

Title Page

IgE-binding potencies of three peach Pru p 1 isoforms

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Abstract

Scope: Pru p 1, the Bet v 1 homologue from peach, has been identified as a clinically relevant allergen. Three isoforms have been described, two in peach fruit (Pru p 1.0101, Pru p 1.0201) and one in pollen (Pru p 1.0301). The present study aimed to compare their IgE binding potencies.

Methods and results: Three Pru p 1 isoforms were cloned and expressed as soluble proteins with His-tags in *Escherichia coli*. Protein identity was confirmed by mass spectrometry, circular dichroism, and RNase activity. IgE binding capacity by ELISA and ImmunoCAP was compared. Three Pru p 1 isoforms had quite similar IgE-binding potencies for 60% of the sera, but more than two-fold between any two isoforms among 40% of the 47 sera. The mean IgE binding of Pru p 1.0201 was slightly higher than other two isoforms. In a sera pool, homologous ImmunoCAP inhibition was higher than other two heterologous isoforms. Individual serum with diverse IgE values of three isoforms demonstrated the higher IgE inhibition of specific isoform with higher IgE value.

Conclusion: A similar and variable pattern of IgE recognition was observed among three Pru p 1 isoforms. The two new isoforms can be used as more accurate diagnostic reagents.

Keywords: component-resolved diagnosis, cross-reactivity, peach allergy, isoallergen, Pru p 1

Abbreviations: CD, circular dichroism; CRD, component-resolved diagnosis; PR, pathogenesis related; IUIS, International Union of Immunological Societies

1 Introduction

Peach (*Prunus persica* L. Batsch) is amongst the fruits most frequently reported to cause food allergy, both in China [1, 2, 3] and Europe [4]. To date, five allergens have been reported for peach (www.allergome.org): Pru p 1 (Bet v 1-homologous PR-10), Pru p 2 (thaumatin-like protein), Pru p 3 (lipid transfer protein), Pru p 4 (profilin) and Pru p 7 (anti-microbial peptide). Of these, the lipid transfer protein Pru p 3 has been identified as a major allergen in Northern China [1]. The clinical relevance of Pru p 1 in China has recently been evaluated and confirmed [2]. Pru p 1, a fairly labile 18 kDa protein [5], accumulates in

both peach peel and pulp, varying in quantity in different peach and nectarine cultivars [6]. In genomic studies, different isoforms of the peach PR-10 gene family have been given isoform designations *Pru p 1.01*, *Pru p 1.02* and *Pru p 1.06*, but these were not supported by IgE-binding data using sera of peach-allergic patients [7-9]. These designations were therefore not accepted by the IUIS allergen nomenclature subcommittee. Unfortunately, this has created some confusion. Currently, the IUIS subcommittee lists three isoforms: *Pru p 1.0101* (identical to *Pru p 1.01* in [8]), *Pru p 1.0201* (identical to *Pru p 1.06* [6-8]) and *Pru p 1.0301* (identical to *Pru p 1.02* in [7]). The expression level of *Pru p 1.0101* and *Pru p 1.0201* has been shown to differ during growth stages of the fruit, with high levels in the fruit skin at the pit hardening stage [8,9], while *Pru p 1.0301* is especially abundant in the pollen and leaf [9]. Protein identification in the mature peach fruit has also provided solid evidence of two main PR-10 (*Pru p 1*) proteins corresponding to *Pru p 1.0101* and *Pru p 1.0201* [10].

Bet v 1 is a primary sensitizer for most fruit allergic patients with oral allergy syndrome in northern Europe [11-12]. Research on the allergenicity of different *Bet v 1* isoallergens and variants has been in progress since 1996. Natural iso-allergens of *Bet v 1* have been reported with different IgE-binding capacity and T cell activation capacity [13]. Two *Bet v 1* isoforms (*Bet v 1.0401* and *Bet v 1.1001*) have been shown to behave as poor IgE binders for birch pollen allergic individuals, i.e. as naturally occurring hypo-allergens [14, 15]. The low pH in the gastric environment generally reduces the allergenicity of *Bet v 1* homologues in food, but there is some variation among different sources [16]. In carrot, *Dau c 1* isoforms and variants have been compared for their IgE epitope heterogeneity and a new isoform identified and recommended in the component-resolved diagnosis (CRD) [17]. Although genomic and proteomic data supporting the presence of peach *Pru p 1* isoforms or variants is accumulating, very little is known about differences in allergenicity. In CRD, it is of course important to select the most relevant isoforms. For *Pru p 1*, the ImmunoCAP system uses *Pru p 1.0101* but this choice was probably not based on an in-depth comparison of *Pru p 1* isoforms. In the present study, we report recombinant production of the three known isoforms of *Pru p 1*, allowing us to compare their IgE-binding capacity and cross-reactivity using sera of peach-sensitized patients.

2 Materials and Methods

2.1 Patient sera

Sera used were from four Chinese subjects with a convincing history of birch pollen-associated peach allergy (Table 1) and from 47 patients, from eight European cities, with a convincing history of birch pollen allergy and IgE antibodies against Bet v 1 and cross-reactive IgE antibodies to peach. Specific IgE antibodies to rPru p 1 (i.e. Pru p 1.0101) and rBet v 1 (i.e. Bet v 1.0101) were measured by ImmunoCAP™ (ThermoFisher, Uppsala, Sweden) according to the manufacturer's instructions. The forty seven sera from European patients were used to measure specific IgE titers of three recombinant Pru p 1 isoforms by ImmunoCAP [18]. The use of the Chinese serum samples was approved by the local ethics committee (authorization No. 2011-R-1, the Second Affiliated Hospital, School of Medicine, Zhejiang University, China), and written informed consent for the use of the sera was obtained from all patients. For the sera from Europe, ethical approval and informed consent was obtained within the European CREATE project [19]. The approval and consent included future use of serum samples from the CREATE serum bank for allergy-related research.

