族**

➤ **Pru p 3 – Art v 3**

➤ **Profilin?**

➤ **which one is the primary sensitizer**

谁是初始致敏原？

Peach 桃

Mugwort 蒿

Research Design 研究方案

Patients 招募患者

History
患者病史

桃过敏peach   交叉过敏both   艾蒿花粉过敏mugwort
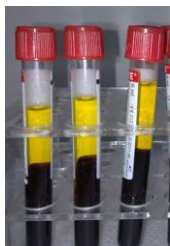
体外诊断
（ImmunoCAP)

| P. 桃果实提取物 | M.艾蒿花粉提取物 | Extracts |

↓+   ↓+

过敏原组分

| Pru p 1 | Pru p 3 | Pru p 4 | Art v 3 | Art v 1 | Single components |

P 桃单一致敏   P/M交叉过敏   **M**艾蒿花粉单一致敏

sIgE values comp.比值 Art v 3 /Pru p 3   Art v 3/ **Pru p 3**

IgE Inhibition 抑制实验

Corresponding to different primary sensitizer

Patients info 患者信息

➲ **Places 血清采集地**

➢ **Zhejiang（杭州），Shanxi（太原、大同）**

➢ **88 samples**

➢ **（5 negatives,含5份阴性对照）**

➢ **13 excluded 排除13例阴性血清（只有桃毛反应, Neg. sIgE）**

Allergy group	Nr.	M/F (男/女)	N北方	S南方
P. and M. 桃和艾蒿花粉	24	13/11	24	0
Peach 桃	15	11/4	10	5
Mugwort 艾蒿花粉	31	17/14	31	0
Total 总计	70	41/29	70	5

Symptoms

⮊ **Peach 桃过敏症状**

➢ **OAS**口腔症状**(25/39)**, **Urt.**荨麻疹**(16/39)**, **G-I** 消化道症状**(7/39)**

➢ **Conj.**结膜炎，**Dys.**呼吸困难，**Ana.**过敏性休克

⮊ **Mugwort 蒿草花粉过敏症状**

➢ **Rhinitis**季节性鼻炎伴随打喷嚏 **(49/55)**

➢**Asthma 哮喘(27/55)**

IgE (ImmunoCAP) test 检测结果

sIgE levels of peach allergenic subjects

Peach UniCAPs

桃过敏患者

sIgE levels of mugwort allergenic subjects

Mugwort UniCAPs

蒿花粉过敏患者

➢ **Main allergens主要过敏原：Pru p 3, Art v 1 and Art v 3**
➢ **sIgE浓度：蒿提取物 > 桃提取物，Art v 3 (20kU$_A$/L)> Pru p 3 (8kU$_A$/L)**

