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Allergy to apple cultivars among patients with birch pollinosis and oral allergy syndrome

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ABSTRACT

Background: Oral allergy syndrome (OAS) is caused by cross-reacting allergens found in pollen, raw fruits, vegetables, and some tree nuts. The major apple allergen, Mal d 1, is a cause of food allergic reactions in birch pollen sensitized patients.

Objective: To explore the allergenicity of the most popular and commonly consumed apple cultivars in Poland in patients with birch pollen allergy with or without OAS.

Methods: Data were obtained from 46 adults with clinical symptoms of birch allergy and allergic rhinitis or rhinoconjunctivitis. Patients were divided into 2 groups according to the occurrence of OAS to apple. Skin prick tests (SPTs) were performed with pulp from the 11 most popular apple cultivars in Poland. Specific IgE (sIgE) to Bet v 1 was measured by radioallergosorbent test.

Results: Patients with OAS had more positive responses to apple SPT vs patients without OAS (odds ratios, 4.8–11.96). Patients with OAS had distinctive responses to apple cultivars. Szara Reneta and Cortland induced positive responses in 50% and 83% of patients, respectively. Patients with OAS vs patients without OAS who were allergic to apples vs nonallergic had 2-fold ($P = .008$) and 7-fold ($P = .03$) higher blood concentrations of sIgE Bet v 1, respectively. There were different profiles of correlations of sIgE Bet v 1 with wheal diameter for low and high allergenic cultivars in patients without and with OAS.

Conclusion: We noted a substantial role for Bet v 1 sensitization in the allergic response based on evaluation of the allergenicity of 11 apple cultivars. The sIgE Bet v 1 and SPT results of patients with and without OAS allowed differentiation between low and high allergenic cultivars.

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Introduction

The term *oral allergy syndrome* (OAS) was first proposed in 1987 by Amlot et al¹ and referred to conventional IgE-mediated allergic reactions in which oral symptoms were observed. It has also been described under various names, including *pollen-food allergy syndrome*, *pollen-food syndrome*, and *pollen-associated food allergy syndrome*. Today, approximately 60% of all food allergies are cross-reactions between food and inhaled allergens, and OAS accounts for most allergic reactions caused by food.² Unlike other food allergies, OAS is the reaction limited to the oral mucosa, lips, tongue, and throat. The underlying pathophysiologic mechanism of OAS may play a role in clinical presentation and outcome, depending on whether the cross-reactive protein is a heat-labile PR-10 protein, a

partially labile profilin, or a relatively heat-stable lipid transfer protein. Diagnostic testing is variable based on the underlying food tested, but the fresh food skin prick test (SPT) typically has the highest sensitivity.³ According to recent reports, up to 90% of patients allergic to birch pollen or Bet v 1 homologues of other trees closely related to birch have IgE-mediated reactions to plant fruits and vegetables, such as apple, hazelnut, stone fruits, carrot, kiwi, and more.^{4–6} Fruits of the Rosaceae family are foods most frequently involved in allergic reactions. These food allergies are a consequence of Bet v 1 sensitization and subsequent IgE and T-cell cross-reactivity with homologous allergens, such as Mal d 1 (a Bet v 1 homologue).^{4,6} The first clinical descriptions of this condition came from patients from Northern and Central Europe, where apple allergy is mainly associated with birch pollinosis.⁷ Moreover, specific IgE (sIgE) to a Bet v 1-related allergen does not predict clinical manifestation of allergy. The factors that determine the clinical spectrum of Bet v 1-associated plant food allergy are mostly unknown.⁴ The apple-birch syndrome (ABS) is rarely discussed in detail concerning apple cultivars. Apples, oranges,

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bananas, grapefruits, and grapes are the top 5 consumed fruits in the world. In 2008, the annual apple harvest in Poland was estimated at approximately 2.83 million tons, comprising approximately three-fourths of the total fruit production. Apples can be consumed fresh, but they are also a valuable raw material for processing into apple juice and concentrates; canned, frozen, dried, and stewed fruits; jellies; purée; cider; and distillates. The percentage of apples used for processing increased in Poland from 50% in the 1980s to approximately 70% in the 1990s and to 80% at the beginning of the new century. Poland is one of the world leaders in production and export of apple juice concentrate.⁸