2.2 Cloning, heterologous expression and purification of Pru p 1 isoforms

Total RNA from peach peel and pollen was prepared as described previously [7]. First-strand cDNA was synthesized from approximately 1 µg of total RNA using the oligo₁₈ primer and AMV Reverse Transcriptase (TaKaRa, Dalian, China) according to the manufacturer's protocol. The cDNA obtained was then amplified by PCR using the forward primer

(5'-CACCGGTGTCTTCACATATGAGA-3') and reverse primer

(5'-TTAGTTGTAGGCATCGGGGGTG-3') for Pru p 1.01; forward primer

(5'-CACCGGTGTCTTCACATACTCAG-3') and reverse primer

(5'-TTAGTTGTAGGCATCTGGGTTG-3') for Pru p 1.02, and forward primer:

(5'-CACCGGTGTCTTCACGTACGAAA-3') and reverse primer

(5'-TTAGTTGTAGGCATCGGGGTTG-3') for Pru p 1.03. Amplification was carried out with *Pfu* high fidelity DNA polymerase (Promega, USA). PCR products were purified using the QIAquick Gel Extraction kit (Qiagen, Germany), and cloned into the pET 100/D-TOPO vector (Invitrogen, USA). Four clones were sequenced using an ABI 3730 XL sequencer (Invitrogen, Shanghai, China) to ensure the sequence and orientation was correct. Recombinant protein was produced in Rosetta-gami B (DE3) pLysS host cells (Novagen, USA). Protein synthesis

was induced with 1 mM isopropyl- β -D-thio-galactopyranoside (IPTG) for 3-5 hours at 37°C when an OD600 nm optical density of 0.5-0.8 was reached. Subsequently, cells were harvested by centrifugation, resuspended in lysis buffer (20mM Tris-HCl, 0.5M NaCl, 10% glycerol, pH7.9) and disrupted using an Ultrasonic Cell Disruption System (Nanjing, China). The His-tagged fusion protein was purified using an ÄKTA explorer (GE Healthcare) system, on a HisTrap FF crude column (GE Healthcare) according to the manufacturer's instructions.

2.3 Mass spectrometry-based sequencing

For mass determination, recombinant protein was dissolved in 5mM PBS buffer at a concentration of 1 mg/ml and applied to a MALDI-TOF/TOF mass spectrometer (Applied Biosystems, USA). For peptide fingerprinting, bands corresponding to rPru p 1 were excised from the gel (containing at least 1 μ g protein) and then applied to MALDI-TOF/TOF.

2.4 Circular dichroism spectroscopy

The secondary structures of the purified proteins were analyzed by circular dichroism (CD) spectroscopy. The CD spectra were recorded on a Jasco J-810 automatic recording spectropolarimeter at room temperature. The spectral range was 190–250 nm.

2.5 Measurement of RNA hydrolysis activity of three Pru p 1 isoforms

Total RNA from 2g of frozen peach fruit peel was extracted as described above. We basically followed the protocol described by Zubini et al.[8] to assay the three Pru p 1 isoforms. The isoform proteins (10 mM) were incubated in the presence of RNA (10 mg) in 50 mM phosphate buffer at different pH (3, 5, 7.5 and 9) in a total 50 μ l mixture. In addition, in buffer pH 7.5, 200 mM zeatin (Sigma-Aldrich) was added in one treatment, and in another, the isoform proteins were heated for 5 min at 100°C before adding to the mixture. Ten microliter of the reaction mixture was transferred to a separate tube and 2 μ l loading buffer added to stop the reaction [20]. This was then stored at -80°C. The RNA in the resulting reaction mixtures from the assays were analyzed on 1% agarose gels, stained with ethidium bromide.

2.6 ImmunoCAP and ELISA analyses

ImmunoCAP analyses for the three rPru p 1 isoforms were carried out with streptavidin ImmunoCAPs (Phadia, Uppsala, Sweden) to which 0.5 μ g of biotinylated recombinant Pru p 1

isoforms (rPru p 1.01, rPru p 1.02 and rPru p 1.03) was bound [18]. These laboratory-made isoform ImmunoCAPs were only tested with the European Pru p 1 positive sera at the Amsterdam Medical Center, The Netherlands. Four Chinese serum samples were also tested on commercially available ImmunoCAPs with rPru p 1 (i.e. Pru p 1.0101), rPru p 3, rPru p 4 and rBet v 1. IgE-binding of the Chinese serum samples to the three isoforms of Pru p 1 (coated with 0.5 µg) was measured by ELISA.

2.7 ImmunoCAP inhibition assays

A pool of serum samples with equal volumes of 10 sera (similar IgE titers for three isoforms) and three individual serum samples (diverse IgE titers of three isoforms) were used for ImmunoCAP-inhibition for each Pru p 1 isoform. For all ImmunoCAP-inhibitions, 75 µl serum (diluted to 5-10 kU_A/L if necessary) and 75 µl of inhibitor (100 µg/ml, and four 10-fold dilutions) were pre-incubated for one hour at room temperature prior to ImmunoCAP analysis. IgE values were compared to an uninhibited control (75 µl of PBS instead of inhibitor).

3.1 Amino acid sequence and expression and purification of three Pru p 1 isoallergens

The primary amino acid sequences of the three Pru p 1 isoforms were deduced from cDNAs obtained from fruit (Pru p 1.0101 and Pru p 1.0201) and pollen (Pru p 1.0301). The first isoform, Pru p 1.0101, is exactly the same as accession DQ251187 that appeared in the IUIS allergen database. The other two were assigned to new isoallergens (IUIS) and linked to the NCBI accessions KM350692 and KM350693, respectively. These three isoforms shared 74.8%-78.0% amino acid sequence identity, but had much lower identity to the birch pollen Bet v 1.0101: 59.1%, 56.6% and 58.5%, respectively (Figure 1). rPru p 1.0101, rPru p 1.0201 and rPru p 1.0301 were successfully expressed as soluble proteins and purified. They gave the expected protein band at an apparent molecular mass of approximately 21 kDa on SDS-PAGE including His-tag (Figure 2).

3.2 Identification by MS and peptide fingerprinting by MALDI-TOF/TOF

The molecular mass of the three purified intact isoforms, rPru p 1.0101, rPru p 1.0201 and rPru p 1.0301, determined by MALDI-TOF/TOF (Supporting Information Figure S1), is in agreement with the theoretical mass of 21642, 21432, and 21471 Da, with the deviation of

molecular mass within 1%. Further confirmation of the amino acid sequence was obtained after digestion with trypsin, with the resulting peptides analyzed by MALDI-TOF/TOF. Matched peptide fragments covered 43.6%, 43.6%, and 72.8% of the corresponding sequence (Figure 3). Both molecular mass and peptide fingerprinting for each recombinant protein showed the validity of the three isoforms of Pru p 1.

3.3 Secondary structure analysis from circular dichroism spectroscopy

Circular dichroism spectroscopy indicated that all isoforms were properly folded, with a comparable content of secondary structure in Pru p 1.0101, Pru p 1.0201 and Pru p 1.0301 (Figure 4). The peak value of the spectra was approximately 195 nm and the minimum values between 215 nm and 217 nm, indicating an ordered structure with dominant β -sheet content (45.4%, 44.8%, and 46.2% for rPru p 1.0101, rPru p 1.0201 and rPru p 1.0301, respectively). Only the rPru p 1.0201 had 5% amino acids in β -turn.

3.4 RNase activity of three Pru p 1 isoforms

RNase activity was demonstrated to be pH dependent. No hydrolysis of RNA was observed at pH 3 for the three isoforms, and only Pru p 1.0201 was active at pH 5. When the buffer pH was increased to 7.5 and 9, ribonuclease activity was observed for all three isoforms. Pru p 1 belongs to the pathogenesis related protein 10 that has binding potency to plant hormone cytokinin [8]. The presence of zeatin inhibited the RNase reactivity in the case of Pru p 1.0101, while this had no effect on Pru p 1.0201 and even increased reactivity on Pru p 1.0301 in which complete RNA hydrolysis occurred as early as half an hour after incubation. Five min pretreatment of the protein at 100°C slowed down RNA hydrolysis, but did not completely inactivate the enzymatic activity (Figure 5).

