Characterization of Group 1 Major Allergen of *Quercus mongolica*, Que m 1

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Characterization of Group 1 Major Allergen of *Quercus mongolica*, Que m 1

Directed by Professor Jung-Won Park

The Master's Thesis
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June-Yong Lee

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(Directed by Professor Jung-Won Park)

Background

Oak, which belongs to Fagales family and the pollen is the main cause for spring pollinosis in Korea. white oak pollen has been being used for diagnosis and treatment of spring pollinosis, even though it is not native species in Korea. Group 1 major allergen, which belong to pathogenesis-related protein-10 (PR-10) comprises of 80% of total allergenicity of Fagales pollens and it is considered as the most important major allergen for Fagales pollinosis. Therefore, we produced and investigated the allergenicity of group 1 major allergen of mongolian oak, which is the prevalent oak species in Korea.

Methods

A putative Que m 1 was cloned by RT-PCR, and expressed in *Escherichia coli*. IgE binding reactivity to the recombinant proteins, along with commercial Bet v 1, were assessed using enzyme-linked immunosorbent assay (ELISA) with the 50 sera of Korean Fagales pollinosis patients.

Results

Que m 1 consist of 160 amino acids, with calculated molecular weight (MW) of 17,292 Da and the isoelectric point is 5.37. Que m 1 shares 96.2% amino acid sequence identity with Que a 1. However, Que m 1 showed higher specific IgE reactivity than Que a 1 in Korean Fagales pollinosis patients (90.0% vs. 78.0%). For diagnosis of Korean Fagales pollinosis, area under the curve of Que m 1 sIgE was higher than that Que a 1 in ROC analysis. Bet v 1 sIgE was higher than these oak pollen group 1 major allergens and its positive rate (94.0%). In IgE inhibition blotting analysis, recombinant Que m 1 completely inhibited IgE response to about 17 kDa allergen of mongolian oak extract, while it was partially inhibited by recombinant Que a 1. Que m 1 seems to be a major cause for oak allergy in Korea.

Conclusion

We cloned and produced recombinant group 1 major allergen of *Quercus mongolica* (Que m 1), which may be potentially useful for the diagnosis and treatment of Fagales pollinosis in Korea. It showed strong cross-reactivity with other allergenic PR-10 molecules from oak and birch, and had better clinical values than Que a 1 for diagnosis of Korean Fagales pollinosis.

Key words: Allergen; Fagales pollinosis; *Quercus mongolica*; Pathogenesis-related protein-10;

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I. INTRODUCTION

The oak pollen is the main cause of spring pollinosis in Korea.[1] The prevalence of sensitization to oak has been described between 4.1% and 9.1% of allergic patients by skin prick test (SPT),[1-3] and has been considered as one of the most prevalent allergen, although the results of SPT and ImmunoCAP test showed a slight difference.[4] White oak (*Quercus alba*) pollen extracts have been being employed for diagnosis and treatment in Korea. However, *Q. alba* is not a native species in Korea and mongolian oak (*Quercus mongolica*) is the dominant species.[1, 4, 5] In Korea, oak and pine cover about 80% of the forest area and birch occupies about 1% of it [JH Park's thesis, 2009] and six representative oak species (*Quercus mongolica, Quercus aliena, Quercus variabilis, Quercus serrata, Quercus dentata, and Quercus acutissima*) has been

described. Birch distributes rarely in highland and common silver birch trees (*Betula verrucosa*) are planted as ornamental purpose. However, there are many patients showing IgE reactivity to birch pollen. It could be explained by the cross-reactivity of oak and birch allergens, both of them belong to Fagales family. Four allergens have been described from oak; PR-10 protein, profilin, lipid transfer protein (nsLTP), and calcium binding proteins.[6] PR-10 allergens are known as the most potent allergens with strong cross-reactivity from the pollens of trees belongs to order Fagales, such as birch, oak, hornbeam, chestnut, alder and hazel.[7-9] PR-10 is produced against the various environmental stresses and pathogen attacks on plants.[10] It is commonly found in a variety of plants and the highly conserved tertiary structure is responsible for its cross-reactivity, even though its biological function is not well characterized.[11] In order Fagales, with PR-10, allergens from group 2 (Profilin), group 3 (Polcalcin-like protein), group 4 (Polcalcin), group 6 (PhenylCoumaran benzylic ether reductase, Isoflavone reductase homologue), group 7 (Cyclophilin) and group 10 (Luminal binding protein) have been reported. Profilin is an allergen that has a strong cross-reactivity, while its allergenicity is relatively low and clinically less significant than PR-10 allergens. Sensitization to PR-10 and profilin is known to be a cause of oral allergy syndrome (OAS) to several kinds of fruits.[6] It has been reported that co-sensitization to PR-10 proteins and profilins are dominant in northern and central Europe.[9] nsLTP is thought to be one of the main molecule inducing peach and apple allergy, and often described as a marker of severe allergic reaction to plants.[12] However, according to the research on sensitization patterns to nsLTP in Mediterranean patients, diverse clinical expressions are shown from asymptomatic

sensitization to severe anaphylaxis.[13, 14] It is probably considered to be regulated by pollen allergen co-sensitization and the existence of cofactor.[15] In this study, we aimed to characterize a major allergen (PR-10) of mongolian oak, a dominant species in Korea, and produce recombinant protein that is useful not only in diagnosis and treatment for spring pollinosis, but also in researches on pathogenesis of pollinosis in Korea.

II. MATERIALS AND METHODS

1. Serum samples

Serum samples were collected from Fagales pollinosis patients at the Allergy-Asthma Center at Severance Hospital, Seoul, Korea. Patient consent was obtained before blood collection. Fifty sera from allergic patients (31 males and 19 females, mean age 23 yrs, ranging from 6 to 66 yrs) with ImmunoCAP (ThermoFisher scientific, Uppsala, Sweden) higher than 0.7 kU_A/L to white oak (t7) were used in this study (Table 1). Sera from 12 individuals who have no history of allergic reactions and are negative to oak and birch extract on ImmunoCAP assay were selected as negative control. This study was approved by the institutional review board of our institute (4-2013-0397).