According to recent studies, the allergic potential of apple cultivars differs with respect to their Mal d 1 content.^{9–11} Interestingly, intracultivar Mal d 1 content variations are also observed, depending on the location of growing, time of harvesting, duration, and storage conditions.^{9–11} Furthermore, the clinical manifestation of apple allergy may vary among individuals allergic to apple, and those with apple allergy should choose cultivars with known relative low Mal d 1 content.⁷ Finally, some patients with birch pollen rhinitis or conjunctivitis do not have any clinical symptoms at all after eating apples. This lack of symptoms may suggest that other pollen allergens of the Betulaceae-Corylaceae family (eg, Bet v 2, Bet v 4, Cor a 1) rather than Bet v 1 are involved in sensitization.¹² Nevertheless, the search for low and high allergenic apples should be continued and extended to other cultivars that are familiar, frequently consumed, and popular in particular countries. For this reason, fruit growers must continuously improve their orchard agricultural practices to meet the growing consumer demand for better fruit quality and low allergenicity.¹¹

The aim of this work was to assess the association between susceptibility of patients with and without OAS and particular apple cultivar allergies and to relate this to birch pollen sIgE Bet v 1 blood levels in patients from Poland. Identification of concomitant factors associating allergy to apples may have implications for medical diagnostics, immunotherapy, and breeding schemes for new hypoallergenic cultivars in Central-Eastern Europe.

Methods

Study Participants

Patients were eligible for participation in the study based on a positive history of birch allergy, the occurrence of clinical manifestations of allergic rhinitis or rhinoconjunctivitis in the period of tree pollen season, positive SPT result, and sIgE to birch pollen (Bet v 1). The patients were divided into 2 groups according to the occurrence of OAS to apples. The mean age of the patients with OAS was 37 years, compared with 40 years in the group without OAS symptoms. All the patients were interviewed by questionnaire about typical OAS reactions after fruit and vegetable consumption. The study was approved by the Bioethics Committee of the Regional Medical Chamber in Lodz.

SPT Procedure

A total of 46 adults with clinical symptoms of birch allergy were confirmed by SPT. Tests were performed according to a standard procedure with standard birch extract (*Betula alba*, 50,000 SBE/mL; Allergopharma, Reinbek, Germany) with histamine dihydrochloride (1.7 mg/mL) as a positive control and SPT diluents (0.9% sodium chloride) as a negative control. The SPT results were regarded as positive when the diameter of the wheal was 3 mm or larger. Tests were performed after pollen season in Poland (end of September). All participants confirmed that antihistamine treatment was stopped no less than 7 days before testing.

sIgE Measurement

The sIgE antibodies were measured by the radioallergosorbent test using the Pharmacia ImmunoCAP 250 analyzer (Pharmacia, Stockholm, Sweden). ImmunoCAP was used as in vitro diagnostics to assess sensitization for each individual. Commercial recombinant Bet v 1 allergen extract was used to measure the concentration of IgE antibodies.

Prick by Prick Test Procedure

Tests were performed with the 11 most popular apple cultivars in Poland: Golden Delicious, Jonagold, Idared, Gloster, Szampion, Cortland, Ligol, Lobo, Jonagored, Jonika, and Szara Reneta. Apples were from the same harvest in autumn and stored under the same conditions. Tests were performed shortly after harvesting using apple material obtained from the pulp of the apple. Apple material was obtained from the middle area of the fruit, approximately 3 cm below the stem. Tests were performed similarly as described above for the SPT procedure, and the results were regarded as positive when diameter of the wheal was 3 mm or larger.

Statistical Analysis

The normal distribution of continuous variables was verified with the Shapiro-Wilk test. The strength and the direction of the linear association among these variables were evaluated using the Spearman correlation coefficient. The Pearson χ^2 test was used to compare the distribution of discrete variables. The strength of the association between discrete variables was assessed on the basis of the odds ratio (OR) calculated in the linear regression model. All calculations were performed with Statistica software, version 7 (Dell Statistica, Tulsa, Oklahoma), or GraphPad Prism 5 (GraphPad Software Inc, La Jolla, California), at the assumed level of significance $P \leq .05$.