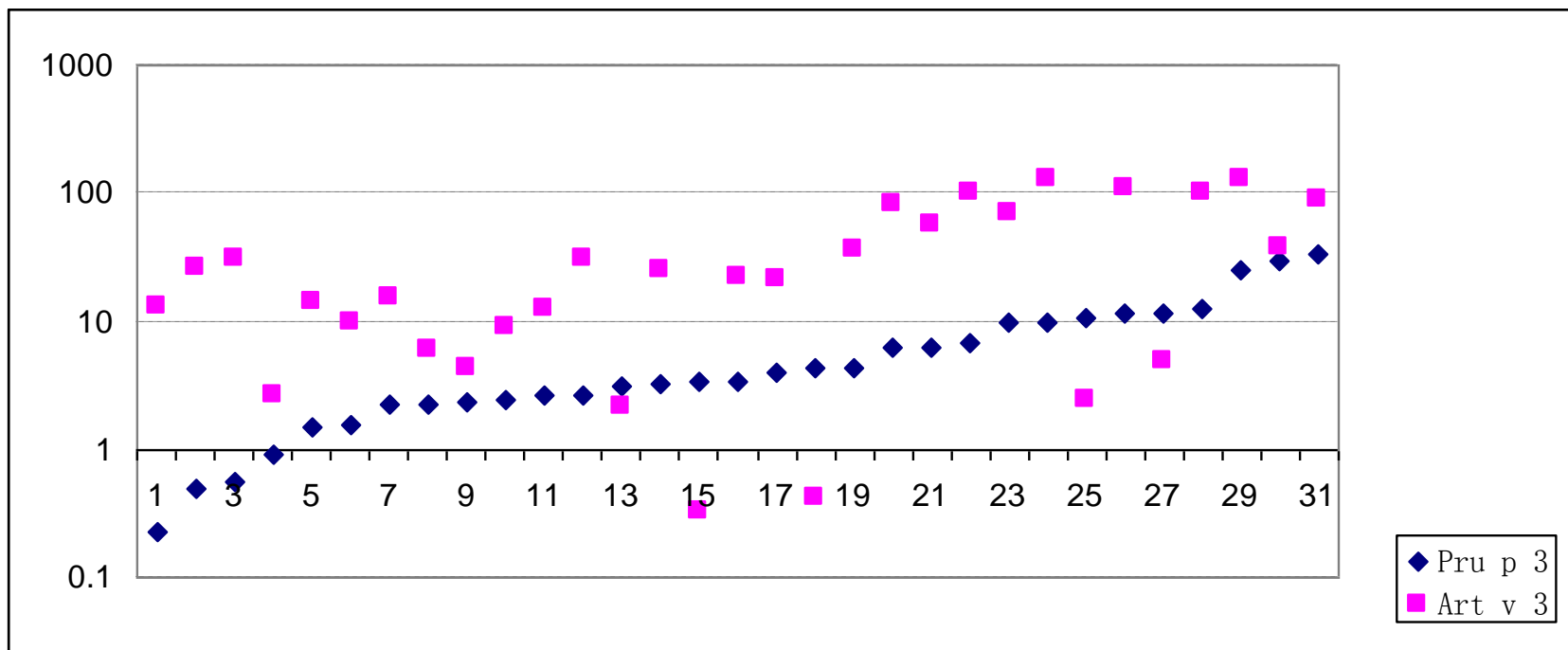# Extract and Single component sIgE Positive distribution

按患者单独过敏/交叉过敏分组（提取物和单一组分IgE阳性数量）

过敏组 Group	Nr.	Peach 桃				Mugwort 蒿		
		E提取物	rPru p 1	rPru p 3	rPru p 4	E提取物	nArt v 1	nArt v 3
P&M 桃和蒿	24	24	2	23	7	24	22	24
P 桃	15	15	1	14	0	6	0	5
M 蒿	31	17	0	9	4	31	25	12
Tot总计	70	56	3	46	11	61	47	41

➤ **Some positive Art v 3 or Pru p 3 IgE，no symptom yet, only IgE binding cross-reactive**