Table 1. Clinical features of enrolled subjects

Subject	Gender/age	*Diagnosis	**Cosensitization profile	sIgE to birch	sIgE to oak	Total IgE (kU_A/L)
S01	M/10	AR	d2, w1	12.00	12.70	220
S02	F/7	AR	w1, d2, f13, f256	26.50	18.70	ND
S03	F/13	AR	e5	61.70	67.50	498
S04	M/38	AR	t2, d2, g5, g6	18.00	3.59	166
S05	M/6	AR	d2, f1, f11, f13, f14, f20, f24, f253, f256	0.97	2.06	427
S06	M/16	AR	w1, m6, d2, e5	71.00	30.70	ND
S07	F/13	AR	w1, m6, d2, i6, e5	19.90	16.90	456
S08	M/9	AR	d2	0.80	0.94	462
S09	M/13	AR	w1, e5	39.50	19.70	492
S10	M/16	AR	g6, d2, e1	4.01	3.80	38
S11	M/9	AR	w1, f13	3.34	1.27	52.4
S12	M/13	AR	w1	25.10	20.80	53.5
S13	M/8	AR	w1, i6	0.83	1.12	ND
S14	M/8	AR	w1	1.89	1.83	ND
S15	F/45	AR, AS	d2, e1, e5	25.00	10.90	ND
S16	F/7	AR	w1, d2	2.63	5.22	2059
S17	M/14	AR	d2, e5	3.60	7.29	ND

S18	M/17	AR	d2, e1, e5	1.49	0.88	ND
S19	M/8	AR	w1, d2	1.86	3.80	ND
S20	M/6	AR	w1, d2	0.76	1.33	ND
S21	M/8	AR	w1, d2	2.38	3.07	ND
S22	M/7	AR	w1, m6, d2, e4	54.10	35.10	ND
S23	F/8	AR	i6, f1, d2, f1, f2, f95	1.21	3.03	681
S24	M/11	AR	g6, w1, w22, d2, f13	22.80	33.20	ND
S25	F/20	AR	d2, e5	2.06	1.97	ND
S26	M/11	AR	w1, d2, f13	15.70	19.50	ND
S27	M/25	AR	d2	5.46	4.09	ND
S28	M/15	AR	m6, d2	9.83	5.30	203
S29	M/17	AR	d2, e5	66.30	39.60	ND
S30	F/66	AR	t2	16.50	6.21	59
S31	M/16	AR	w1	14.70	16.70	ND
S32	M/23	AR	w1, d2	19.80	35.00	ND
S33	F/61	AR	w1, w6, e1, e2	4.28	4.56	ND
S34	M/28	AR	w1, w6, w22, d2, i6	15.90	50.60	ND
S35	M/38	AR	d2	9.40	1.71	35.1
S36	M/30	AR	w1, d2	79.20	62.00	871
S37	M/21	AR, CU	f4	5.97	5.31	ND
S38	F/46	AR	i6	16.70	12.70	306

S39	F/54	AR	-	19.10	3.24	42.6
S40	F/26	AR, CU	f44, f84, f95, f242	37.40	13.10	ND
S41	F/26	AR, AC, OAS	e1, e5, g6, w1, w6, w22	34.40	15.50	240
S42	M/39	AR, OAS	e1, e5, d2	12.40	9.71	248
S43	F/29	AR, AC	e5, d2, i6	2.14	2.71	715
S44	F/35	AR	d2, e5	20.00	11.70	ND
S45	M/44	AR, AS	w6, d2	4.29	11.20	ND
S46	F/31	AR, AS	t2	36.80	21.80	179
S47	M/30	AR	d2, e5	0.42	9.90	ND
S48	F/54	AR, AC	-	32.80	6.36	ND
S49	M/34	AR, AC	t1, g5, d2	4.94	3.29	ND
S50	F/21	AR, OAS	d2	5.41	1.03	351
	M (32), F (18); Mean age = 23	AR (50), OAS (3), AS (3), AC (3), CU (2)		Mean value = 17.87	Mean value = 13.60	

*****AR, allergic rhinitis; OAS, oral allergy syndrome; AS, asthma; AC, allergic conjunctivitis; CU, chronic urticarial.

******All subjects listed here showed positive response to oak and birch. d2, *D. farinae*; w1, ragweed; w6, sagebrush; w22, chrysanthemum; e1, cat dander; e2, dog epithelium; e4, cow dander; e5, dog dander; t1, Acer; t2, alder; f1,egg white; f2, milk; f4, wheat; f11, buckwheat; f13, peanut; f14, soya bean; f20, almond; f24, shrimp; f44, strawberry; f84, kiwi; f95, peach; f242 cherry; f253, pine nut; f256, walnut; g5, rye grass; g6, timothy grass; i6, cockroach; m6, *Alternaria alternata*.

2. Expression and purification of recombinant group 1 major allergens of Fagales

Quercus mongolica pollen was collected by a botanist. Total RNA was extracted from the pollen using Trizol reagent (Invitrogen, Carlsbad, CA, USA) and used as a template for cDNA synthesis. Degenerate oligonucleotide primers (forward: 5'-ATgggWgTTDTYRVWYAYgA-3', reverse: 5'-BTHRTAKDSAKCDgDRTKTgMC-3', W, A+T; D, A+T+G; Y, C+T; R, A+C+G; B, T+C+G; H, A+T+C; K, T+G; S. C+G; M, A+C) were designed on the basis of PR-10 cDNA sequences of order Fagales. Subsequently, cDNA was PCR-amplified and cloned into pEXP5NT/TOPO vector (Invitrogen). After transformation into *E. coli* Rosetta 2 (DE3), expression of recombinant proteins was induced by adding 1 mM of isopropyl-1-thio-β-galactopyranoside. Ni-NTA agarose (Qiagen, Hilden, Germany) was used to purify recombinant PR-10 protein under denaturing conditions with 6 M urea. Recombinant Que a 1, group 1 major allergen of *Quercus alba* was also produced for comparison. The open reading frame of Que a 1 codon-optimized for recombinant protein production and was obtained from Bioclone Inc (CA, USA). Subsequently, PCR was carried out using oligonucleotide primers (forward: 5'-CCATGGCATCCACTACTCACC-3', reverse: 5'-CTCGAGCTATTTGTACAGA-3'). The underlined sequences indicates to *Nco* I and *Xho* I restriction sites and the PCR products were ligated into the pCR4-TOPO vector (Invitrogen). After restriction digests, it was subcloned into the corresponding restriction sites of pET28a vector (Merck Millipore, Darmstadt, Germany). It was transformed into BL21 Star™ (DE3) (Invitrogen). Subsequently, recombinant Que a 1 was expressed and purified as

described above. Recombinant Bet v 1, group 1 major allergen of *Betula verruca* was purchased from Indoor biotechnologies (Virginia, USA).