3.5 IgE-binding capacity by ELISA and ImmunoCAP assays

Direct IgE-binding capacity of the three isoforms was studied by ELISA, using sera of four Chinese peach-sensitized subjects. For P1, rPru p 1.0201 and rPru p 1.0301 gave higher IgE antibody binding than rPru p 1.0101, with very similar binding observed for the other three sera (Supporting information Figure S2). The IgE binding potency of the three isoforms tested was very similar for 30 of the 47 European Pru p 1 positive sera, but there was a more than two fold difference among 17 sera. The three sera with more than 10-fold difference to the three isoforms are highlighted in Figure 6. Median IgE titer values of each

isoform (Figure 6A) and the ratio of IgE titers pairwise comparison (Figure 6B), showed that the titer was slightly higher for Pru p 1.02 than the other two isoforms, but not significantly different. However, it is clear that the IgE response of individual serum is different for more than half of the tested sera, which was reflected by the IQR (Inter-Quartile Range) values, which ranged from 0.53 to 0.79 (Figure 6B).

3.6 Cross-inhibition analysis

This was to assess IgE cross-reactivity amongst the three isoforms and compare their IgE-binding potencies with a sera pool from 10 European patients with quite similar IgE titers for the three isoforms (indicated in red in Figure 6B). IgE-binding to each Pru p 1 isoform can be fully inhibited by its homologous isoform at a high concentration, while less so by the other two isoforms (Figure 7). There was little difference in Pru p 1.01 CAP and Pru p 1.03 CAP inhibitions among the three inhibitors (Figure 7A and C), but this was not the case for Pru p 1.02 CAP inhibition, Pru p 1.01 was the weakest (Figure 7B). Three individual serum samples with a diverse specific IgE profile to the three isoforms gave a different inhibition pattern to the sera pool in Figure 6, the IgE inhibition effect was strongly dependant on the isoform with the highest IgE titers in the serum (Figure 8A-C). For example, a serum with high Pru p 1.02 titer, lower Pru p 1.01 and the lowest Pru p 1.03 titer (serum in green in Figure 6), Pru p 1.02 can fully inhibit IgE in all three isoform CAP inhibition assays (Figure 8B).

4 Discussion

Three isoforms of Pru p 1 were successfully produced as recombinant reagents. Their identity was confirmed by mass spectrometry and their structural integrity by CD spectroscopy (secondary structures dominated by β - sheets) and by their ability to effectively function as RNase. It was reported that the RNase activity of peach PR-10 proteins is affected by pH [8]. Indeed, the three isoforms of Pru p 1 could all catalyze the hydrolysis of RNA within the reported pH range of 7.5-9 (Figure 5), with no hydrolysis of RNA at pH 3, and only Pru p 1.02 being active at pH 5. In addition, we confirmed that zeatin inhibited RNA hydrolysis by Pru p 1.01, but not by Pru p 1.02 (corresponding to Pru p 1.06D in the original paper) [9]. Zeatin seemed to increase RNA hydrolysis in Pru p 1.0301 (Figure 5F), indicating cytokinin binding to this isoform should have a positive effect on RNase activity in contrast to the previous assumption that zeatin binding inhibited RNase activity [8].

Further isothermal titration calorimetry with zeatin and purified recombinant or natural Pru p 1 isoforms are proposed to verify this hypothesis. The immunogenicity of rPru p 1.01 and rPru p 1.02 was demonstrated by the successful production of three monoclonal antibodies in mice, which bind to both the recombinant proteins and their natural fruit counterparts in peach extract at pH 7.5. In agreement with the loss of RNase reactivity, recognition of natural Pru p 1 in the extract was lost at lower pH (≤ 5) (data not shown here).

All together, these results support that the three isoforms of Pru p 1 were correctly folded and functional recombinant reagents. Direct ELISA and ImmunoCAP analyses also supported structural integrity, with all three isoforms binding IgE to a similar extent but variable pattern depending on patients, although Pru p 1.0101 tended to be a slightly less potent IgE binder than the other two isoforms. With the ImmunoCAP inhibition assay, there was higher and complete inhibition homologous inhibitions with all three isoforms when using a sera pool with similar IgE titers of three isoforms. In contrast, for those sera with diverse IgE titers of the three isoforms, homologous isoform inhibition reached complete inhibition only at the highest concentration. This indicates that the isoform with stronger IgE binding potency in a serum also reflected on the inhibiting potency.

It seems safe to conclude that Pru p 1.0201 is a comparable or even more potent allergen than Pru p 1.0101. One clue is that Pru p 1.0201 more closely resembles the Bet v 1-like fold region (109-105 aa, boxed region in Figure 1) [21], as reported in carrot, with a different inhibition pattern between Dau c 1.0201 and Dau c 1.0104 [22]. A very recent report on tomato Bet v 1 homologous PR-10 allergen also demonstrated the difference in IgE binding potency of two recombinant isoforms, Sola l 4.02 is higher, its amino acid sequence in the short stretch of Bet v 1-like region (Figure 1) is closer to Bet v 1.0101 than Sola l 4.01 [23]. Similar IgE binding pattern for half of the patients indicated the conserved IgE and isoform binding epitope, while the variable IgE binding capacity of three isoforms reflected a diverse allergen epitope recognition pattern for individual patients.

With Pru p 1.0301 being located mainly in pollen and leaves, Pru p 1.0101 and Pru p 1.0201 together is probably better for diagnostic testing of birch pollen-related peach fruit allergy. Further research is being conducted to quantify the Pru p 1 isoforms in fruits with sensitive mAbs, to understand the proportion in the peach fruit peel and pulp, and variation amongst different peach cultivars.

Author contribution

ZSG and XZ drafted the manuscript. XZ, ZWY, WYF and LG performed the gene cloning and expression experiments. HYW, JYZ were involved in the clinical study of patients. SAV and JHA tested the recombinant proteins with ImmunoCAPs and reviewed the manuscript. RvR and ZSG designed the research and reviewed the whole manuscript.

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Conflict of interest statement

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Figure legends

Figure 1 Primary sequences of three rPru p 1 isoallergens and comparison with Bet v 1.0101.

Pru p 1.0101 (accession: DQ251187), Pru p 1.0201 (KM350692), Pru p 1.0301 (KM350693), Bet v 1.0101 (X15877). Amino acid sequence identity are 74.8% (Pru p 1.01/Pru p 1.02), 78.0% (Pru p 1.01/Pru p 1.03) and 76.1% (Pru p 1.02/Pru p 1.03); with Bet v 1.01 are 59.1%, 56.6% and 58.5%, respectively. Short stretch of Bet v 1-like fold is boxed (reference [21]: Wallner et al. JACI, 2011; 127:1571-8).