➤ **有些桃或者蒿草LTP sIgE 阳性担并没有表现症状，仅仅是IgE结合交叉反应**

Pru p 3 and Art v 3 sIgE values

Large group: Art v 3 sIgE is greater
Small group: Pru p 3 sIgE is greater

LTP sIgE inhibition method
过敏原组分免疫抑制

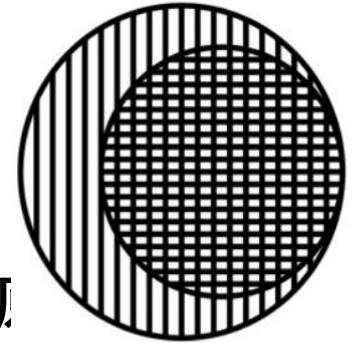➲ **Sera: two pools and individuals**

➲ **Inhibitors抑制剂：**

  ➲ **Both rPru p 3, rArt v 3 同源和异源过敏[原]**

  ➲ **Concentration浓度 （0，0.25，2.5，25，250μg/ml ）**

➲ **ImmunoCAPs 测试血清sIgE**

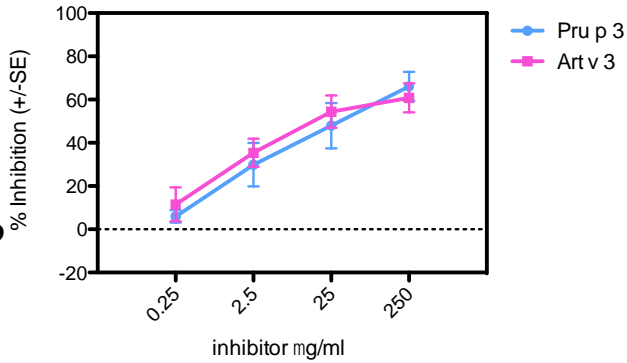 ➢ **1: Inhibitor and serum 75 μl mix, incubation 2 hours**

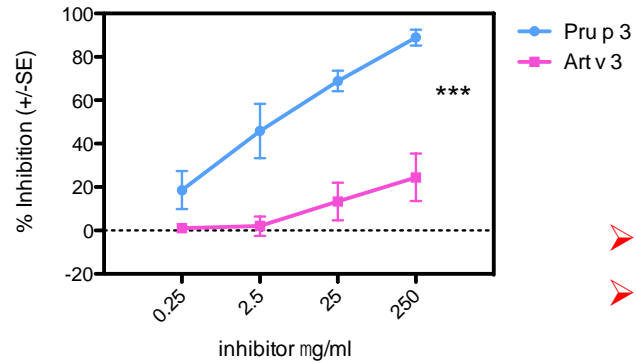 ➢ **2: sIgE measuring by Art v 3 and Pru p 3 CAPs respectively**

H 高 Art v 3/ Pru p 3   L 低 Art v 3 /Pru p 3
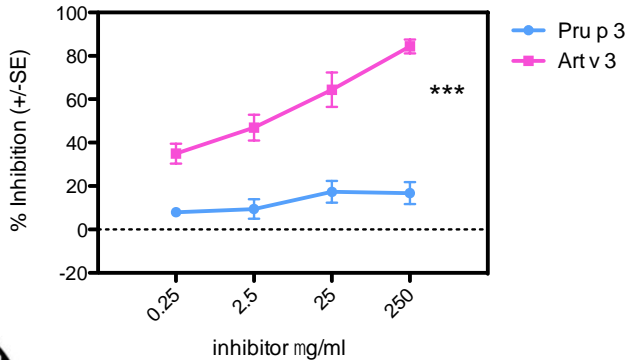
Pru p 3 CAP Inhibition
sera: high Art v 3/ low Pru p 3

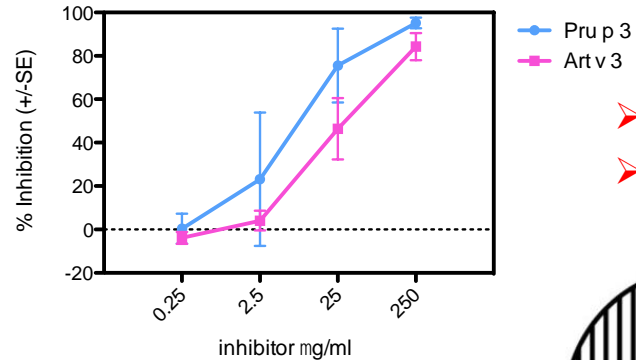Pru p 3 CAP Inhibition
sera: lower Art v 3/ positive Pru p 3

➤ **Pru p 3 CAP**
➤ 异源抑制效果接近同源抑制

➤ **Pru p 3 CAP**
➤ 异源抑制效果弱于同源抑制
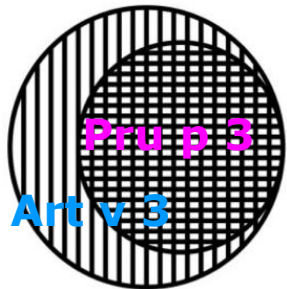
Art v 3 CAP Inhibition
sera: high Art v 3/ low Pru p 3

