



Short communication

Cloning, expression in *E. coli* and immunological characterization of Par j 3.0201, a *Parietaria* pollen profilin variantA. Bonura^a, A. Trapani^a, L. Gulino^a, V. Longo^a, R. Valenta^b, R. Asero^c, P. Colombo^{a,*}^a Istituto di Biomedicina ed Immunologia Molecolare, "Alberto Monroy" del Consiglio Nazionale delle Ricerche, Palermo, Italy^b Christian Doppler Laboratory for Allergy Research, Division of Immunopathology, Department of Pathophysiology, AKH, Vienna, Austria^c Allergy Department of the Clinica San Carlo, Paderno Dugnano, Italy

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ABSTRACT

Parietaria judaica pollen is one of the main sources of allergens in the Mediterranean area. Its allergenic composition has been studied in detail showing the presence of two major allergens (Par j 1 and Par j 2) and two minor allergens belonging to the profilin and calcium binding protein families of allergens (Par j 3 and Par j 4, respectively). Clinical reports support the hypothesis of a limited cross-reactivity between profilin from *Parietaria* and unrelated sources. We screened a *P. judaica* cDNA library to identify novel forms of profilins with allergenic activity. This strategy allowed us to isolate a 767 bp cDNA containing the information for a 131 amino acids protein with homology to profilins from unrelated sources greater than that observed with the already published *Parietaria* profilins. This profilin was expressed in *Escherichia coli* as a recombinant protein and its immunological prevalence was studied in a population of *Parietaria* allergic patients from Southern Europe. Immunoblotting analysis showed that the *Parietaria* profilin was recognized by IgE from 6.5% of the allergic population. Finally, a selected population of profilin allergic patients was enrolled to demonstrate the cross-reactivity of this novel variant with other profilins from grass and date palm. In conclusion, molecular cloning and immunological studies have allowed the isolation, expression and immunological characterization of a novel cross-reactive profilin allergen from *P. judaica* pollen named Par j 3.0201.

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1. Introduction

Allergies affect growing numbers of people living in industrialized countries. The Skin Prick Test (SPT) represents a widely used method to diagnose this kind of pathology although it has the drawback of being performed with natural extracts that are heterogeneous mixtures of several allergenic proteins whose standardization is difficult to achieve. Sensitization to plant pan-allergens (i.e. Profilins, Calcium Binding Proteins, and Lipid Transfer Proteins) poses relevant diagnostic problems as they show highly conserved amino acid sequences that are widely distributed in the plant kingdom making the interpretation of diagnostic test based on natural extracts difficult due to their IgE cross-reactivity.

The development of in vitro diagnostic tests based on the use of purified natural and/or recombinant allergens gives a well-defined picture of a patient's IgE recognition (Hiller et al., 2002), therefore, knowledge of the complete spectrum of allergens contained in

an allergenic source and the identification of markers of sensitization represents a key issue for a correct diagnosis and for the subsequent prescription of allergen specific immunotherapy vaccines (Mothes et al., 2006; Valenta and Niederberger, 2007).

Profilins are small cytosolic proteins found in all eukaryotic cells as part of the cytoskeleton. Plant profilins are usually minor allergens (Anto et al., 2012) and cause IgE cross-reactivity not only among botanically unrelated pollens (Valenta et al., 1992) but also between pollens and foods (van Ree et al., 1992) as well as pollen and latex (Ganglberger et al., 2001). Most studies have confirmed the immunological equivalence of profilins from different sources (Ganglberger et al., 2001; van Ree et al., 1992), but some reports found limited cross-reactivity of some specific profilins, such as that from *Parietaria*, with birch and grass profilins (Asero et al., 2003).

Parietaria is one of the most relevant causes of pollen allergy in people living in the Mediterranean basin (D'Amato et al., 2007). About 30% of all allergic subjects living in southern Italy are positive on SPT with *Parietaria judaica* (Pj) pollen extract (D'Amato, 2000). The composition of allergenic extracts of Pj pollen has been studied by several methods showing the presence of two major allergens belonging to the Lipid Transfer Protein (LTP) family (Par j 1 and Par j 2) (Colombo et al., 2003) as well as the presence of two highly cross-reactive allergens, namely Calcium Binding Protein (Par j 4)

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(Bonura et al., 2008) as well as two profilin isoforms (Par j 3.0101 and Par j 3.0102) (Asturias et al., 2004).