		*	20	*	40	*	60																																																	
Bet v 1.0101 :	GVFN	YE	ET	TS	VI	EA	AR	LF	RA	ET	LD	GN	LF	PK	VA	PQ	AI	SS	VE	NI	EG	NG	GG	ET	IK	KS	IF	EE	:	60																										
Pru p 1.0101 :	GVFT	YE	SE	ET	SE	IP	PP	RL	FR	AE	VL	DA	DN	IV	PK	IA	PQ	AI	KH	SE	TE	LE	GD	GG	GG	ET	IK	KS	IT	FG	:	60																								
Pru p 1.0201 :	GVFT	YS	DE	ST	SV	IP	PP	RL	FR	AE	VL	EA	DT	IL	PK	IA	PQ	SV	KS	AE	IV	EG	DG	GG	VG	TI	IK	KS	IF	EE	:	60																								
Pru p 1.0301 :	GVFT	YE	ET	ET	SV	IP	PP	RL	FR	AE	IL	DA	DN	IV	PK	IA	PT	AV	RD	TE	LE	GD	GG	VG	TI	IK	RV	TF	GE	:	60																									
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Bet v 1.0101 :	GF	PF	KY	VR	DR	VE	VD	HT	NE	KY	YS	VT	EG	GP	IC	DT	LE	KI	SN	ET	KI	VA	TE	D	GG	S	IL	KI	SN	KY	:	120																								
Pru p 1.0101 :	GS	Q	Y	GV	KH	R	I	D	S	I	D	R	N	H	S	Y	S	T	L	I	E	G	D	A	L	G	I	N	L	E	KI	S	Y	E	T	KI	V	A	S	E	S	G	G	S	I	L	K	S	T	S	H	Y	:	120		
Pru p 1.0201 :	GS	H	Y	S	Y	V	K	H	R	I	D	G	L	D	R	N	E	V	N	Y	T	L	I	E	G	D	A	L	S	D	R	V	E	KI	T	Y	E	T	KI	V	A	S	E	D	GG	S	I	L	K	S	T	S	N	Y	:	120
Pru p 1.0301 :	GS	Q	Y	GV	KH	R	I	D	G	L	D	R	N	L	T	S	Y	T	L	I	E	G	D	A	L	S	D	V	I	E	KI	V	Y	D	I	KI	V	A	S	E	N	G	G	S	I	V	K	T	S	H	Y	:	120			
		*	140	*																																																				
Bet v 1.0101 :	HT	K	G	D	H	E	V	R	A	E	C	V	R	A	S	K	B	M	G	E	T	L	L	R	A	V	E	S	Y	L	L	A	H	S	D	A	Y	N	:	159																
Pru p 1.0101 :	HT	K	G	D	V	E	I	K	E	E	C	V	R	A	G	K	E	R	A	S	N	L	F	K	L	I	E	T	Y	L	K	G	H	P	D	A	Y	N	:	159																
Pru p 1.0201 :	HT	K	G	D	V	E	I	K	E	E	C	V	R	A	G	K	E	K	A	T	G	L	F	K	L	I	E	N	Y	I	V	A	N	P	D	A	Y	N	:	159																
Pru p 1.0301 :	HT	K	G	D	V	E	I	K	E	E	C	V	R	A	G	K	E	R	A	C	L	F	KI	V	E	G	Y	L	L	A	N	P	D	A	Y	N	:	159																		

Figure 2 SDS-PAGE of recombinant Pru p 1 isoallergens.

M, protein marker; 1, purified rPru p 1.0101; 2, rPru p 1.0201 and 3, rPru p 1.0301.

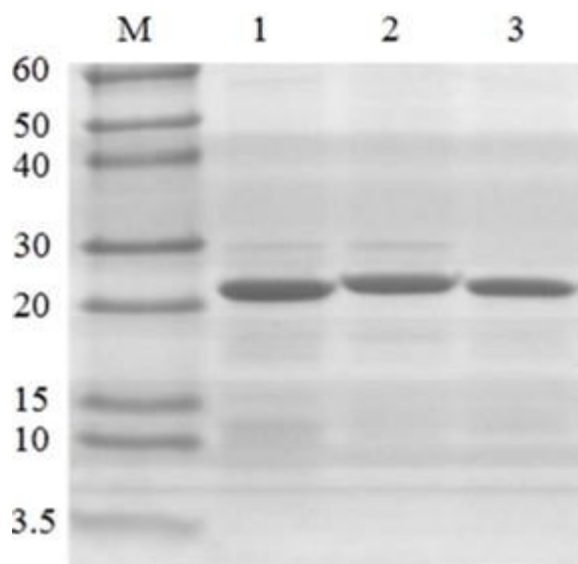


Figure 3 MALDI-TOF mass spectrum of digested peptides for three Pru p 1 isoallergens and peptide amino acid matching.