3. Specific IgE reactivity to recombinant group 1 major allergens of Fagales

IgE binding reactivity to the recombinant proteins were measured by ELISA. A microtiter plate was coated with recombinant protein (2 μg/mL in 50 mM sodium carbonate, pH 9.6) and kept at 4℃ overnight. After washing with phosphate buffer saline (PBS) containing 0.05% Tween 20 (PBST), plate was blocked with 3% skim milk in PBST. Serum samples diluted 1:4 in PBST containing 1% bovine serum albumin (BSA) were dispensed in the plate and then incubated for 1 hr. Subsequently, IgE antibodies were detected by biotinylated goat anti-human IgE (1:1,000) (Vector, Burlingame, CA, USA) and streptavidin peroxidase (1:1,000) (Sigma-Aldrich, St. Louis, Mo., USA). Color development was conducted by 3,3′,5,5′ -tetramethylbenzidine (Kirkegaard and Perry Laboratories, Gaithersburg, MD, USA). After the addition of 0.5 M H_2SO_4, the absorbance was measured at 450 nm. The cutoff value was determined as the mean absorbance plus double standard deviation of the negative controls. The cut off values for Que m 1 and Que a 1 positive rates were validated and compared by receiver operating characteristic (ROC) analyses with Area Under the Curves (AUC).

4. Mongolian oak pollen extraction

Mongolian oak pollen was defatted with ethyl ether with slow stirring. After ethyl ether dry out completely, it was sonicated in PBS with 10-second pulse on and off on ice and kept at 4℃ overnight. The supernatant was collected by centrifugation at 13,000g for 15 min at 4℃. Subsequently, the extract was extensively dialyzed against water (Spectrum labs, CA, USA) at 4℃ overnight. The extract was centrifuged again and filtered through syringe filter (0.22 μm, PVDF membrane filter) (Merck Millipore) to remove insoluble matter. The samples were lyophilized and aliquoted and then kept on 4℃. Before use, the extract was dissolved in PBS and protein concentration was measured by Bradford assay (Bio-Rad, Hercules, CA, USA).

5. Inhibition immunoblotting

Mongolian oak pollen extract (10 μg) was separated on 15% polyacrylamide gel containing sodium dodecyl sulfate under reducing condition, transferred onto polyvinylidene fluoride (PVDF) membrane (0.45 μm, GE Water & Process Technologies, Trevose, PA, USA). After blocking with 3% skim milk, the membrane was immunoprobed with sera (1:4, pooled from four patients positive to recombinant protein), which had been pre-incubated with 40 μg of recombinant protein. Subsequently, Allergen-specific IgE antibodies were detected in a 1:1000 dilution of alkaline phosphatase-conjugated goat anti-human IgE (1:1000-diluted) (Sigma-Aldrich, St. Louis, MO, USA) and the color was developed with nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate (Promega, Madison, WI, USA).

III. RESULTS

1. Molecular cloning of Que m 1

The open reading frame was predicted to encode 160 amino acids with a calculated molecular weight (MW) of 17,292 Da and the isoelectric point is 5.37 (Fig. 1). The MW of recombinant protein is 19,761 Da and the isoelectric point is 5.90 due to 22 additional amino acids derived from tagging protein at the N-terminus. The translated amino acid sequence of Que m 1 shared 56.9% to 96.2% identity with previously known allergenic PR-10 molecules from various plants (Fig. 2). In particular, it showed the highest identity with Que a 1. However, Bet v 1 shares 56.9% identity with Que m 1.

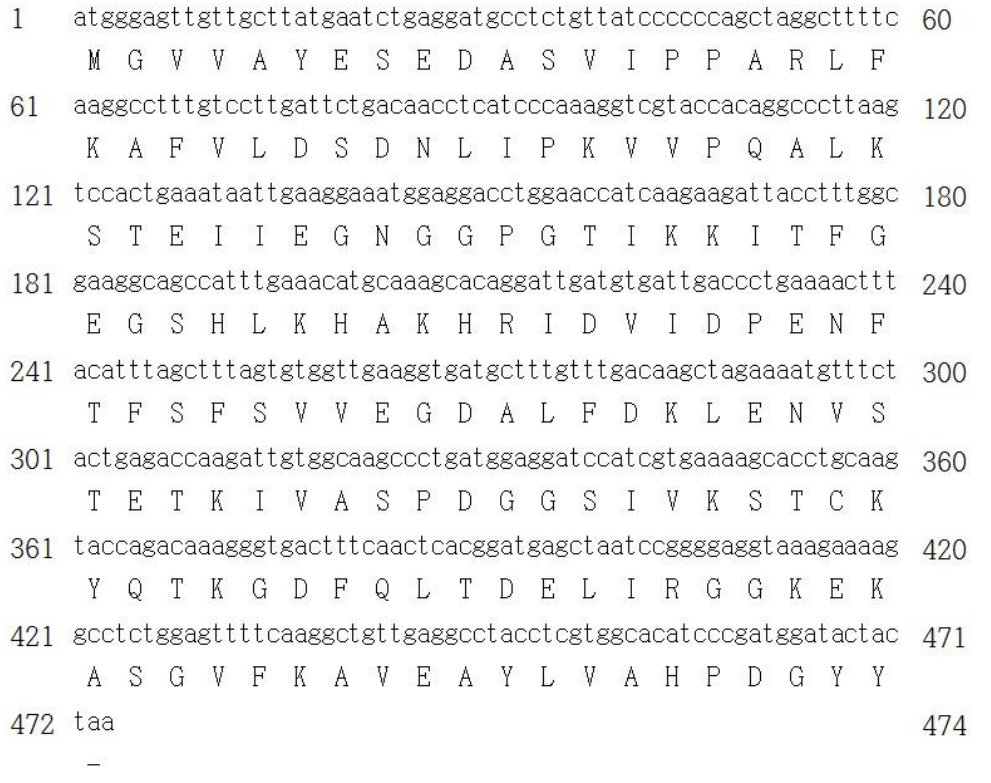

Fig. 1. Nucleotide and deduced amino acid sequence of PR-10 allergen from the mongolian oak. The full-length sequence encoded 160 amino acids with an estimated molecular mass of 17.2 kDa.