Results

We studied the allergy reactions of patients to 11 common apple cultivars available in Poland. Initially, 46 patients qualified for inclusion in the study group based on a positive history of birch allergy, the occurrence of clinical manifestations of allergic rhinitis in the period of tree pollen season, and positive SPT and IgE test results to birch pollen. A total of 22 men and 24 women were studied. The mean age of patients was 38 years (range, 20–65 years). We examined 46 patients and analyzed the incidence of OAS to apple within this group. Twenty-eight patients (61%) were diagnosed as having OAS on detailed interview (Fig 1). It has previously been reported on the basis of SPTs and gene expression studies that apple cultivars differ in

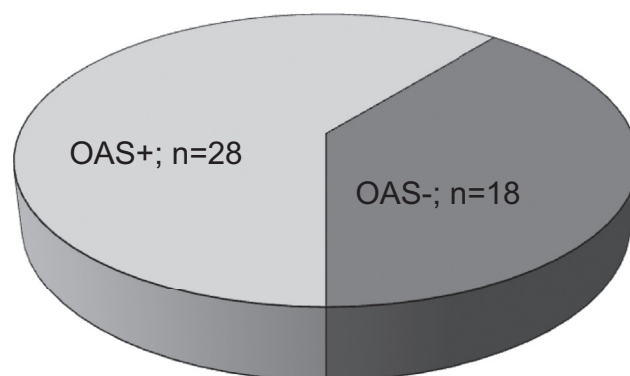


Figure 1. Percentage of patients with (+) and without (–) oral allergy syndrome (OAS) included in the study.

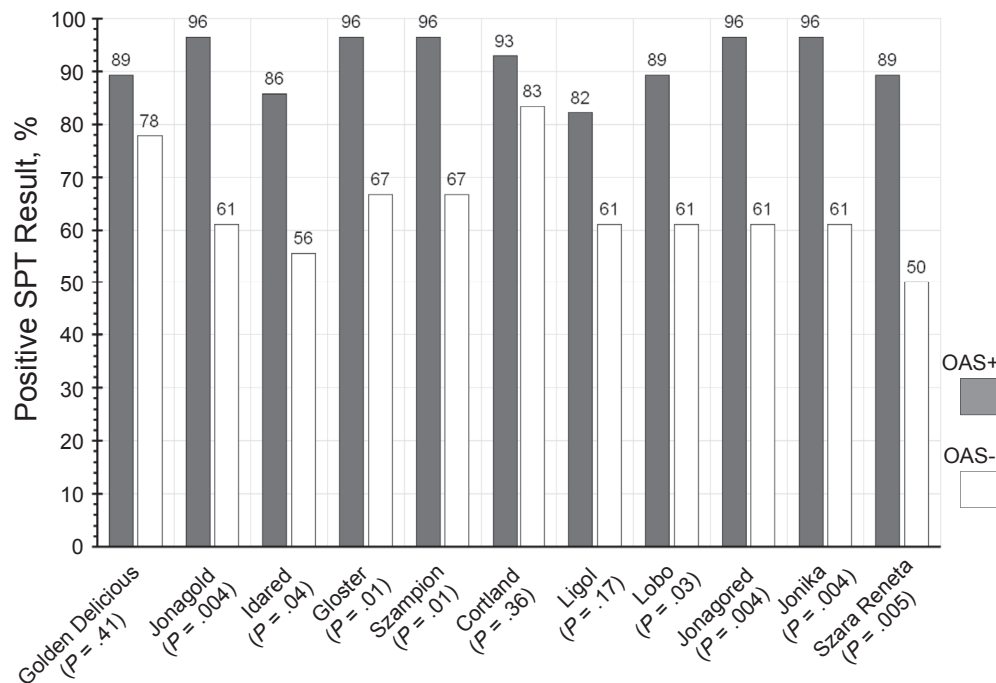


Figure 2. Percentage of patients who responded positively to apple skin prick by prick test in the groups with (+) and without (–) oral allergy syndrome (OAS).

their allergenic potential.⁵ We performed the prick by prick test procedure using the battery of apple cultivars commonly consumed in Poland to assess allergy to apple cultivars. We have found that patients with OAS more often responded significantly to apple prick by prick test for 8 of 11 cultivars tested (Fig 2 and Table 1). Indeed, the presence of OAS predisposed patients to a positive response to the apple test (OR, 4.8–11.96) for 8 cultivars tested. Although the incidence of a positive apple test result did not appear to differ among tested apple cultivars in patients with OAS, particular cultivars were found to induce distinctly lower or higher rates of positive responses in patients without OAS. As seen in Figure 2 and Table 2, Szara Reneta, representing an old cultivar having originated in Europe in the 18th century, induced 50% of the positive responses in patients with OAS, whereas the relatively new cultivars Golden Delicious and Cortland, which originated in the United States in the late 19th century, induced 78% and 83% of the positive responses in patients without OAS, respectively. On the basis of the strategy of apple allergenicity categorization described previously by Vlieg-Boerstra et al¹³ and Bolhaar et al,¹⁴ apple cultivars can be ranked according to SPT results. Both authors classified cultivars according to SPT values to 2 groups (high or low allergenic cultivar) or 3 groups (low, moderate, or high allergenic cultivar).^{13,14} We proposed preliminary classification of the tested apple cultivars into 2 groups with high or low allergenic potential according to incidence of SPT positive results in the group without OAS. The cutoff value was set at 61% to separate both groups. We categorized 4 cultivars, Golden Delicious, Gloster, Szampion, and Cortland to the high allergenic apple group, whereas the cultivars Jonagold, Idared, Ligol, Lobo, Jonagored, Jonika, and Szara Reneta were in the low allergenic group.