In this report we describe the isolation, expression in *Escherichia coli* and immunological characterization of a novel variant of *Parietaria* pollen profilin with allergenic activity that, according to IUIS nomenclature, has been named Par j 3.0201.

2. Materials and methods

2.1. Screening of the cDNA library and sequence analysis

A *P. judaica* cDNA library was prepared as previously described (Bonura et al., 2008). The library was screened with a Phl p 12 cDNA clone labelled with dCTP³² (Valenta et al., 1994). P³² labelled phages were picked, plaque-purified, and stored at 4 °C. Inserts from the selected phages were amplified with T3 and T7 primers by PCR (94 °C 1 min, 52 °C 1 min, 72 °C 1 min for 30 cycles) and phages with the largest inserts ($n = 10$) were further analyzed. PCR products were purified and cloned in the pCR4-TOPO vector (Invitrogen, Milan, Italy). Similarity searches and alignments of deduced amino acid sequences were performed using the BLAST 2.0 program (www.ncbi.nlm.nih.gov/BLAST/). Predictions of secondary structure were performed using www.predictprotein.org web site. A 3D model was obtained by using the services of the Swiss-Model Protein Modelling server (<http://swissmodel.expasy.org>) relying on the structure of a profilin from *Arabidopsis thaliana* (entry 1a0kA) as a template (Arnold et al., 2006). Estimation of the 3D model was performed using the Global Model Quality Estimation (QMEAN4) algorithm available through the Swiss-Model web site (Benkert et al., 2011).

2.2. Human sera

Prevalence study with *Parietaria* allergic patients was performed enrolling patients fulfilling the following criteria: (1) clinical history of seasonal respiratory allergy and (2) positive SPT to *P. judaica* commercial extracts. Donors signed an informed consent before undergoing sampling. In particular, 31 consecutive sera were collected by the Unità di Allergologia dell'Ospedale Civico (Palermo, Italy). In addition, sera from 18 profilin-hypersensitive patients seen at the Allergy Department of the Clinica San Carlo (Paderno Dugnano, Northern Italy) were studied. In this region *Parietaria* allergy shows a low prevalence. Profilin hypersensitivity was detected by SPT using a commercial profilin-enriched date palm pollen extract (50 mg protein/ml; ALK-Abellò, Madrid, Spain). These patients underwent SPT with commercial *Parietaria* pollen extract (50,000 SPU/ml; Allergopharma, Reinbeck, Germany) and the detection of IgE to *Phleum pratense* Phl p 1, Phl p 5, Phl p 7, Phl p 12; *P. judaica* Par j 2; *Betula verrucosa* Bet v 1; *Olea europea* Ole e 1; *Ambrosia artemisiifolia* Amb a 1; *Artemisia vulgaris* Art v 1 and *Cupressus arizonica* Cup a 1 allergens was performed by ImmunoCAP (Termo-Fischer/Phadia, Uppsala, Sweden) in order to detect the primary sources of sensitization.

2.3. Recombinant *P. judaica* profilin expression and purification

Recombinant Pj profilin was expressed as a His-tagged fusion protein using the pQE30 expression vector (QIAGEN, UK). In particular, the *Parietaria* profilin coding region was amplified using the following primers: profilin forward (Pj for) 5'-cgcGGATCCATGTCGTGGCAGACC3' and profilin reverse (Pj rev) 5'-cgcTTCGAACTTAGAGGCCAGTCTC3' (underlined letters indicate the BamH I and Hind III restriction enzyme sites introduced for cloning in the expression vector, bold letters the first methionine and the stop codon respectively) (see Fig. 1 for details). Coding sequence was confirmed by sequencing.