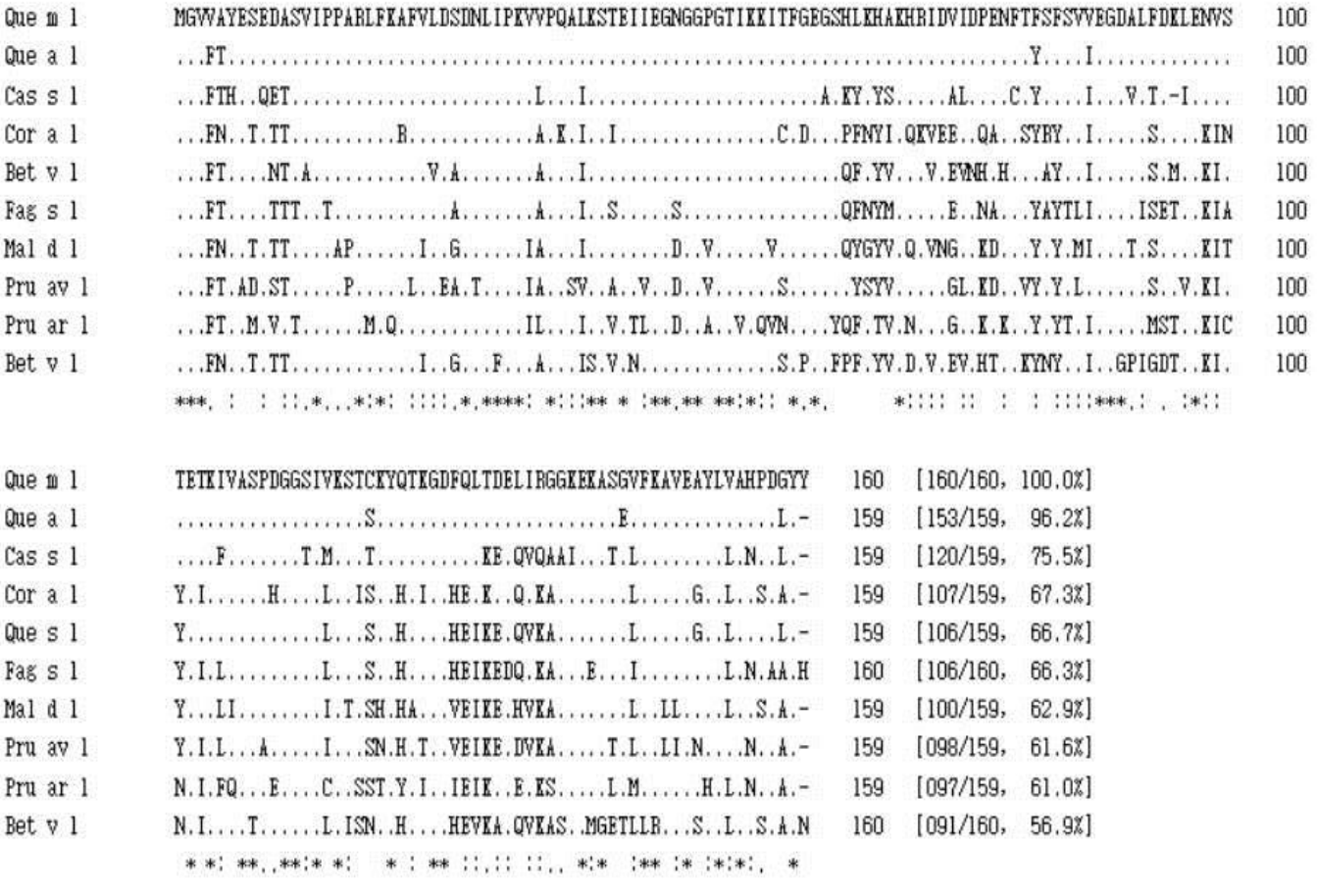
Que m 1 MGWVAYESEDASVIPPARLFKAFVLDSDNLIPKVVPQALKSTEIIEGNGGPGTIKKITFGEGSKHLKHAKHRIDVIDPENFTFSFSVVEGDALFDKLENVS 100
Que a 1 ...FT.....Y....I..... 100
Cas s 1 ...FTH..QET.....L...I.....A.KY.YS.....AL....C.Y....I...V.T.-I.... 100
Cor a 1 ...FN..T.TT.....B.....A.K.I..I.....C.D...PFNYI.QKVEE..QA..SYEY..I.....S....KIN 100
Bet v 1 ...FT....NT.A.....V.A.....A...I.....QF.YV...V.EVNH.H...AY..I.....S.M..KI. 100
Fag s 1 ...FT....TTT..T.....A.....A...I..S.....S.....QFNYM.....E..NA...YAYTLI....ISET..KIA 100
Mal d 1 ...FN..T.TT....AP.....I..G.....IA...I.....D..V.....V.....QYGYV.Q.VNG..KD...Y.Y.MI...T.S....KIT 100
Pru av 1 ...FT.AD.ST.....P.....L..EA.T....IA..SV..A..V..D..V.....S.....YSYV.....GL.KD..VY.Y.L.....S..V.KI. 100
Pru ar 1 ...FT..M.V.T.....M.Q.....IL...I..V.TL..D..A..V.QVN....YQF.TV.N...G..K.K..Y.YT.I.....MST..KIC 100
Bet v 1 ...FN..T.TT.....I..G...F...A...IS.V.N.....S.P..FPF.YV.D.V.EV.HT..KYNY..I..GPIGDT..KI. 100
Que m 1 TETKIVASPDGGSIVKSTCKYQTKGDFQLTDELIRGGKEKASGVFKAVEAYLVAHPDGYY 160 [160/160, 100.0%]
Que a 1 .....S.....E.....L.- 159 [153/159, 96.2%]
Cas s 1 ....F.....T.M...T.....KE.QVQAAI...T.L.....L.N..L.- 159 [120/159, 75.5%]
Cor a 1 Y.I.....H....L..IS..H.I..HE.K..Q.KA.....L.....G..L..S.A.- 159 [107/159, 67.3%]
Que s 1 Y.....L...S..H....HEIKE.QVKA.....L.....G..L....L.- 159 [106/159, 66.7%]
Fag s 1 Y.I.L.....L...S..H....HEIKEDQ.KA...E...I.....L.N.AA.H 160 [106/160, 66.3%]
Mal d 1 Y...LI.....I.T.SH.HA...VEIKE.HVKA.....L..LL....L..S.A.- 159 [100/159, 62.9%]
Pru av 1 Y.I.L...A.....I...SN.H.T..VEIKE.DVKA.....T.L..LI.N....N..A.- 159 [098/159, 61.6%]
Pru ar 1 N.I.FQ...E....C..SST.Y.I..IEIK..E.KS.....L.M.....H.L.N..A.- 159 [097/159, 61.0%]
Bet v 1 N.I....T.....L.ISN..H....HEVKA.QVKAS..MGETLLR...S..L..S.A.N 160 [091/160, 56.9%]