After this stage, we analyzed patients' blood sIgE Bet v 1 levels, OAS incidence, and status of allergy to apple cultivars, as a consequence of Bet v 1 sensitization and subsequent IgE and T-cell cross-reactivity with homologous apple allergens (Mal d 1). We have found that patients with OAS have an almost 2-fold higher level of

sIgE Bet v 1 in the blood compared to patients without OAS (33.36 vs 18.16 kU/L, $P = .004$) (Fig 3A). Furthermore, the group of patients with allergy to apple (to ≥ 1 apple cultivars according to the prick by prick test) exhibited levels of sIgE Bet v 1 comparable to the levels in patients with OAS, whereas nonallergic patients had 7-fold lower sIgE Bet v 1 levels (30.29 vs 3.78 kU/L, $P = .003$) (Fig 3B). Interestingly, this association was observed for each particular apple cultivar tested (Fig 4 and Table 3). Patients allergic to particular apple cultivars had markedly higher concentrations of sIgE Bet v 1 in the blood (29.88–34.5 kU/L) compared with nonallergic patients (7.34–17.79 kU/L). Statistical analysis confirmed the difference between sIgE Bet v 1 levels in the blood of allergic and nonallergic patients for 9 apple cultivars and found the most pronounced difference for Szara Reneta (34.5 vs 7.34 kU/L, $P < .001$) and Jonika (31.09 vs 9.95 kU/L, $P < .009$).

Subsequently, we performed Spearman rank correlation analysis to assess the strength and the direction of the linear association between sIgE Bet v 1 concentration and wheal diameter in patients with and without OAS tested with different apple cultivars. We found evidence of a correlation between sIgE Bet v 1 concentration and wheal diameter in patients with and without OAS tested for cultivars classified in the high allergenic category, namely, Gloster, Szampion, and Cortland (Table 4). Surprisingly, the results of the analysis obtained for the group consisting of Jonagold, Idared, Ligol, Lobo, Jonagored, Jonika, Szara Reneta, and Golden Delicious cultivars, categorized as low allergenic cultivars (except for Golden Delicious), have different associations in the groups with and without OAS. We observed a 2-fold weaker correlation between sIgE Bet v 1 and wheal diameter in the group with OAS compared with the group without OAS. Importantly, the resultant correlation data (summarized in Table 4) reproduced the same categorization pattern of cultivar allergenicity as ranking created using the SPT strategy, which thus supports the use of the latter approach. Finally, the obtained results suggest that observed differences in correlations between sIgE Bet v 1 and wheal diameter might be related to the interplay of different apple allergens in patients with and without OAS during allergic reactions to apple.

Table 1
Skin Prick Test Results in Patients With and Without Oral Allergy Syndrome (OAS)