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47
cggcacgagatcaaatcaaaatccccaaatctccattctctccaacc
48
Pj for
ATGTCGTGGCAGACCTACGTCGATGATCACCTCATGTGCGAAATCGAGGGCAACCACCTC
108
ACCGCCGCCGCATCCTCGGCCAGGACGGCAGCGTCTGGGCTCAGAGCGCTCCTTCCCG
168
CAGTTTAAGCCTGAGGAAATGCGCGAATCGTAAAAGATTTCGAGGAGCCTGGTACTCTT
228
GCCCGACTGGGTATTCTCTCGGGGGCGGAAGTACATGGTCATTAGGGCGAGGCCGGA
288
GTTGTTATCCGTGGCAAGAAGGGATCGGGTGGTGTCACTGTTAAGAAAACCGGGCAAGCT
348
CTGGTAATAGGAATTTACGACGAGCCGATGGCACCTGGACAGTGCACATGATCGTCGAG
408
Pj rev
AGGTTGGGAGACTACCTTATTGAGACTGGCTCTAAGTtttctctctgctcgtatttta
468
caaaactggtgattttctttttctttttctctgtgctgtttggcgttgactgctgttttc
528
ctgctatggcctcgaacttgttggtatcgtagagtcgttctcctgaaataatcgttttc
588
agacacaagaaaagattgtgtgtgttagagtcgttctttttctgtcggtttttccg
648
ttgtggttcctggaattgtgtatttaaacgcctccacagtttctcctgttgatgagtc
708
Atgattgtgtaattcttaagtatccatgagtttcatttggttaaaaaaaaaaaaaaaaaaa

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B

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1
MSWQTYVDDH LMCEIEGNHL TAAAILGQDG SVWAQSASFQ QFKPEEIAAI 50
51
VKDFEPEPTL APTGLFLGGA KYMVIQGEAG VVIRGKKGSQ GVTVKKTGQA 100
101
LVIGIYDEPM APGQCNMIVE RLGDYLIETG L 131

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Fig. 1. (A) Nucleotide sequence of the cDNA encoding for a novel profilin from *Parietaria* pollen. Lower case letters indicate 5' and 3' untranslated regions. The first methionine and the STOP codon are marked in bold letters. Arrows indicate the oligonucleotides used for cloning and expression of the Pj profilin in the pQE30 vector. (B) Deduced amino acid sequence of the Pj profilin.

A single recombinant pQE30-Pj3 XL1-Blue colony was grown over night at 37 °C in 10 ml of 2YT broth (Bactotryptone 16 g/l, Bacto-yeast 10 g/l, NaCl 5 g/l, pH 7.0) + AMP 100 µg/ml. A 1:40 dilution was made and the culture was grown for 2 h at 37 °C, then recombinant protein expression was induced with 1 mM isopropyl-D-thiogalactopyranoside (IPTG) for 3 h at 37 °C. The recombinant proteins were purified by affinity chromatography as previously described (Bonura et al., 2008). Briefly, the cells were harvested, resuspended in 20 ml of Buffer I (20 mM phosphate buffer pH 7.4, 0.5 M NaCl and 6 M urea) and lysed with mild sonication. Cell debris was removed by centrifugation at 10,000 rpm for 15 min at 4 °C and the supernatant was filtered using a 5 µm disc and then loaded on a HiTrap™ Chelating HP column (GE Healthcare Biosciences AB, Sweden) following the manufacturer's instructions. The recombinant proteins were eluted using a buffer containing 20 mM phosphate buffer pH 7.4, 0.5 M NaCl, 6 M urea and 500 mM imidazole; the fractions were analyzed by 12% SDS-PAGE and Coomassie brilliant blue staining. The fractions containing the recombinant profilin were dialyzed against a buffer containing 20 mM phosphate buffer pH 7.4 and 0.5 M NaCl to allow refolding of the proteins. Buffer exchange was performed using a Sephadex G-25 column (GE Healthcare Bio-sciences AB, Sweden) in 1× PBS. The purity and concentration of the proteins were determined by Coomassie brilliant blue staining and densitometric analysis (Quantity ONE software, Biorad, USA).

2.4. Western blot analysis

Recombinant Pj profilin was fractionated on a 16% SDS-PAGE and transferred onto a PVDF transfer membrane (Millipore, USA) using a semi-dry Trans-Blot Transfer (Millipore, USA). Sera were

Table 1
Percentage of identity and homology between the Par j 3.0201 and other profilins with allergenic activity.