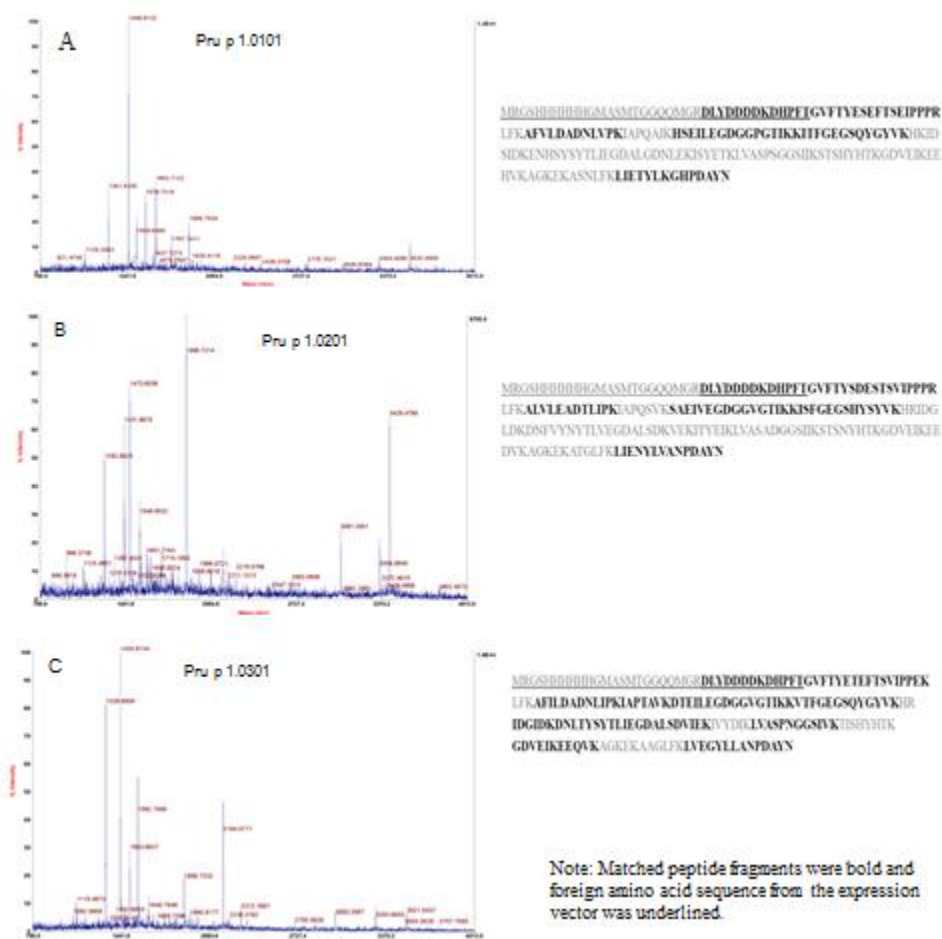


Figure 4 Far-UV CD spectrum of three recombinant Pru p 1 isoallergens.

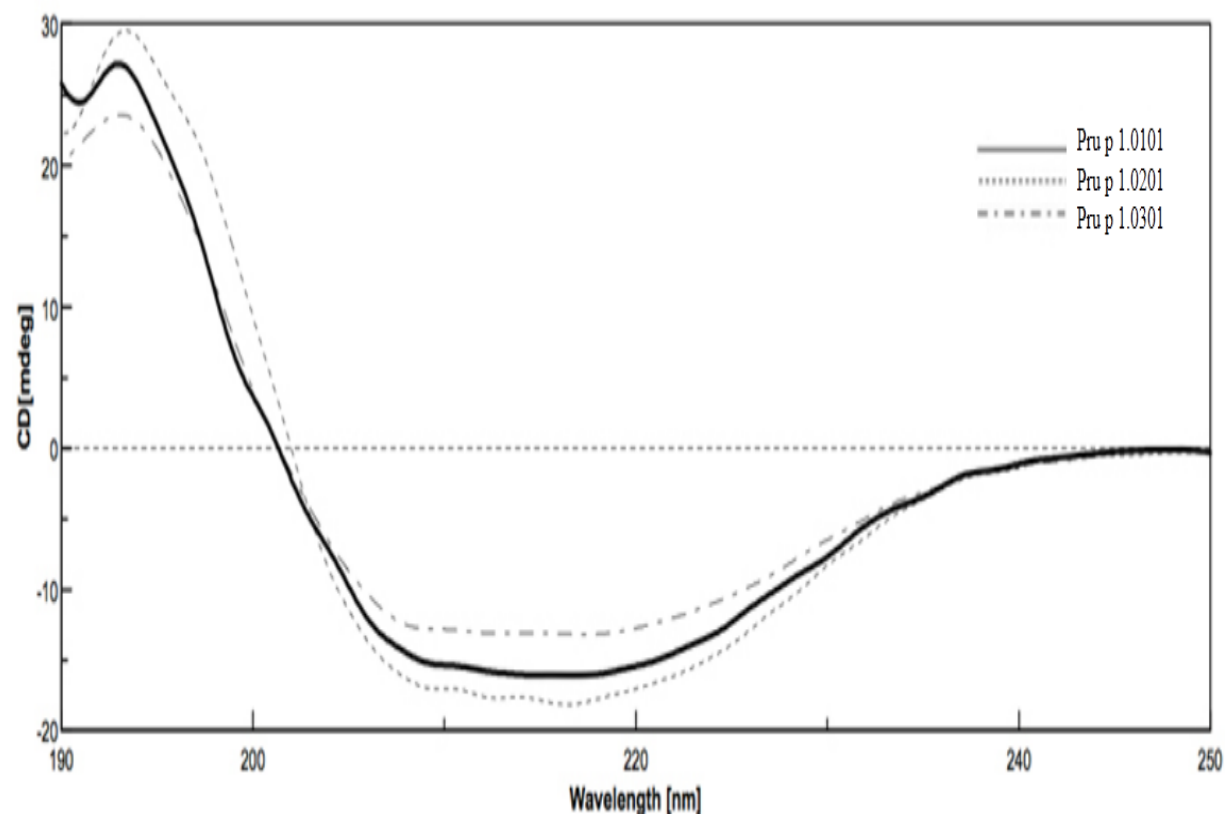


Figure 5 RNA hydrolyzing activity of peach recombinant rPru p 1.0101, rPru p 1.0201 and rPru p 1.0301 in different pH buffer and additional treatments at pH7.5.

(A) pH 3.0; (B) pH 5.0; (C) pH 9; (D) pH 7.5. (E) pH 7.5, boiled at 100°C for 5min; (F) pH 7.5 with 200 μ M zeatin. CK-refer to control, without the addition of Pru p 1 recombinant protein.