Apple cultivar	Wheal diameter, mm		
	Mean (SD)	Median (95% CI)	Range
Golden Delicious			
With OAS	6.23 (3.06)	5.5 (4.99–7.47)	2–16
Without OAS	4.71 (1.54)	4.5 (3.82–5.60)	3–7
Total	5.70 (2.71)	5 (4.83–6.57)	2–16
Jonagold			
With OAS	6.04 (2.93)	5 (4.88–7.19)	3–14
Without OAS	4.64 (1.21)	4 (3.83–5.45)	3–7
Total	5.63 (2.61)	4.5 (4.77–6.49)	3–14
Idared			
With OAS	5.72 (2.96)	5 (4.50–6.94)	2–14
Without OAS	4.50 (1.51)	4 (3.42–5.58)	3–7
Total	5.37 (2.67)	4 (4.45–6.29)	2–14
Gloster			
With OAS	5.56 (2.26)	5 (4.66–6.45)	3–12
Without OAS	4.75 (2.60)	4 (3.10–6.40)	3–12
Total	5.31 (2.36)	5 (4.54–6.07)	3–12
Szampion			
With OAS	5.52 (1.84)	5 (4.80–6.24)	3–9
Without OAS	5.25 (1.76)	5 (4.13–6.37)	3–9
Total	5.44 (1.79)	5 (4.86–6.02)	3–9
Cortland			
With OAS	5.63 (2.34)	5 (4.70–6.55)	2–12
Without OAS	4.27 (1.44)	4 (3.47–5.06)	3–7
Total	5.14 (2.15)	5 (4.47–5.81)	2–12
Ligol			
With OAS	5.54 (3.16)	5 (4.26–6.82)	2–16
Without OAS	4.46 (2.07)	4 (3.21–5.71)	2–8
Total	5.18 (2.86)	5 (4.25–6.11)	2–16
Lobo			
With OAS	5.52 (2.48)	5 (4.49–6.55)	3–12
Without OAS	4.36 (2.31)	3 (3.02–5.69)	2–9
Total	5.10 (2.46)	4 (4.31–5.90)	2–12
Jonagored			
With OAS	6.18 (2.67)	6 (5.13–7.24)	3–14
Without OAS	4.92 (2.29)	4 (3.54–6.31)	2–9
Total	5.77 (2.60)	5.5 (4.94–6.60)	2–14
Jonika			
With OAS	6.15 (2.21)	6 (5.27–7.03)	3–12
Without OAS	4.54 (1.98)	4 (3.34–5.74)	1–7
Total	5.62 (2.25)	5 (4.90–6.34)	1–12
Szara Reneta			
With OAS	5.36 (1.80)	5 (4.62–6.10)	3–10
Without OAS	5.00 (2.05)	4.5 (3.53–6.47)	2–8
Total	5.26 (1.85)	5 (4.62–5.89)	2–10

Abbreviations: % CI, confidence interval; OAS, oral allergy syndrome.

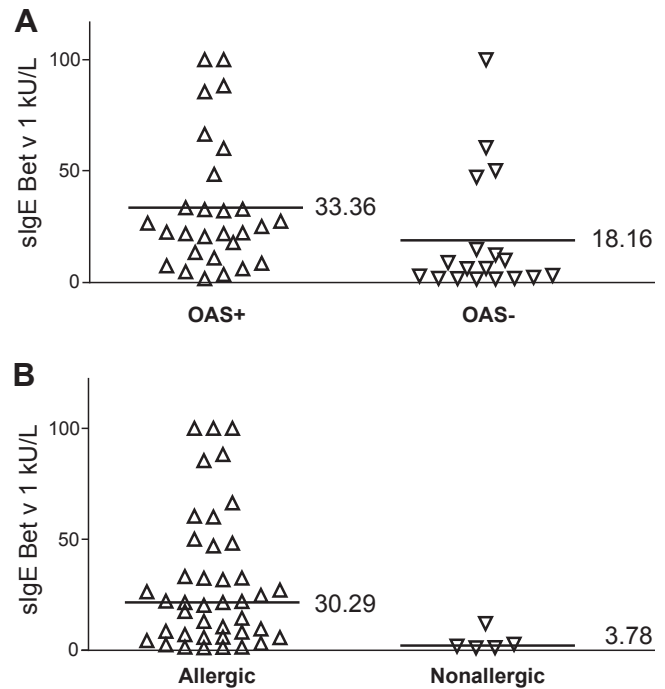
Discussion

Allergy to apple and other plant foods is the most common food allergy across Europe. In Northern and Central Europe, apple allergy symptoms are rather mild and related to birch pollen allergy,

Table 2
Incidence and ORs of Positive SPT Results (Wheal ≥ 3 mm) for Particular Apple Cultivars in Patients With and Without OAS

Apple cultivar	No. (%) of patients		OR (95% CI)	P value
	With OAS	Without OAS		
Golden Delicious	25 (89.29)	14 (77.78)		.41
Jonagold	27 (96.43)	11 (61.11)	11.96 (1.82–78.62)	.004
Idared	24 (85.71)	10 (55.56)	4.80 (1.22–18.64)	.04
Gloster	27 (96.43)	12 (66.67)	9.53 (1.43–63.55)	.01
Szampion	27 (96.43)	12 (66.67)	9.53 (1.43–63.55)	.01
Cortland	26 (92.86)	15 (83.33)		.36
Ligol	23 (82.41)	11 (61.11)		.17
Lobo	25 (89.29)	11 (61.11)	5.30 (1.30–22.50)	.03
Jonagored	27 (96.43)	11 (61.11)	11.96 (1.82–78.62)	.004
Jonika	27 (96.43)	11 (61.11)	11.96 (1.82–78.62)	.004
Szara Reneta	25 (89.29)	9 (50.00)	8.33 (1.94–35.11)	.005

Abbreviations: CI, confidence interval; OAS, oral allergy syndrome; OR, odds ratio; SPT, skin prick test.