Allergen name	% identity	% homology
Pru p 4.0101	86.3	93
Fra a 4.0101	84.7	91
Hev b 8.0101	84.0	91
Mal d 4.0101	82.0	91
Cyn d 12	82.4	91
Tri a 12.0101	80.8	90
Phl p 12.0101	80.2	91
Ara h 5	79.0	90
Ole e 2.0101	78.4	87
Bet v 2	78.2	88
Par j 3.0101	75.8	84
Par j 3.0102	75.0	84

diluted 1:5 in a buffer containing 1× PBS, 0.25% BSA and 0.1% Tween 20. IgE complexes were detected using a goat anti-human IgE-HRP conjugate (Biosource International, USA). Reactive bands were visualized by using SuperSignal West Pico Maximum Sensitivity Substrate (Pierce Biotechnology Inc., Rockford, USA) and subsequent exposure to Kodak X-OMAT X-ray film (Kodak, New York, NY).

2.5. *P. judaica* profilin immuno slot-blotting

Recombinant Pj profilin (2 µg/well) was spotted on nitrocellulose membrane using a slot blot manifold apparatus (GE Healthcare Bio-sciences AB, Sweden). The spots on the membrane were cut, equilibrated in 1× PBS-T (1× PBS pH 7.4, 0.1% Tween 20) and then incubated in Blocking Solution (1× PBS pH 7.4, 0.1% Tween, 3% BSA, w/v) for 1 h at room temperature under agitation. Membranes were rinsed three times in 1× PBS-T for 15 min, and then incubated overnight with single sera (1:5 in 1× PBST, 0.02% Na₂S₂O₃) from 18 Phod 2 and Phl p 12 allergic patients, one serum from a profilin positive *Parietaria* patient and a serum from a non-allergic subject (negative control). After incubation, membranes were rinsed three times in 1× PBS-T for 15 min and incubated for 1 h with conjugated Goat α-Human IgE-HRP (Southern Biotech, USA) diluted 1:5000 in α-IgE dilution Buffer (1× PBS pH 7.4, 0.1% Tween, 0.25% BSA, w/v). Reactive bands were visualized using the Amersham™ ECL PLUS Western blotting Detection System (GE Healthcare, Sweden) and subsequent exposure to Kodak X-OMAT X-ray film (Kodak, New York, NY).

3. Results

3.1. Cloning and sequencing of a novel variant of profilin from *P. judaica* pollen

Ten purified plaques were analyzed for the size of the inserts by PCR using the M13 forward and reverse primers. All PCR products showed the same size and 3 out of 10 were purified and cloned in the pCR4-TOPO vector. Sequence analysis showed a 767 bp insert with short 5' and 3' untranslated regions (47 and 300 bp, respectively) (Fig. 1 panel A). In silico analysis showed the presence of a coding region of 396 bp capable of expressing a 131 amino acid protein (Fig. 1 panel B). All the inserts contained a canonical poly-A tail and did not show any polymorphism at the nucleotide level. A homology search performed with the 131 amino acid long coding region showed a high percentage of homology with profilins from several plant species (data not shown). Table 1 indicates the range of identity (from 75 to 86.3%) and homology (from 84% to 93%) to some of them with allergenic activity detected by the FASTA algorithm (<http://www.ebi.ac.uk/fasta33>). In addition, sequence comparison between the previously published *Parietaria* profilins Par j 3.0101, Par j 3.0102 and the new variant described in this

manuscript showed a percentage of amino acid identity of around 75%, less than that detected between the two already published sequences (97%) (Fig. 2). The in silico structural analysis showed that the novel *Parietaria* profilin displays a canonical 4 α-helices and 7 β-sheet structure common to other profilins. In a similar way, using the X-ray structure from a profilin from *Arabidopsis thaliana* (PDB 1a0ka) as a template we showed that the 3 *Parietaria* profilin isoforms display a similar 3D structure. The QMEAN4 global scores of the three models were 0.865 (for Par j 3.0101), 0.825 (for Par j 3.0102) and 0.84 (for Par j 3.0201) (Fig. 2).