Fig. 2. Amino acid sequence alignment of PR-10 allergens from various plants. Que a 1, *Quercus alba* (White oak, Accession no. EU283863.1), Cas s 1, *Castanea sativa* (Sweet chestnut, FJ390843.1), Cor a 1, *Corylus avellana* (Common hazel, Z72440.1), Que s 1, *Quercus suber* (Cork oak, JQ290115.1), Fag s 1, *Fagus sylvatica* (European beech, FJ390847.1), Mal d 1, *Malus domestica* (Apple, Z72426.1), Pru av 1, *Prunus avium* (Sweet cherry, AY540508.1). Pru ar 1, *Theobroma cacao* (Cacao tree, XM007035636.1), Bet v 1, *Betula pendula* (Common silver birch, X15877.1).

2. Production of recombinant Que m 1, Que a 1, and Bet v 1

Recombinant proteins were produced with 22 additional amino acids with 6-histidine residues at the N-terminus. The yield of Que m 1 and Que a 1 were 2.715 and 1.876 mg/L of *E. coli*. SDS-PAGE analysis of recombinant proteins showed the apparent bands about 17 kDa (Fig. 3).

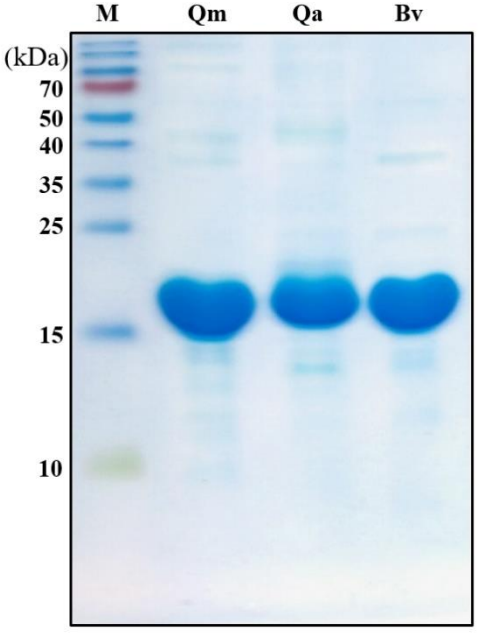

Fig. 3. Purification of the recombinant Que m 1 and Que a 1. The proteins were separated on 15% SDS-PAGE gel under reducing conditions. M, molecular mass standard; Qm, recombinant Que m 1; Qa, recombinant Que a 1; Bv, recombinant Bet v 1.

3. Prevalence of IgE reactivity to Que m 1

Specific IgE antibodies for the recombinant proteins were measured by ELISA (Fig. 4). Fifty serum samples from Fagales pollinosis patients were examined. The Que m 1, Que a 1 and Bet v 1 specific IgE antibodies were detected in 90.0%, 78.0% and 94.0% of allergic subjects (n=50). IgE reactivity to Que m 1 showed a high correlation with reactivity to Que a 1 (Pearson's correlation coefficient = 0.84), whereas strong correlation of IgE reactivity was not detected between Que m 1 and Bet v 1 (0.34), nor between Que a 1 and Bet v 1 (0.28) allergens. Pearson's correlation coefficients of the IgE values between Que m 1, Que a 1 and Bet v 1 were shown in Figure 5. ROC curve analysis for diagnosis of Fagales pollinosis patients using recombinant Que m 1 or Que a 1 sIgE was done. The AUCs of the scores for Que m 1 and Que a 1 were 0.980 (95% CI: 95-100%) and 0.924 (95% CI 86-99%) respectively (Fig. 6).

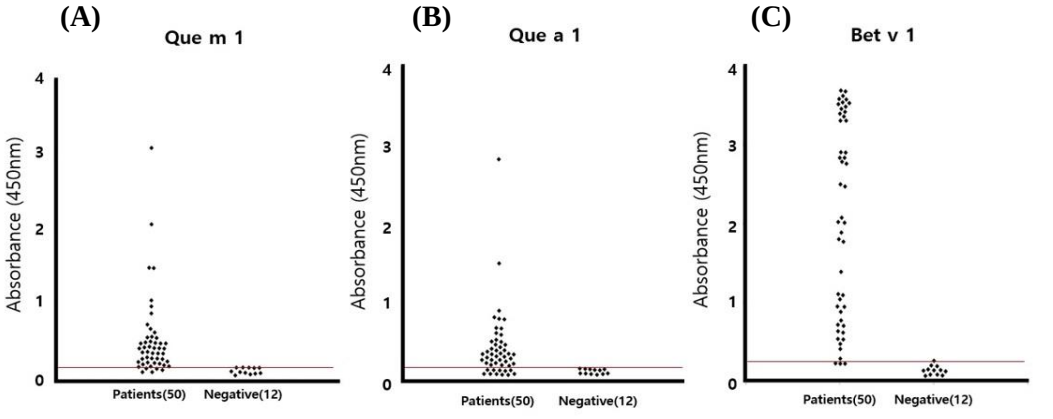

Fig. 4. IgE reactivity of the recombinant PR-10 molecules from Fagales.