**Figure 3.** Comparison of specific IgE (sIgE) Bet v 1 concentration in blood between patients with (+) and without (–) oral allergy syndrome (OAS) (A) and patients allergic to apples and those nonallergic (B). Values are presented as mean (SD) (OAS+ vs OAS–, $P = .004$; allergic vs nonallergic, $P = .003$; Mann-Whitney test).

sensitization to Bet v 1 allergen, and cross-reactivity with apple homologue Mal d 1. The clinical manifestations of local IgE-mediated allergy reaction to ingested apple include itching and swelling of lips, tongue, and throat and are usually described as OAS. The aim of this work was to assess the association between susceptibility of patients with and without OAS to particular apple cultivar allergies and its relation to birch pollen sIgE Bet v 1 levels in the blood of Polish patients.

Various methods have been used in practice to quantify allergy to apples (eg, Radioallergosorbent test, SPT, or oral provocation tests).⁶ A common recent approach for testing apple allergenicity is combined testing (eg, SPT and food challenge test).^{13,14} Although oral provocation tests enhance the evaluation of apple cultivar allergenicity, these methods still suffer from inconsistency in model standardization, labor intensiveness of the procedure, subjective evaluation of OAS symptoms (eg, visual analog scale), occasional development of tachyphylaxis, and lack of correlation with other methods.¹³ Thus, in our study, we performed prick by prick SPTs to test the allergenicity of apple cultivars available and consumed in Poland among patients with OAS. The SPT is widely accepted as a clinical tool for the assessment of sensitization to apple in patients with OAS and to differentiate the allergenic potential of apples.^{10,13,14} We have found that patients with OAS more often responded positively to apple prick by prick tests. At least 82% of patients with OAS responded positively to each apple cultivar (the maximum being 96%). Although tested cultivars were found to induce a positive response in a mean of 92% patients with OAS, patients without OAS partially responded to tested apple cultivars. The relatively new cultivars Golden Delicious, Gloster, Szampion, and Cortland induced 78%, 67%, 67%, and 83% positive responses in patients without OAS, respectively, and the results did not differ statistically from those of patients with OAS. By contrast, Szara Reneta, representing an old cultivar that originated in Europe in the 18th century, induced only 50% positive responses in patients without OAS ($P = .005$ for patients with vs without OAS). Thus, we