3.2. Recombinant expression in *E. coli* and prevalence study

Intracellular protein expression of the *Parietaria* profilin protein in *E. coli* was studied by means of SDS-PAGE and Coomassie brilliant blue staining (Fig. 3 panel A). The protein, expressed as His-tagged protein, was purified by one-step affinity chromatography yielding about 0.5 mg/l of protein. The immunological activity of the purified profilin was analyzed by immunoblotting. Thirty-one sera from consecutive Pj-allergic subjects were studied demonstrating that the expressed profilin displayed an allergenic activity in 2/31 cases (6.5%) (Fig. 3 panel B). No signal was observed when a serum from a non-allergic subject was tested as a negative control. Official WHO-IUIS allergen nomenclature has been requested and the name Par j 3.0201 has been proposed for the identified Pj profilin.

3.3. Profilin-specific IgE recognize Par j 3.0201

To study the cross-reactivity between the Par j 3.0201 allergen and profilins from other allergenic sources, eighteen sera from Phod 2 reactors living in the Northern Italy were selected. Within this population IgE reactivity to profilin was further confirmed by the presence of specific IgE to Phl p 12, a grass pollen profilin (Table 2). These patients showed a heterogeneous pattern of skin reactivity to the Pj pollen extract in vivo: strong in 3 cases, moderate in 3, weak in 9, and absent in 3. However, these patients did not show any IgE reactivity to the marker of primary *Parietaria* sensitization Par j 2 (Stumvoll et al., 2003) suggesting that their skin reactivity to *Parietaria* was the result of cross-reactivity between profilins. However, immunoblot experiments performed with sera from these patients showed that all of them reacted to Par j 3.0201, thus demonstrating that this allergen is able to bind profilin-specific IgE antibodies elicited by profilins from other allergenic sources (Fig. 4).

4. Discussion

Profilin is a plant pan-allergen found in almost all eukaryotic cells that is largely responsible for IgE cross-reactivity between botanically unrelated allergen sources. The presence of two allergenic profilins in *Parietaria* pollen has been reported showing the presence of two isoforms with 97% homology at the amino acid level but quite different prevalence of sensitization in the population (18% and 6%, respectively) and partial cross-reactivity with other profilins (Asturias et al., 2004). The latter point has been confirmed by some clinical reports supporting the hypothesis of a limited cross-reactivity between *Parietaria* profilin and birch and grass profilins (Asero et al., 2004). These observations opened the way to further discussion about the identification of a proper tool for the diagnosis of profilin sensitization in the *Parietaria* allergic population.

The first goal of this study was the identification of a 131 amino acid long variant with about 75% homology to the Par j 3.0101 and Par j 3.0102 profilins that represents a novel variant named Par j 3.0201 according to IUIS guidelines. Structural predictions showed that the Par j 3.0201 variant displays a secondary structure common to this family of proteins and, despite a different homology at

A

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1                                     60
Par j 3.0101 MSWQAYVDDHLMCDVGDGNTPASAAIIGHDGSVWQAQSANFPQLKPEEVTGIMNDFNEAGF
Par j 3.0102 MSWQAYVDDHLMCDVGDGNTLASAAIIGHDGSVWQAQSANFPQLKPEEVTGIMNDFNEGGF
Par j 3.0201 MSWQTYVDDHLMCEI-EGNHLTAAAILGQDGSVWQAQSAFPQFKPEEIAAIVKDFEEPGT
*****:*****: :** : :***: :*****:***:*****: :* : :***: *
61                                     120
Par j 3.0101 LAPTGLFLGGTKYMVIQGESGAVIRGKKGGGATLKKTGQAIVIGIYDEPMTPGQCNLVV
Par j 3.0102 LAPTGLFLGGTKYMVIQGESGAVI-GKKGGGATLKKTGQAIVIGIYDEPMTPGQCNLVV
Par j 3.0201 LAPTGLFLGGAKYMVIQGEAGVVIRGKKGGGVTVKKTGQALVIGIYDEPMAPGQCNMIV
*****:*****:***:*** *****: :*****:*****:*****: :* : :
121      132
Par j 3.0101 ERLGDYLLEQGL
Par j 3.0102 ERLGDYLLEQGM
Par j 3.0201 ERLGDYLIETGL
*****: * *:

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B**Alignment data:**

Alignment length: 132 aa
 Identity (*): 98 (74.24%)
 Strongly similar (:): 23 (17.42%)
 Weakly similar (.): 4 (3.03%)
 Different: 7 (5.30%)

C**Overall homology between the *Parietaria* profilins**

	Par j 3.0101	Par j 3.0102	Par j 3.0201
Par j 3.0101		97%	75.8%
Par j 3.0102	97%		75.0%

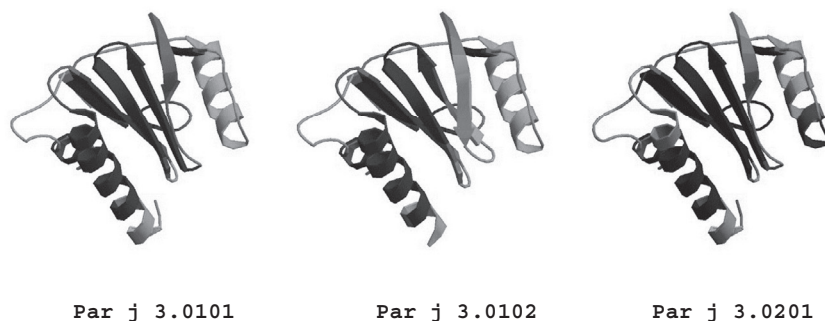
D**3D modeling**

Fig. 2. (A) Alignment of the amino acid sequences of Par j 3.0101 and Par j 3.0102 with the novel Pj profilin. The entries displayed are: Par j 3.0101 (#Q9XG85), Par j 3.0102 (#Q9TOM8) and Par j 3.0201 (#L8BTD8). Asterisks indicate amino acid identity, colons show strong amino acid similarity and dots weak amino acid similarity. Gaps have been introduced to achieve best alignment. (B) Alignment data between the three Pj profilins. (C) Percentage of identity between the three Pj profilins. (D) Backbone ribbon representation of the Par j 3.0101, Par j 3.0102 and Par j 3.0201 allergens determined using the services of the Swiss-Model Protein Modelling Server and the X-ray data from a profilin from *Arabidopsis thaliana* (entry 1a0kA) as a template.

the amino acid level, in silico modelling highlighted that the three *Parietaria* profilins display a similar 3D structure. Interestingly, this new variant showed a percentage of homology to profilins from unrelated sources greater than that detected between the other *Parietaria* profilins. To study the prevalence of the Par j 3.0201 variant, this profilin was expressed in *E. coli* and the immunological relevance of the recombinant allergen was analyzed in a *Parietaria* pollen allergic population by means of Western blot. We were able to detect IgE reactivity to the new variant in 6.5% of South Mediterranean *Parietaria* allergic subjects demonstrating that, in this geographical area, Par j 3.0201 is a minor allergen.

A second point was addressed by this study in the analysis of the IgE cross-reactivity of the Par j 3.0201 variant to two well-known

profilins, the date palm pollen profilin (Pho d 2) and the grass pollen profilin (Phl p 12). Sera from a selected population of 18 Pho d 2-sensitized subjects living in an area where profilin sensitization is the consequence of primary sensitization to airborne allergen sources other than *Parietaria* (Villalta and Asero, 2010) were studied. These patients showed great variability in their degree of skin reactivity to *Parietaria* extract: strong in 3 cases, moderate in 3, weak in 9, and absent in 3, respectively (Table 2). Nonetheless, all the sera were negative to the *Parietaria* marker of sensitization Par j 2 and positive to the Par j 3.0201 variant reaffirming (i) the unreliability of such a reagent for a correct diagnosis of *Parietaria* profilin sensitization and (ii) demonstrating the cross-reactivity of this novel *Parietaria* variant to profilins

