

Allergen-families (n=50)	Allergens (n=85)	Route of exposure	1997/2007 *	Prolamin/Seed storage protein	Tri a gliadin		0/0	
Acidic glycoprotein	Alt a 1		1/1	nsLTP - food	Cor a 8 Pru p 3		0/0	
Mitogillin family	Asp f 1		0/0	nsLTP - pollen	Par j 1 Par j 2		1/0	
Mannitol dehydrogenase	Cla h 8		0/0	Legumin-like protein	Ana o 2		0/0	
PR-10	Api g 1	orange	1/1	CCD marker	Ana c 2		0/0	
	Act d 8	orange	1/0	Ovomucoid	Gal d 1		0/0	
	Aln g 1	green	7/9	Ovalbumin	Gal d 2		0/0	
	Bet v 1	green	7/9	Ovotransferrin (Conalbumin)	Gal d 3		0/0	
	Cor a 1	green	6/8	C-type lysozyme/alpha-lactalbumin family	Bos d 4 Gal d 4		0/0 4/0	
	Dau c 1	orange	0/0	Beta-lactoglobulin	Bos d 5		0/0	
	Mal d 1		7/8	Serum albumin	Bos d 6 Can f 3 Gal d 5		2/0 1/1 0/0	
Profilin	Bet v 2	green	3/5	Immunoglobulins	Bos d 7		0/0	
	Cyn d 12	green	4/3	Casein	Bos d 8		0/1	
	Hel a 2	green	3/3	Transferrin	Bos d Lactoferrin		2/0	
	Hev b 8	green	5/5	Lipocalin	Can f 1		2/3	
	Mer a 1	green	5/5	Fel d 1 family	Fel d 1		7/5	
	Ole e 2	green	5/4	Group 1 mite allergens	Der f 1 Der p 1 Der s 1		0/0 1/0 0/0	
	Par j 3	green	2/2	Group 2 mite allergens	Der f 2 Der p 2		5/5 5/5	
	Phl p 12	green	3/2	Tropomyosin	Ani s 3 Der p 10 Hel as 1 Pen i 1 Pen m 1 Per a 7		0/0 0/0 0/0 0/0 0/0 0/0	
	Pho d 2	green	3/4	Aspartic protease	Bla g 2		0/0	
	Pectate-lyase	green	0/0	Glutathione S-transferase	Bla g 5		0/0	
Ole e 1-related protein	Pla a 1	green	0/0	Phospholipase A2	Api m 1		1/0	
Polygalacturonase	Pla a 2	green	2/1	Melittin	Api m 4		7/0	
Common olive group 1	Ole e 1	green	5/6	Serine protease inhibitor	Ani s 1		0/0	
Group 1 grass pollen allergens	Lol p 1 Phl p 1	green	10/11 9/11	Positive: n=25	n=42			
Group 2/3 grass pollen allergens	Phl p 2	green	7/8	* Number of individuals with IgE reactivity in 1997/2007				
Group 5/6 grass pollen allergens	Phl p 5 Phl p 6	green	9/9 8/8					
Ca-binding 2-EF-hand protein	Phl p 7	green	3/3					
Barley, Alpha amylase/Beta amylase	Hor v 16 Hor v 17	orange	0/0 0/0					
Class A pollen coat protein	Art v 1	green	4/3					
Rubber elongation factor	Hev b 1 Hev b 3	green	0/0 0/0					
Acidic protein	Hev b 5	green	0/0					
Hevein-like domain	Hev b 6	green	1/0					
	Hev b 11	green	0/0					
	Tri a 18	orange	0/0					
Patatin	Hev b 7	green	0/0					
Enolase	Alt a 6	green	0/0					
	Hev b 9	green	0/0					
Fe/Mn superoxide dismutase	Hev b 10	green	0/0					
Papain-like cysteine protease	Act d 1	orange	0/0					
Thaumatin-like protein	Act d 2	orange	0/1					
Expansin N-terminal domain	Act d 5	orange	0/0					
Plant invertase/Pectin methylesterase inhibitor	Act d 6	orange	1/0					
Pectin methylesterase	Act d 7	orange	0/0					

FIG E1. Families of tested allergens and summary of reactivities. Allergens belonging to the same cross-reactive allergen family (*left column*) were grouped in individual boxes (route of exposure: green, Airborne allergens; orange, food allergens; and yellow, venom allergens). The *right column* displays the numbers of positive sera in 1997 and 2007. Total numbers of allergen families and allergens recognized in 1997, 2007, or both are shown (*bottom line*, purple boxes). nsLTP, Nonspecific lipid transfer protein; PR-10, pathogenesis-related protein family 10.