(A), Que m 1; (B), Que a 1; (C), Bet v 1. The red line indicates the cutoff value.

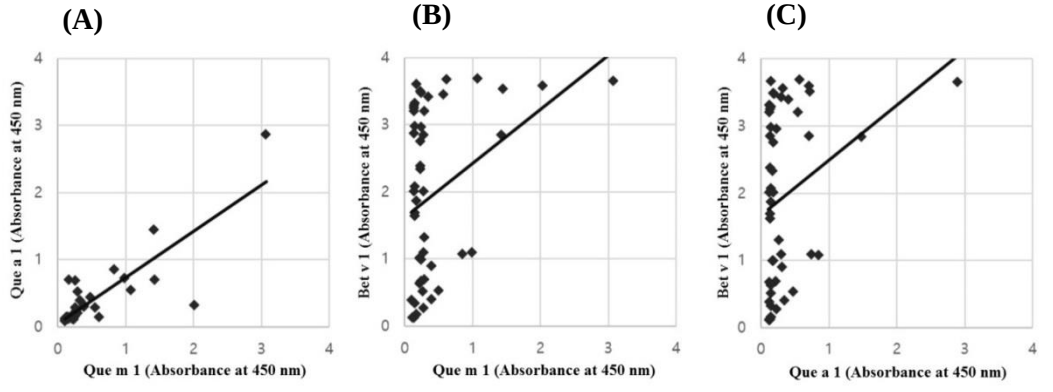

Fig. 5. Correlation of IgE reactivity between PR-10 allergens. IgE reactivity between Que m 1 and Que a 1 (A), Que m 1 and Bet v 1 (B) and Que a 1 and Bet v 1 (C).

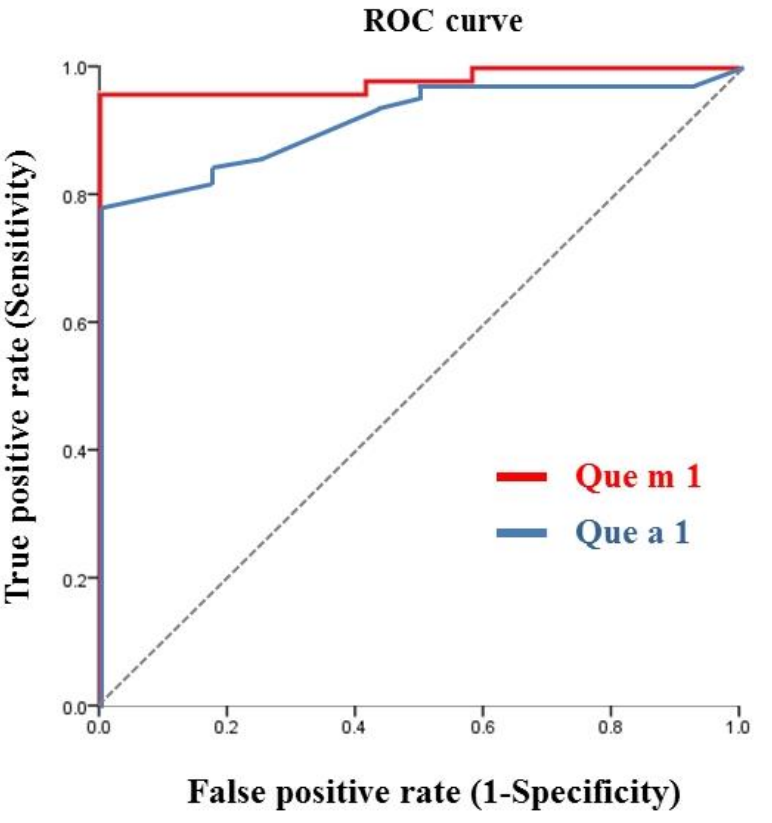

Fig. 6. ROC curves of ELISA results for measuring IgE against recombinant Que m 1 and Que a 1 in Fagales pollinosis patients. The test was performed with sera from 50 Fagales pollinosis patients and 12 negative controls.

4. Inhibition analysis

For the inhibition immunoblotting, 10 μg of mongolian oak extract was run on 15% SDS-PAGE (Fig. 7). Inhibition analysis was performed using serum samples from four patients who have strong IgE sensitivity to recombinant Que m 1 and Que a 1. A strong band of about 17 kDa was completely inhibited in by pre-incubation with recombinant Que m 1. In the case of Que a 1, the inhibition was less effective. In addition to 17 kDa allergen of mongolian oak, apparent band was detected at approximately 12 kDa in the lane of no inhibitor and pre-incubated serum with Que m 1 or Que a 1.

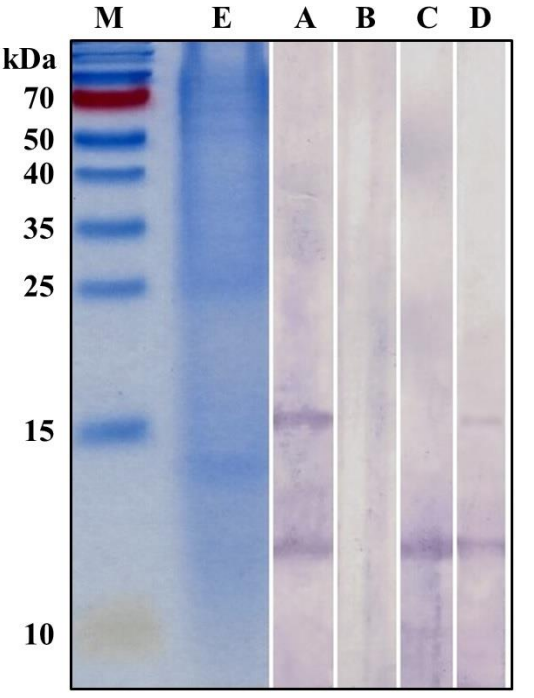

Fig. 7. IgE immunoblot inhibition analysis. Mongolian oak extract was separated by 15% SDS-PAGE gel and transferred onto a PVDF membrane. M, molecular mass standard; E, mongolian oak extract stained with Coomassie blue; IgE-reactive components probed with the sera from patients pre-incubated with A, no inhibitor; B, mongolian oak pollen extract; C, recombinant Que m 1; D, recombinant Que a 1.