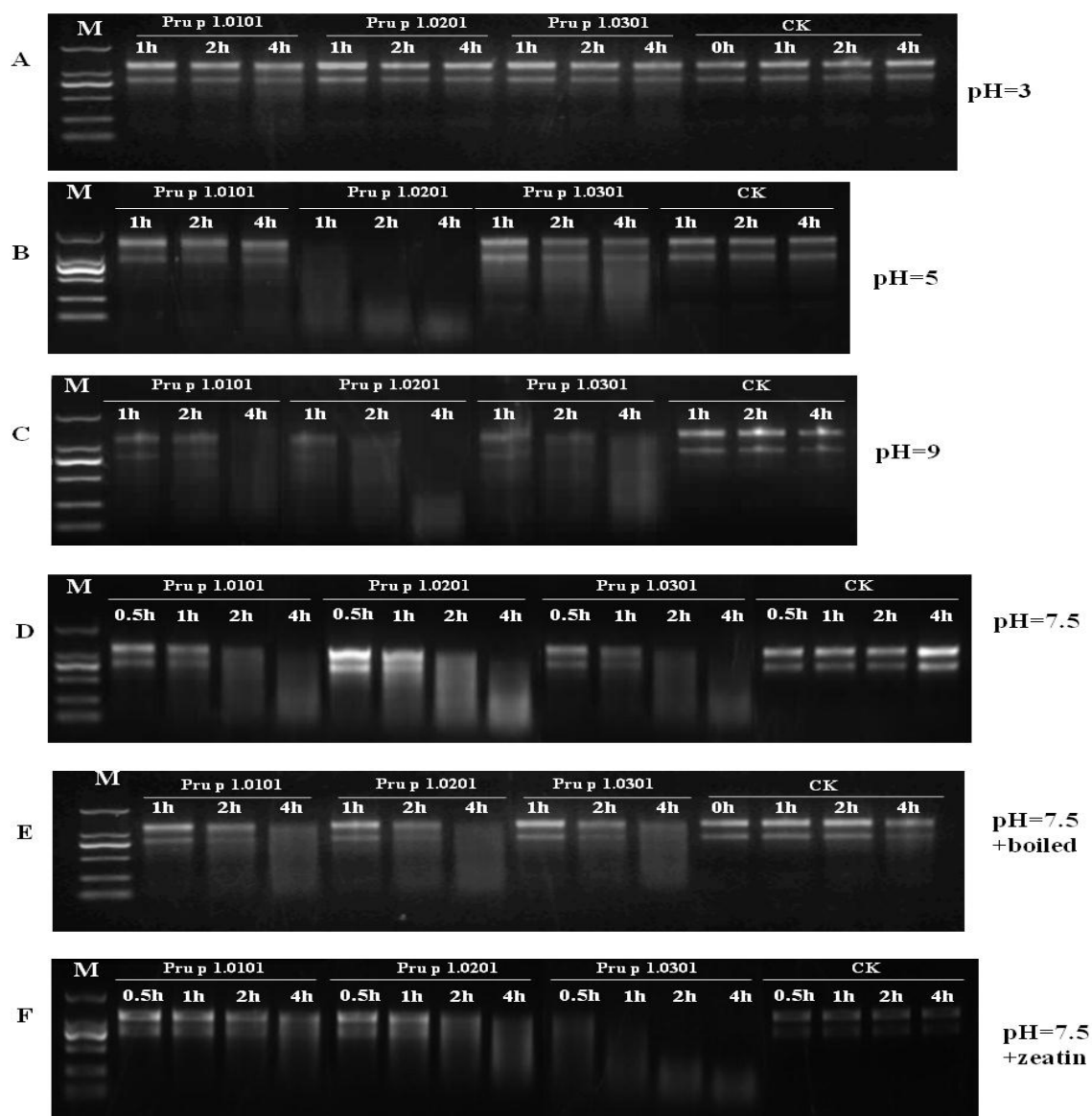


Figure 6 Comparison of IgE titers of three Pru p 1 isoforms on 47 Pru p 1 positive patients.

Median IgE titer and IQR (Inter-Quartile Range) for each isoform (A) and pairwise ratio comparison (B). Ten red spots illustrate 10 sera with similar IgE titer to three isoforms. The blue, green and yellow spots indicate the diverse IgE titers to the three isoforms.

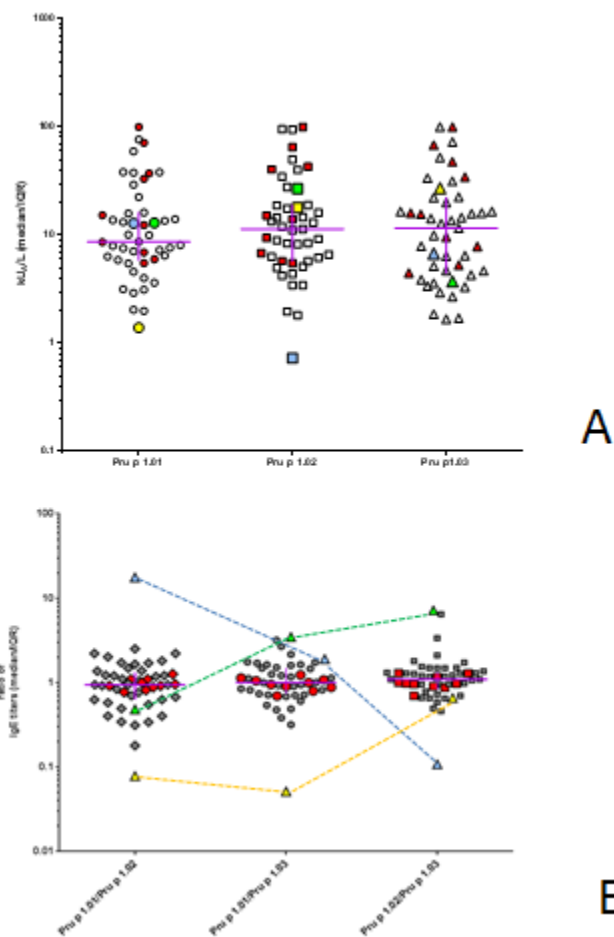


Figure 7 ImmunoCAP inhibition of three Pru p 1 isoforms with a sera pool of similar IgE to the three Pru p 1 isoforms.

A-CAP bound with rPru p 1.0101; B- CAP bound with rPru p 1.0201; C- CAP bound with rPru p 1.0301. Composition of single serum are indicated in red in Figure 6.

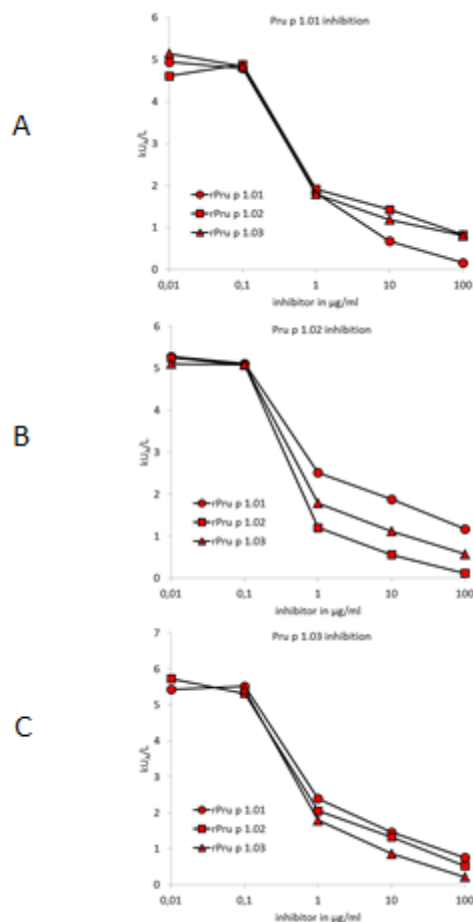


Figure 8 Isoform ImmunoCAP inhibition experiments of single serum with three Pru p 1 isoallergens. (A) serum with higher Pru p 1.01 IgE value as indicated by blue spots in Figure 6, (B) serum with higher Pru p 1.02 IgE value as indicated by green spots; and (C) serum with higher Pru p 1.03 IgE value as indicated by yellow spots in Figure 6.