Art v 3 CAP Inhibition
sera: lower Art v 3/ positive Pru p 3

➤ **Art v 3 CAP**
➤ 异源抑制效果弱于同源抑制

➤ **Art v 3 CAP**
➤ 异源抑制效果接近同源抑制

Conclusions 结论

- **Identify main allergens 明确主要过敏原**
  - **peach 桃：Pru p 3，Mugwort 蒿草：Art v 1, -3**

- **Primary sensitizer for peach 初始致敏原**
  - **S China 南方：桃患者以 Pru p 3 为主**
  - **N China 北方：蒿花粉 Art v 3 为主，与桃Pru p 3交叉致敏**

- **Lipid transfer protein is potential risk food allergen in China, environmental pollen attributing occurrence of food allergy**
  - **脂质转移蛋白是中国食物过敏的潜在危险组分**

Recent progress and plan

- **Antibody against Peach LTP (Pru p 3)**
  - **mAb (IgG1, IgG2) (桃主要致敏蛋白的单抗)**
- **Measurement of LTP content of various peach cultivars (不同桃品种果实过敏蛋白含量测试)**
- **Genome survey of putative LTP sequences from mugwort species in China**
  - **Putative LTP amino acids sequences**
- **Recombinant isoallergens and variants**
  - **重要过敏原异构体的重组蛋白**
- **Cell biological analysis of allergenicity of mugwort and peach LTP (蒿和桃LTP过敏原致敏性的细胞生物学机理)**

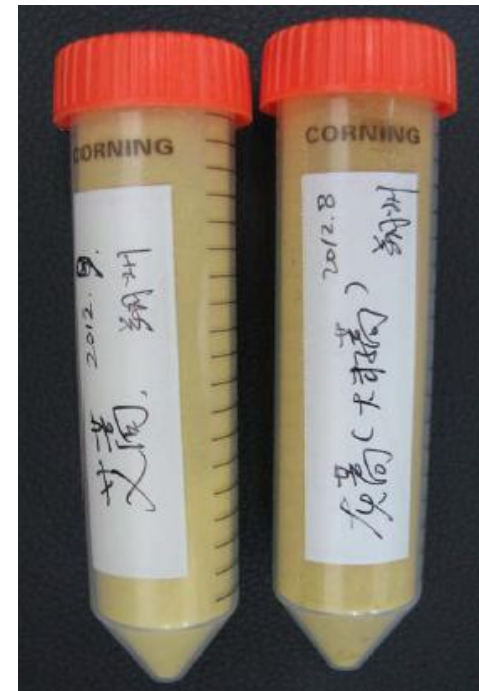# Diversity of the lipid transfer proteins in peach and mugwort pollen

- ☐ All peaches (including nectarine, flat peach) have the same amino acid sequences by genome survey

- ☐ China has different mugwort species and putative different LTP sequences by homologous gene sequencing

Protein analysis of pollens from different Artemisia spp.

- ☐ **Collection of pollens**
- ☐ **Protein extract**
- ☐ **mRNA and proteomics**
- ☐ **Allergens /isoallergens**
- ☐ **Variants**

Recent papers 论文

- ☐ Gao ZS, Yang ZW, Wu SD, Wang HY, Liu ML, Mao WL, Wang J, Gadermaier G, Ferreira F, Zheng M, van Ree R, Peach allergy in China: A dominant role for mugwort pollen lipid transfer protein as a primary sensitizer, J Allergy Clinical Immunology, 2013, 131, 1: 244－246

- ☐ Wang HY, Gao ZS, Yang ZW, Shao JX, Zhao XZ, van Ree R. Anaphylaxis and generalized urticaria in a woman eating Chinese bayberry fruit. Zhejiang University Sciences B 2012, 13（10）851－854

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