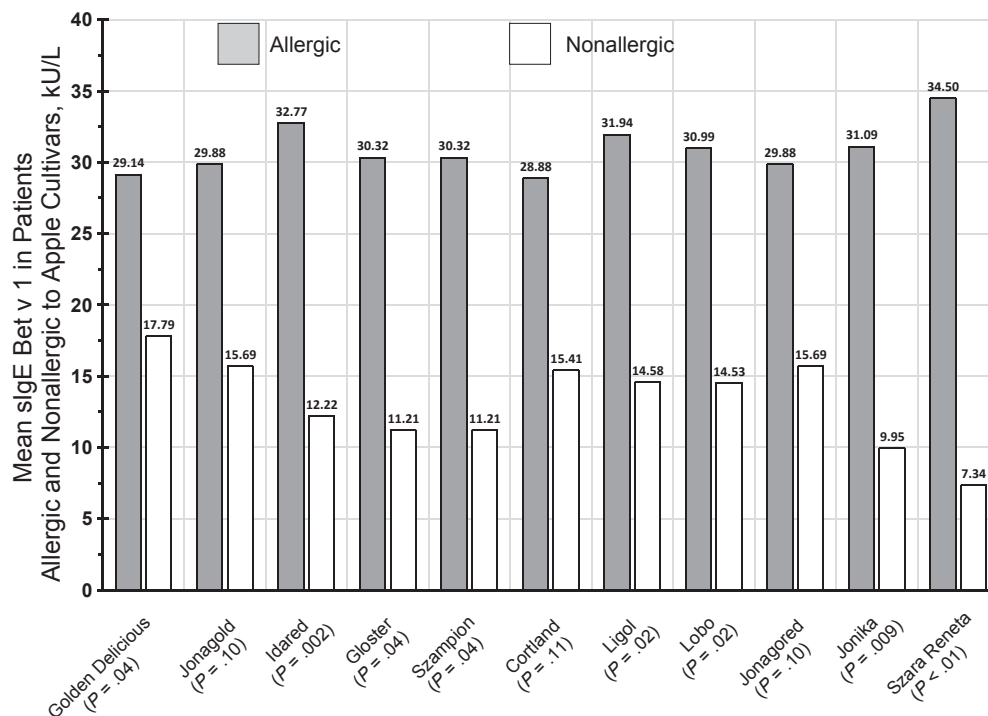


Figure 4. Mean concentration of specific IgE (slgE) Bet v 1 in blood of patients allergic to apple cultivars in the group with (+) vs without (–) oral allergy syndrome (OAS).

Table 3

Concentration of slgE Bet v 1 in Blood of Patients Allergic or Nonallergic to Tested Apple Cultivars

Allergy	slgE Bet v 1, kU/L		
	Mean (SD)	Median (95% CI)	Range
Golden Delicious			
With allergy	29.14 (28.29)	21.50 (19.97–38.31)	1.12–100.00
Without allergy	17.79 (36.45)	2.74	
Jonagold			
With allergy	29.88 (30.68)	21.49 (19.80–39.96)	1.12–100.00
Without allergy	15.69 (20.67)	6.17	1.08–50.00
Idared			
With allergy	32.77 (30.70)	22.03 (22.06–43.48)	1.35–100.00
Without allergy	12.22 (19.79)	2.94	1.08–60.00
Gloster			
With allergy	30.32 (30.40)	21.50 (20.47–40.17)	1.12–100.00
Without allergy	11.21 (17.65)	2.74	1.08–50.00
Szampion			
With allergy	30.32 (30.40)	21.50 (20.47–40.17)	1.12–100.00
Without allergy	11.21 (17.65)	2.74	1.08–50.00
Cortland			
With allergy	28.88 (29.91)	21.49 (19.44–38.32)	1.12–100.00
Without allergy	15.41 (25.33)	2.74	1.08–60.00
Ligol			
With allergy	31.94 (31.22)	21.70 (21.05–42.83)	1.30–100.00
Without allergy	14.58 (19.82)	5.63 (1.99–27.17)	1.08–60.00
Lobo			
With allergy	30.99 (30.59)	21.70 (20.64–41.34)	1.30–100.00
Without allergy	14.53 (21.79)	4.19	1.08–60.00
Jonagored			
With allergy	29.88 (30.68)	21.49 (19.80–39.96)	1.12–100.00
Without allergy	15.69 (20.67)	6.17	1.08–50.00
Jonika			
With allergy	31.09 (30.42)	21.70 (21.09–41.09)	1.30–100.00
Without allergy	9.95 (16.73)	2.30	1.08–50.00
Szara Reneta			
With allergy	34.50 (30.52)	23.42 (23.85–45.15)	1.38–100.00
Without allergy	7.34 (13.00)	2.30	1.08–47.00

Abbreviations: CI, confidence interval; slgE, specific IgE.

could draw the conclusion that Cortland represents the most allergenic potential among tested apple cultivars, whereas Szara Reneta represents the least allergenic cultivar for patients without OAS. Indeed, it is well known that apple cultivars differ in their allergenic potential because of variable levels of Mal d 1 content.⁵ Bolhaar et al¹⁴ found that apple SPT reaction values can be successfully translated into cultivar allergenicity ranking. In contrast, the study of Vlieg-Boerstra et al¹³ suggested that the results of such an approach might be inconclusive. Nevertheless, in both rankings, Golden Delicious represented a highly allergenic apple cultivar and has been established as a referential high allergenic cultivar. On the basis of our results, we suggest a simple classification of the tested apple cultivars into groups exhibiting either high or low allergenic potential according to the incidence of SPT positive results. The high allergenic cultivars group included Golden Delicious, Gloster, Szampion, and Cortland, whereas the low allergenic cultivars were Jonagold, Idared, Ligol, Lobo, Jonagored, Jonika, and Szara Reneta. A nonallergenic cultivar could not be identified.

Table 4

Spearman Correlation (r_s) Between slgE to Bet v 1 and Wheal Diameter in Patients With and Without OAS

Apple cultivar	Patients without OAS		Patients with OAS	
	r_s	P value	r_s	P value
Gloster	0.358		0.320	
Szampion	0.437	.03	0.518	.005
Cortland	0.461	.02	0.479	.01
Golden Delicious	0.461		0.263	.02
Jonagold	0.212		0.146	
Idared	0.491	.02	0.247	
Ligol	0.393		0.068	
Lobo	0.380		0.205	
Jonagored	0.342		0.141	
Jonika	0.483	.02	0.282	
Szara Reneta	0.525	.01	0.279	

Abbreviations: OAS, oral allergy syndrome; slgE, specific IgE.