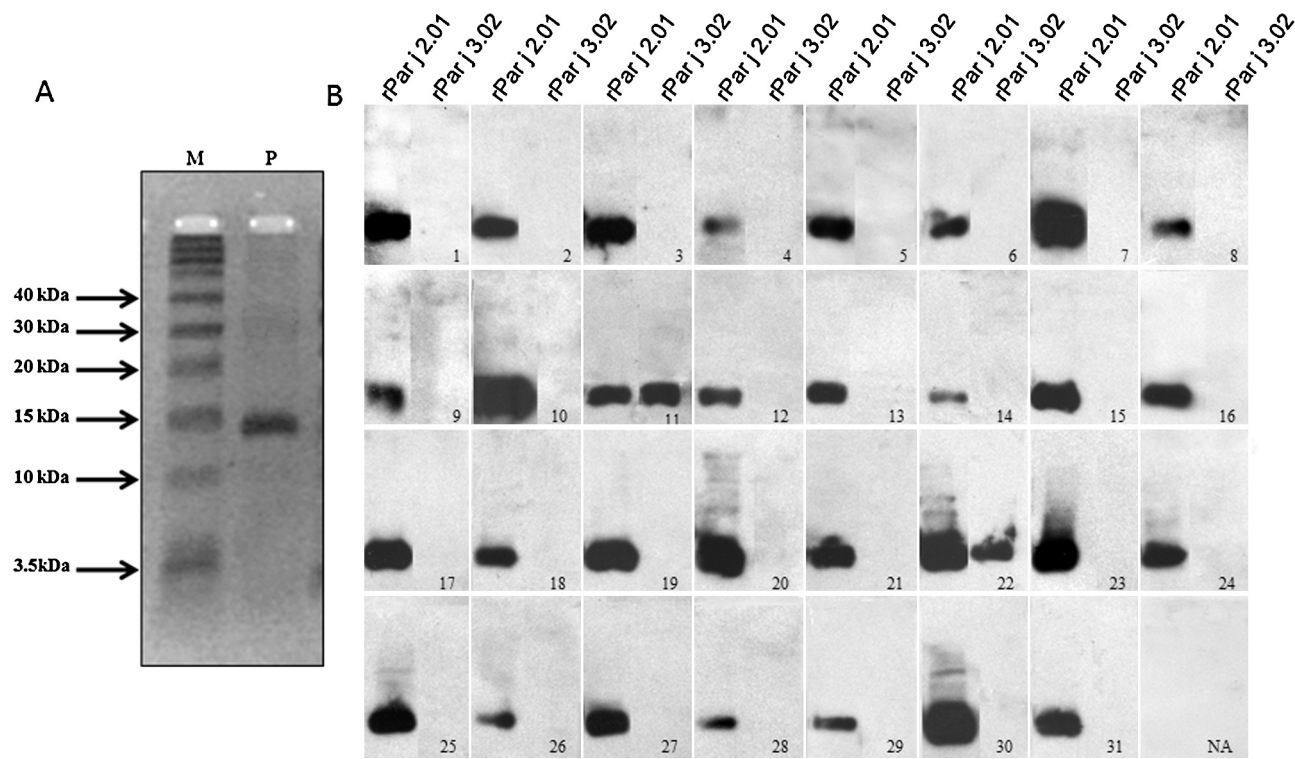


Fig. 3. (A) Lane P Coomassie brilliant blue staining of the purified recombinant *Parietaria judaica* profilin rPar j 3.0201, Lane M: standard Molecular Weight. (B) IgE immunoblotting analysis of 31 sera from *Parietaria* allergic patients with purified rPar j 2.01 and rPar j 3.02 allergens. NA shows a control blot incubated with a non-allergic serum.

such as those from date palm and grass pollen. The latter data are somewhat in contradiction with previous reports raising a question about the characterization of Pj profilins for diagnosis. One possible explanation for these controversial results could be that Pj profilins may display different levels of IgE cross-reactivity due to differences in the primary sensitization agent. Following this line of evidence, it could be relevant to mention it has been reported that the profilin content of pollen extracts is highly variable between species (Ruiz-Garcia et al., 2011) and the amount of natural profilin

contained in the crude extract of *P. judaica* is extremely low (Asturias et al., 2004). These studies could support the hypothesis that Pj profilin sensitization may have been influenced by the presence of other pollens in the area. In addition, we do not know the relative concentration of the three identified profilins in the Pj extract and whether these isoforms are co-expressed in the pollen. A final point of discussion might be the different geographical areas where the mRNAs were extracted suggesting the possibility of different profilins in plants from different areas.