Individual	Sex	Year of birth	Allergies	Symptoms	Immunotherapy	Symptomatic therapy
A1	m	1949	t,g	r	Yes (1975)	ah, nc
A2	m	1953	t,g,w,mi,a,f	r, c, d, oas	No	ah
A3	m	1962	t	r,c, oas	No	-
A4	f	1949	t,g	r	Yes (1980-1985)	ah
A5	m	1970	t,g	r,as,d	No	ah, tc
A6	f	1970	t,g,mi,a	r,c	No	ah
A7	f	1967	t	r,c,as	No	ah
A8	f	1949	t,g, f	r,as, oas	Yes (1980)	ah
A9	m	1961	g	r,c	Yes (1975)	ah
A10	f	1953	t, g, f	r, c	Yes (1983, 1987)	ah, nc
A11	f	1963	t,g	r,c,as	No	ah
A12	m	1962	t, g, f	r, c	No	ah
N1	f	1967	-	-	-	-
N2	f	1964	-	-	-	-
N3	f	1967	-	-	-	-
N4	f	1971	-	-	-	-
N5	f	1969	-	-	-	-
N6	f	1968	-	-	-	-
N7	m	1970	-	-	-	-
N8	f	1964	-	-	-	-
N9	f	1947	-	-	-	-
N10	m	1963	-	-	-	-

FIG E2. Demographic and clinical characterization of allergic patients (A1-12) and nonallergic subjects (N1-N10). f, Female; m, male. Allergies: a, animals; f, food; g, grass pollen; mi, mites; t, tree pollen; w, weed pollen. Symptoms: as, asthma; c, conjunctivitis; d, dermatitis; oas, oral allergy syndrome; r, rhinitis. Symptomatic therapy: ah, antihistamine; nc, nasal corticosteroid; tc, topical (dermal) corticosteroid. Dashes indicate lack of allergy, symptoms, or therapy.

Individual	Reactive with allergen-families (allergens)				Allergen-family (allergen molecule)			
	1997	2007	Difference 2007 vs. 1997		Acquired IgE-reactivities		Lost IgE-reactivities	
			+	-	Genuinely new IgE-reactivity	New IgE-reactivity but pre-existing reactivity to cross-reactive allergens	Loss of detectable IgE-reactivity	Loss of detectable IgE-reactivity but remaining reactivity to cross-reactive allergens
A1	9 (23)	7 (20)	0	-2 (-3)			C-type lysozyme (Gal d 4) Melittin (Api m 4)	PR10 (Act d 8)
A2	15 (22)	8 (14)	+1 (+1)	-8 (-9)	Caseins (Bos d 8)		Serum albumins (Bos d 6) C-type lysozyme (Gal d 4) Transferins (Bos d Lactoferrin) Melittin (Api m 4) Papain-like Cys-proteases (Der p 1) Barwin-family (Hev b 6.02) nsLTP - pollen (Par j 2) nsLTP - food (Pru p 3)	PR10 (Api g 1)
A3	8 (8)	4 (9)	+1 (+6)	-5 (-5)	PR10 (AIn g 1, Api g 1, Bet v 1, Cor a 1, Mal d 1)	Group 1 grass pollen allergens (Phl p 1)	C-type lysozyme (Gal d 4) Transferins (Bos d Lactoferrin) Phospholipase A2 (Api m 1) Melittin (Api m 4) Plant invertase (Act d 6)	
A4	5 (12)	3 (9)	0 (+1)	-2 (-4)		Profilins (Pho d 2)	C-type lysozyme (Gal d 4) Melittin (Api m 4)	Profilins (Cyn d 12, Par j 3)
A5	12 (20)	12 (20)	+1 (+3)	-1 (-3)	Thaumatin-like prot. (Act d 2)	PR10 (Cor a 1) Profilins (Bet v 2)	Melittin (Api m 4)	Profilins (Ole e 2, Phl p 12)
A6	7 (15)	6 (15)	0 (+1)	-1 (-1)		Profilins (Bet v 2)	Class A pollen coat prot. (Art v 1)	
A7	1 (1)	2 (3)	+1 (+2)	0 (0)	Group 1 grass pollen allergens (Lol p 1, Phl p 1) PR10 (AIn g 1, Bet v 1) Ole e 1-related prot. (Ole e 1) Group 2/3 grass pollen allergens (Phl p 2)			
A8	5 (8)	8 (11)	+3 (+4)	0 (-1)				Serum albumins (Bos d 6)
A9	9 (19)	9 (20)	+1 (+2)	-1 (-1)	Lipocalins (Can f 1)	Profilins (Par j 3)	Polygalacturonases (Pla a 2) Melittin (Api m 4)	
A10	6 (11)	5 (11)	0 (0)	-1 (-1)			Fel d 1-family (Fel d 1) C-type lysozyme (Gal d 4) Melittin (Api m 4)	
A11	7 (12)	4 (9)	0 (0)	-3 (-3)				
A12	4 (10)	4 (10)	0 (0)	0 (0)				
N1	0 (0)	1 (1)	+1 (+1)	0 (0)	Fel d 1-family (Fel d 1)			
N2	0	0	0	0				
N3	0	0	0	0				
N4	0	0	0	0				
N5	0	0	0	0				
N6	0	0	0	0				
N7	0	0	0	0				
N8	0	0	0	0				
N9	0	0	0	0				
N10	0	0	0	0				

FIG E3. Detailed analysis of IgE reactivities and their changes for each subject. Numbers of recognized allergen molecules and allergen families in 1997 and 2007, as well as changes in 2007 versus 1997, are shown. Allergens that were negative by means of chip but positive by means of CAP in 1997 are printed in red. PR10, Pathogenesis-related protein family 10.

Patient	CAP	kUA/l
A2	nBos d 8	1.53
A3	rBet v 1	0.05
A7	rPhl p 1	0.49
	rBet v 1	1.06
A8	nOle e 1	1.72
	rPhl p 2	1.44
A9	rCan f 1	1.49
N1	rFel d 1	0.15

FIG E4. Results of CAP measurements for sera that were negative to certain allergens in ISAC in 1997 but positive in 2007. *kUA/l*, Kilounits of antigen-specific IgE per liter.