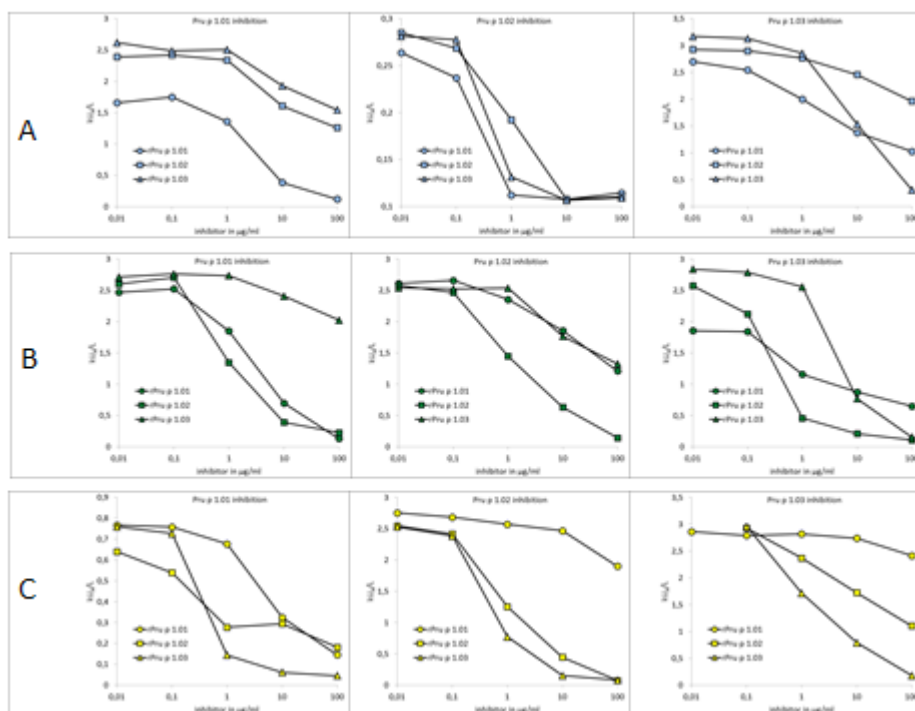
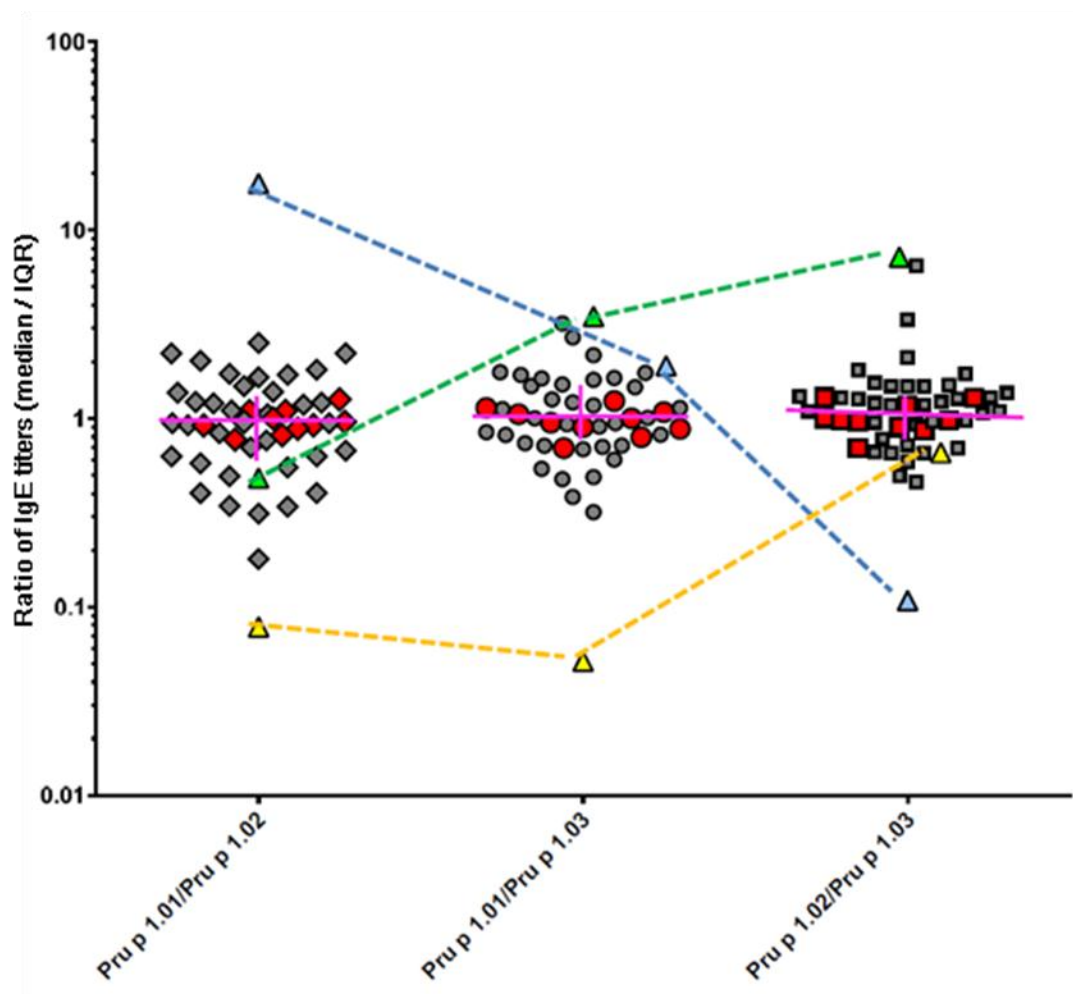