Recently, it has been reported by Matthes et al.⁵ that Mal d 1 content in the apple, and consequently its related allergenicity, can be affected by the cultivation method, harvesting period, and storage. Although we strove to minimize intracultivar variations and followed standardized procedures for sample preparation (see prick by prick test procedure in the Methods section), we could not rule out that intrinsic apple characteristics (eg, accelerated post-harvest maturation) can modify apple allergen content and subsequent allergenic potential. In addition, although results of our study seem to be conclusive and are statistically significant, they should be interpreted with caution with regard to the limited number of patients enrolled in the study.

In addition to the approach described above, we also analyzed blood levels of sIgE Bet v 1, OAS incidence, and status of allergy to apple cultivars, as a consequence of Bet v 1 sensitization and subsequent IgE and T-cell cross-reactivity with homologous apple Mal d 1 allergen. Ciprandi et al.¹⁵ previously found that patients with OAS had higher Bet v 1 levels and more severe symptoms than patients without OAS but with birch allergy. Indeed, we found that patients with OAS have an almost 2-fold higher level of sIgE Bet v 1 in the blood compared with patients without OAS. Furthermore, the group of patients without an allergy to apples had 7-fold lower sIgE Bet v 1 levels than allergic patients, and this association was observed for each particular apple cultivar tested. These findings were expected and in line with numerous studies demonstrating the association of Bet v 1 sensitization and susceptibility to apple allergy.^{4,6,7,11,14}

In our study, we tried to find a correlation between sIgE Bet v 1 concentration and apple allergy intensity (wheal diameter) in patients with and without OAS. We found pronounced correlations for most high allergenic cultivars in patients without OAS (4 of 4 positive correlations) and those with OAS (3 of 4 positive correlations) groups. Weaker correlation in patients with OAS was seen for Golden Delicious, although the significance of this is unclear and will need further detailed analysis. Similar correlations between food allergy and sIgE against panallergens, such as Bet v 1 and Bet v 2, was reported by Ricci et al.¹⁶ Moreover, a positive association of Mal d 1 sensitization among Bet v 1–positive patients was reported by Hao et al.¹⁷ in a Chinese patient population. Surprisingly, we obtained a markedly different profile of correlations for low allergenic cultivars. Although in the group without OAS we observed a pronounced correlation between sIgE Bet v 1 concentration and wheal diameter, the group with OAS had weaker associations for all low allergenic cultivars tested. The observed differences might be related to the interplay of different apple allergens, namely, Mal d 1, Mal d 2, Mal d 3, and Mal d 4, and associated homologues in patients with and without OAS. In light of these results, we might hypothesize that concurrent sensitization to other plant allergens responsible for OAS might predispose patients with OAS to apple allergy that does not strictly depend on Mal d 1 content and its cross-reactivity with sIgE Bet v 1. Indeed, it is widely accepted that plant allergens (eg, nonspecific lipid transfer proteins or profilins) have a high degree of homology and cross-reactivity even among distantly related species. Thus, it is not unexpected that sensitization to peach major allergen Pru p 3 or birch pollen Bet v 2 is associated with subsequent allergic reaction to apples due to cross-reactivity with respective apple allergens Mal d 3 (lipid transfer

protein) and Mal d 4 (profilin).^{7,12} In contrast, the severity of allergic reaction induced by low allergenic apples, containing less Mal d 1, seems to be related to the level of sIgE Bet v 1 in patients without OAS. In other words, patients without OAS but not patients with OAS might benefit from eating low allergenic apples to prevent the development of OAS.

In summary, this study provides, for the first time, clinical evidence of allergy to apple among Polish patients with OAS exposed to birch pollens. We evaluated the allergenicity of various apple cultivars popular in Poland using prick by prick tests and found a substantial role of Bet v 1 sensitization in the allergic response to tested apple cultivars. By plotting sIgE Bet v 1 and SPT results of patients with and without OAS, we could differentiate between low and high allergenic cultivars consumed in Poland. Results from this study may have implications for medical diagnostics, immunotherapy, clinical research, and breeding schemes for new hypoallergenic cultivars in Central-Eastern Europe.

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