IV. Discussion

In Korea, the sensitization rates to inhalant allergens have steadily increased for the last decade. Fagales tree pollens, which include birch and oak pollen, are the main causes of spring pollinosis in Korea and PR-10 protein has been recognized as the major allergen of Fagales pollen. PR-10 proteins have strong IgE reactivity with cross-reactivity over order Fagales. In this study, Que m 1 allergen was cloned and expressed in *E. coli*. Que m 1 shared 56.9% to96.2% sequence identity with other PR-10 allergens. It showed the highest identity (96.2%) with Que a 1 and low sequence identity with Bet v 1 (56.9%). Recombinant PR-10 proteins were well expressed and purified with apparent bands at 17 kDa. The positive rate of IgE to Bet v 1 is highest among the studied PR-10 allergens, but we could not concluded that Bet v 1 is the most prevalent culprit allergen. Birch trees occupy only 1% of forest in Korea, and exposure to birch pollen to spring pollinosis patients may not be substantial. Strong cross-reactivity between Que m 1 and Bet v 1 may affect the high positive rate and high concentration of Bet v 1 IgE. Furthermore post-translational modifications, such as refolding, glycosylation and phosphorylation during production of recombinant allergen markedly influence on its allergenicity. mongolian oak is usually distributed high in the mountains, while sawtooth oak is commonly found around villages. It could lead to difference in exposure intensity to oak pollens. According to the report, other allergens, such as 23 kDa protein and profilin of the oak species could also affect the sensitization even though its allergenicities have been thought to be lower than PR-10.[16] Our IgE immunoblotting features showed strong IgE band to 17 kD

protein which could be Que m 1, but it could not identify 23 kD allergen. Additional 12 kD allergen has identified and it is presumed to be profilin or Ca^{2+} binding protein.[8] Therefore, additional studies for these proteins are needed to understand more precise sensitization patterns for mongolian oak allergy. ROC analysis of the Que m 1 and Que a 1 sIgE ELISA results with 50 Fagales pollinosis patients and 12 negative controls was performed. The AUC values from ROC curves illustrated high specificities and sensitivities of both recombinant allergen specific IgE ELISA. However, Que m 1 sIgE had a relatively higher value than that of Que a 1 sIgE.

Allergenicity of produced Que m 1 was satisfactory. In inhibition immunoblotting, Specific IgE binding to native Que m 1 in mongolian oak extract was completely inhibited by pre-incubated serum with recombinant Que m 1 and incompletely inhibited by recombinant Que a 1, respectively (Fig. 7). This result suggests that recombinant Que m 1 may be more appropriate for diagnosis of spring pollinosis than recombinant Que a 1 in Korea. Moreover, the serum samples used in this study was collected from the patients diagnosed with white oak and common silver birch pollen extracts. Better diagnostic value of recombinant Que m 1 is expected if oak allergy subjects were selected with mongolian oak pollen. Total extract of allergen resources contain not only clinically relevant allergen but also insignificant allergens, such as minor allergens, cross-reactive carbohydrate determinants or easily denatured allergens. Recently component resolved diagnosis (CRD) which using clinically relevant purified native or recombinant major allergen has been successfully

introduced for diagnosis of inhalant, food and insect sting allergic disease.[17-19] CRD provides the detailed information on specific allergen sensitization in patients and helps to select the extracts suitable for immunotheraphy.[20] PR-10 protein of birch pollen (Bet v 1) has been recognized as the most important allergen in birch pollinosis and satisfactory results with immunotherapy using recombinant Bet v 1 have been reported.[21]

V. CONCLUSION

In conclusion, we first cloned, expressed and purified group 1 major allergen of mongolian oak, Que m 1. Its allergenicity is satisfactory and could be useful for the diagnosis and treatment of oak pollen allergy in Korea. However, sawtooth oak tree is also prevalent around villages, IgE reactivity toward Que m 1 and Que ac 1 (group 1 major allergen of sawtooth oak tree pollen) could also vary in localities. Therefore, further studies are needed to understand oak allergy from various regions of Korea.

REFERENCES

1. Lee JW, Choi GS, Kim JE, Jin HJ, Kim JH, Ye YM, et al. Changes in sensitization rates to pollen allergens in allergic patients in the southern part of Gyeonggi province over the last 10 years. Korean J Asthma Allergy Clin Immunol 2011; 31: 33-40.
2. Kim YL, Lee SK, Oh SH, Moon BS, Park HS, Hong CS. A study of allergy skin tests with Korean pollen extracts. Yonsei Med J 1987; 28: 112-8.
3. Kim TB, Kim KM, Kim SH, Kang HR, Chang YS, Kim CW, et al. Sensitization rates for inhalant allergens in Korea: a multicenter study. J Asthma Allergy Clin Immunol 2003; 23: 483-93.
4. Park HJ, Lee JH, Park KH, Ahn HW, Jin MN, Choi SY, et al. A nationwide survey of inhalant allergens sensitization and levels of indoor major allergens in Korea. Allergy Asthma Immunol Res 2014; 6: 222-7.
5. Kim GS, Song HK, Lee CH, Cho HJ, Lee CS. Ecological comparison of Mongolian oak (*Quercus mongolica* Fisch. Ex Ledeb.) community between Mt. Nam and Mt. Jeombong as a Long Term Ecological Research (LTER) site. J Ecol Field Biol 2011; 34: 75-85.
6. Anderson MB, Hall S, Dragsted LO. Identification of European allergy patterns to the allergen families PR-10, LTP, and profilin from Rosaceae fruits. Clin Rev Allergy Immunol 2011; 41: 4-19
7. Mari A, Wallner M, Ferreira F. Fagales pollen sensitization in a birch-free area: a

respiratory cohort survey using Fagales pollen extracts and birch recombinant allergens (rBet v 1, rBet v 2, rBet v 4). Clin Exp Allergy 2003; 33: 1419-28.