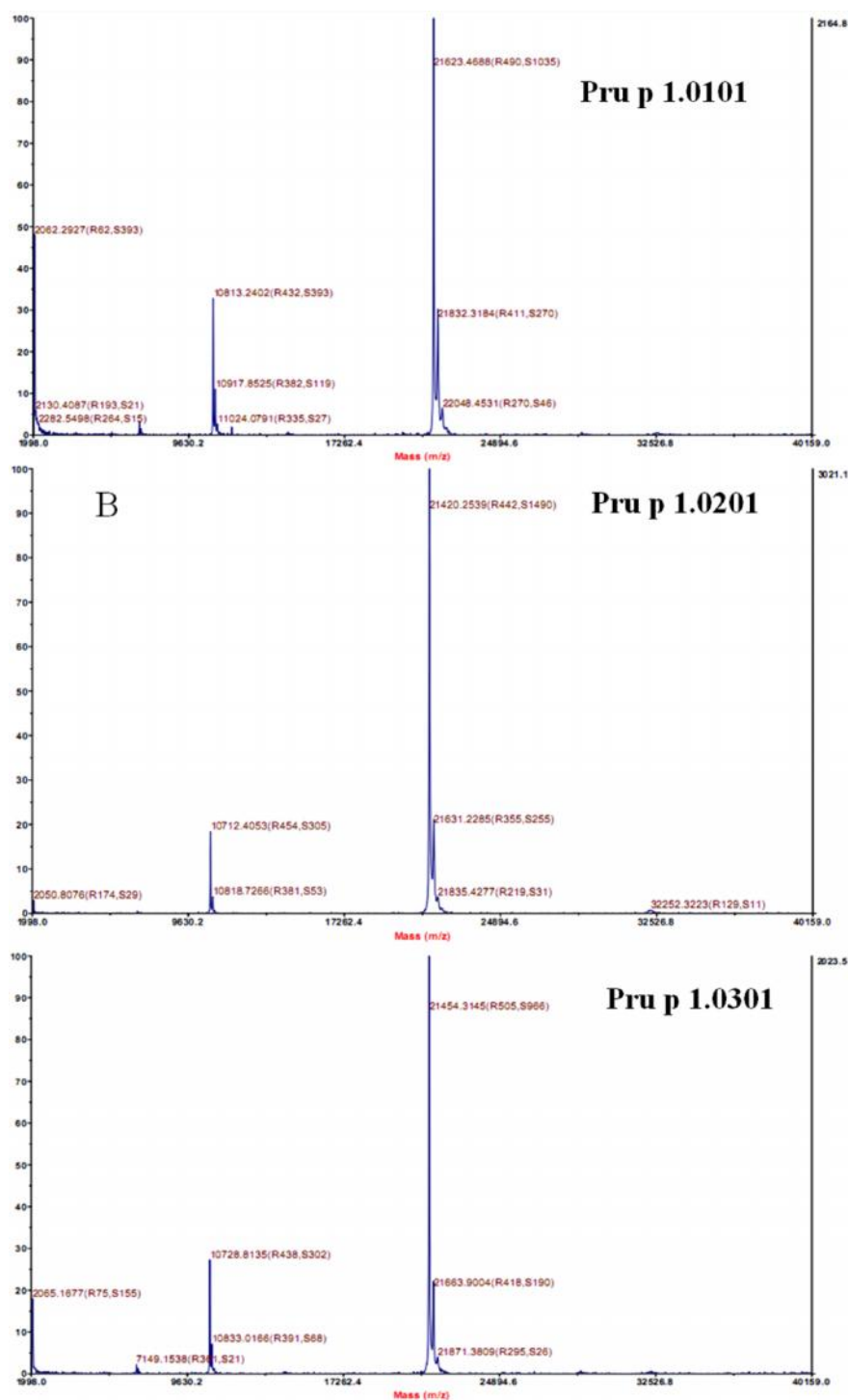
Table 1 Characterization of 4 peach allergic patients and specific IgE test

No	Serum	Country	ImmunoCAP (kU _A /L)			
			rPru p 1	rPru p 3	rPru p 4	rBet v 1
P1		China	5.1	0.1	0.1	16.7
P2		China	1.3	3.16	0.1	4.57
P3		China	1.6	0.55	0.1	4.77
P4		China	2.52	5.54	2.68	68.2

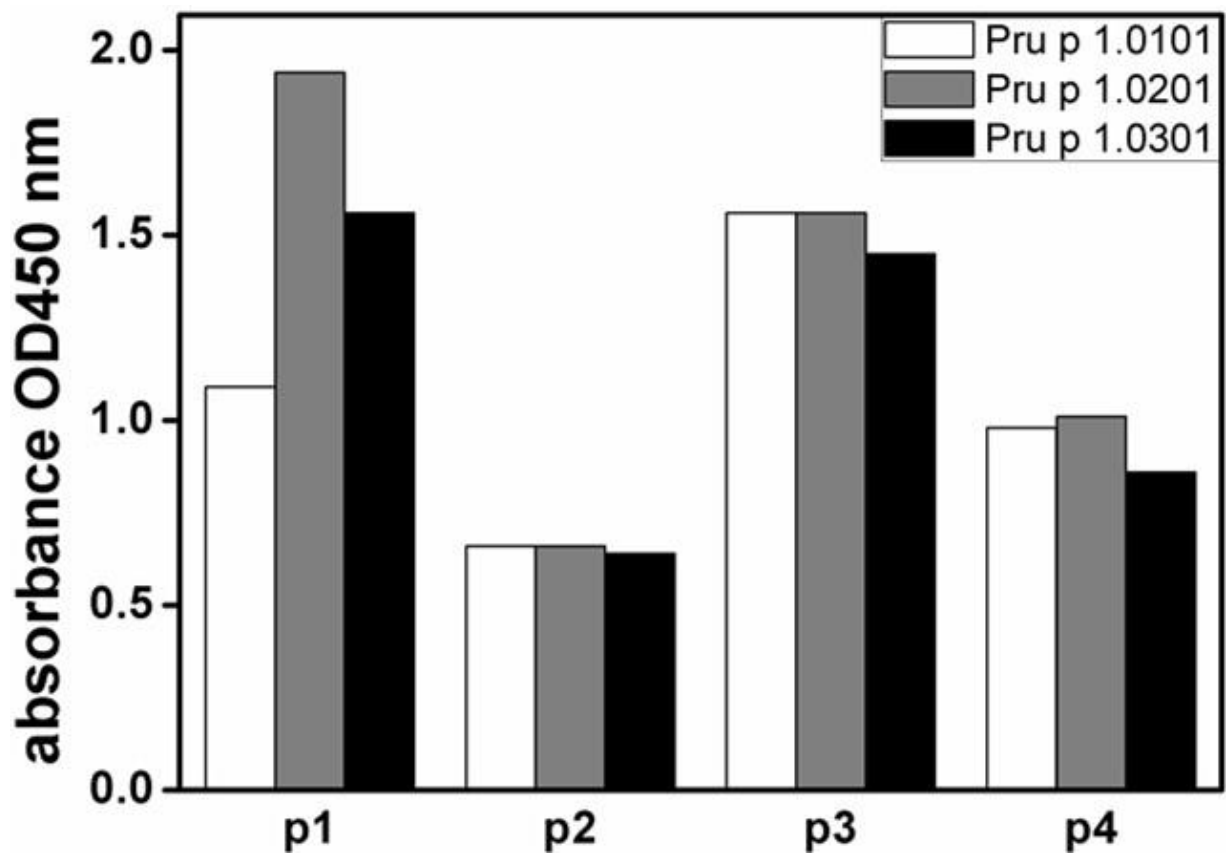
P1, P2 and P3 were the same patients as S1, TY1 and TY14 in the Supplemental Table E1 of Journal of Allergy and Clinical Immunology, 131(1)244-246 (Gao et al., 2013 [1]).



Pru p 1 is a main allergen in peach sensitized patients due to cross reactivity of birch pollen allergy. Three recombinant Pru p 1 isoforms (Pru p 1.01, Pru p 1.02 and Pru p 1.03) were found to have different patterns of IgE binding potency in a group of 47 European Pru p 1 positive patients. There is some similarity, indicated by the ratio of IgE titers around 1, while others are quite different. IQR, represents inter-quartile range.



Supporting Information Figure S1 Mass spectrum of peach recombinant rPru p 1.0101 (A), rPru p1.0201 (B) and rPru p 1.0301 (C).



Supporting Information Figure S2

Immunoassay of recombinant Pru p 1 isoallergens IgE reactivity to recombinant Pru p 1 in ELISA with Chinese patients.