Table 2
IgE reactivity and clinical evaluation of the Pho d 2 positive population.

	Gender	SPT Parietaria	Phl p 1	Phl p 5	Phl p 7	Phl p 12	Par j 2	Bet v 1	Ole e 1	Amb a 1	Art v 1	Cup a 1	Symptoms
Patient 1	F	+++	100	100	0	27.7	0	100	0.81	86.2	0	35	A,G,B,Cip
Patient 2	M	++++	0	0	0	4.37	0	1.48	0.00	43.2	0	0	B,A
Patient 3	M	++	2.01	1.46	0	2.91	0	54.8	4.93	15.6	0	10.6	B
Patient 4	M	++++	76.7	56.7	0	2.51	0	8.27	1.06	100	0	0.21	A,G
Patient 5	M	—	100	12.3	0	5.86	0	0	33.60	7.84	0	8.14	G
Patient 6	M	++	7.44	0	0	96.7	0	0	0.00	100	0	17.3	A
Patient 7	M	—	4.78	3.52	0	3.03	0	0	3.95	80.7	0	5.63	A,G,Cip
Patient 8	M	+	57.5	27.6	0	2.65	0	8.02	0.00	20	0	0.86	G
Patient 9	M	+	75.3	100	0	46.3	0	100					G
Patient 10	M	+	80.1	85.7	0	2.63	0	4.27	0.53	0.62	0	0.85	G
Patient 11	F	++	100	100	0	13.8	0	0.22	0.30	0.57	0	0	G
Patient 12	F	+	41.6	100	0	9.82	0	0	8.71	0.57	0	0	G
Patient 13	M	+	19.3	10.2	0	1.83	0	0	4.22	6	0	39.3	G,A
Patient 14	M	+	15.1	13.4	0	3.11	0	0	3.00	38.5	4.91	0	G,A
Patient 15	M	+	1.22	4.05	0	7.24	0	0	0.00	87.7	0	100	A,Cip
Patient 16	F	—	34.6	63.5	0	2.42	0	17.7	4.94	100	0.33	3.73	G,A
Patient 17	F	+	10	26.8	0	5.04	0	46.8	10.20	100	0	0.42	B
Patient 18	F	+	28.4	43.9	0	6.49	0	39.2	0.00	16.8	0	3.4	B,G,A

B: birch; A: ragweed; G: grass; Cip: cypress. Symptoms means that the patient experienced respiratory allergy symptoms in the flowering period corresponding to the indicated pollen.

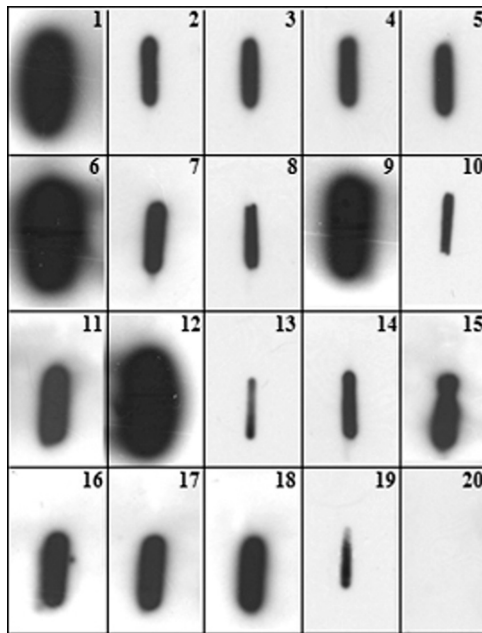


Fig. 4. IgE dot-blot analysis of rPar j 3.0201 with sera from Pho d 2 allergic patients (from #1 to #18); dot #19 displays a previously characterized Pj profilin allergic serum; dot #20 a serum from a non-allergic subject.

5. Conclusion

In this study we reported the identification and immunological characterization of a novel variant of a *Parietaria* profilin with allergenic activity. Since current in vitro diagnostic assays are usually based on single isoforms per species, better definition of the spectrum of cross-reactivities between isoforms and allergens may be of importance to avoid misleading diagnostic results.

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