8. Wallner M, Erler A, Hauser M, Klinglmayr E, Gadermaier G, Vogel L, et al. Immunologic characterization of isoforms of Car b 1 and Que a 1, the major hornbeam and oak pollen allergens. Allergy 2009; 64: 452-60.
9. Hauser M, Asam C, Himly M, Palazzo P, Voltolini S, Montanari C, et al. Bet v 1-like pollen allergens of multiple Fagales species can sensitize atopic individuals. Clin Exp Allergy 2011; 41: 1804-14.
10. Radauer C, Lackner P, Breiteneder H. The Bet v 1 fold: an ancient, versatile scaffold for binding of large, hydrophobic ligands. BMC Evol Biol 2008; 8: 286.
11. Fernandes H, Michalska K, Sikorski M, Jaskolski M. Structural and functional aspects of PR-10 proteins. FEBS J 2013; 280: 1169-99
12. Gomez F, Aranda A, Campo P, Diaz-perales A, Blanca-Lopez N, Perkins J, et al. High prevalence of lipid transfer protein sensitization in apple. 2014; 9: e107304.
13. Pascal M, Muñoz-Cano R, Reina Z, Palacín A, Vilella R, Picado C, et al. Lipid transfer protein syndrome: Clinical pattern, cofactor effect and profile of molecular sensitization to plant-foods and pollens. Clin Exp Allergy 2012; 42: 1529–39.
14. Soto L, Luengo O, Labrador-Horrillo M, Sala-Cunill A, Guilarte M, Cardona V. Recognition pattern to individual allergens in pollen allergic patients sensitised to lipid transfer proteins. Allergy 2012; 67(Suppl. 96): 541A.
15. Asero R, Pravettoni V. Anaphylaxis to plant-foods and pollen allergens in patients with lipid transfer protein syndrome. Curr Opin Allergy Clin Immunol 2013; 13: 379–385.

16. Jeong KY, Son M, Park JH, Park KH, Park HJ, Lee JH, et al. Cross-reactivity between Oak and Birch pollens in Korean tree pollinosis. J Korean Med Sci 2016; 31: In press.
17. Sastre J. Molecular diagnosis in allergy. Clin Exp Allergy 2010; 40: 1442–60.
18. Treudler R, Simon JC. Overview of component resolved diagnostics. Curr Allergy Asthma Rep 2013; 13: 110–117.
19. Canonica GW, Ansotegui IJ, Pawankar R, Schmid-Grendelmeier P, van Hage M, Baena-Cagnani CE, et al. A WAO - ARIA - GA2LEN consensus document on molecular-based allergy diagnostics. World Allergy Organ J 2013; 6: 17.
20. Valenta R, Twaroch T, Swoboda I. Component-resolved diagnosis to optimize allergen-specific immunotherapy in the Mediterranean area. J Investig Allergol Clin Immunol 2007; 17(Suppl 1): 36–40.
21. Klimek L, Bachert C, Lukat KF, Pfaar O, Meyer H, Narkus A. Allergy immunotherapy with a hypoallergenic recombinant birch pollen allergen rBet v 1-FV in a randomized controlled trial. Clinical and Translational Allergy 2015; 5: 28.

Abstract (in Korean)

신갈나무 주요 알레르겐 특성 규명

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배경

국내에서 참나무목에 속하는 참나무의 꽃가루는 봄철 꽃가루 알레르기의 주요한 원인이 되고 있다. 현재 국내에서 봄철 꽃가루 알레르기의 진단 및 치료에 백참나무 꽃가루를 이용하지만, 이는 국내 고유종이 아니다. 병원성관련단백질-10 (PR-10)에 속해있는 group 1 주요 단백질은 참나무목 꽃가루의 전체 알레르기성의 80%를 차지하며, 참나무목 꽃가루 과민성의 가장 중요한 알레르겐으로 여겨진다. 그러므로 우리는 한국의 우점종인 신갈나무의 group 1 주요 알레르겐을 생산하고 그 알레르기성을 조사한다.

방법

역전사중합효소 연쇄반응으로 Que m 1을 클로닝하고, 대장균에서 이를 발현한다. 시중에서 판매한 Bet v 1과 함께 재조합단백질에 대한 면역글로불린 E 의 결합능력은 한국 참나무목 꽃가루 과민성 환자 50명의 혈청을 사용하여 효소면역측정법으로 측정되었다.

결과

Que m 1 은 17,292Da 의 분자량과 5.37의 등전점을 가지고 있는 것으로 예측되며, 160개의 아미노산으로 구성되어있다. Que m 1은 Que a 1과 96.2%의 아미노산 서열을 공유한다. 하지만 국내 꽃가루 과민성 환자에서 Que m 1 (90.0%)은 Que a 1 (78.0%)보다 높은 특이적 면역글로불린 E 반응 능력을 보여주었으며, 수신자 조작 특성 (Receiver Operating Characteristic) 분석에서도 Que m 1이 Que a 1 보다 높은 곡선 아래 면적(AUC)를 보여주었다. Bet v 1 특이적 면역글로불린 E 수치는 참나무 꽃가루 group 1 주요 알레르겐들의 수치보다 높았으며, 양성률 (94.0%) 또한 이들보다 높았다. 면역글로불린 E 억제 면역 블로팅 분석에서는 Que m 1이 신갈나무 추출물의 약 17 kDa 알레르겐의 면역글로불린 E 반응을 완벽하게 억제하였지만, Que a 1은 부분적으로 이를 억제를 하였다. 따라서 Que m 1은 한국에서 참나무 알레르기의 주요한 원인인 것으로 생각된다.

결론

우리는 한국에서 참나무목 꽃가루 과민성의 진단과 치료에 유용한, 신갈나무 재조합 group 1 주요 알레르겐인 Que m 1을 처음으로 클로닝하고 생산하였다. Que m 1은 다른 참나무와 자작나무의 알레르기 PR-10 분자들과 강한 교차 반응 능력을 보여주었고, 한국 참나무목 꽃가루 과민성의 진단에 있어서 Que a 1 보다 좋은 임상적 가치를 가지고 있는 것으로 보인다.

핵심되는 말: 알레르겐; 참나무목 꽃가루 과민성; 신갈나무; 병원성관련단백질-10