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Bet v 1- and Bet v 2-Associated Plant Food Sensitization in Uganda and Germany: Differences and Similarities

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Key Words
Africa · Alder pollen · Atopy · Birch pollen · Food allergy · Pollen allergy

Abstract
Background: Birch pollen allergy and concomitant plant food sensitization are well documented in Europe. However, there are currently no data available on pollen-associated plant food sensitization or even pollen allergy in tropical Africa. Our study aimed to investigate Bet v 1- and Bet v 2-associated plant food sensitization in atopic patients from Uganda and compare it with sensitization rates in German patients. ***Methods:*** Sera from 83 Ugandan and 97 German atopic patients were analysed using UniCAP100™ for allergen-specific IgE against the birch tree pollen allergens Bet v 1 and Bet v 2 as well as the plant foods hazelnut, apple, kiwi, pea, peach, cherry, litchi, peanut, and soy. ***Results:*** As expected, sensitization to Bet v 1 and cross-reactive plant food allergens was more common in German atopic patients. In contrast, the prevalence of sensitization against Bet v 2 was remarkably similar in Ugandan and German patients. Interestingly, in Ugandan patients we found IgE-mediated sensitization against plant foods such as hazelnut, pea, peach, cherry, and litchi that are neither cultivated nor consumed in Uganda. ***Conclusions:*** For Ugandan atopic patients, sensitization against the Bet v 2 allergen (a plant profilin) may explain cross-reactivity to several plant foods which are not consumed in Uganda. Additionally, it is probable that sensitization of Ugandan atopics to alder pollen (*Alnus acuminata*, plant family Betulaceae) caused serological cross-reactivity with *Betula verrucosa*-related allergens.

Introduction

The global prevalence of allergic diseases is reported to be in a range of 20–30% of the world's population [1]. In Europe, epidemiological studies have shown an increasing prevalence of IgE-mediated allergy and nowadays up to 25% of the European population suffer from some form of atopic diseases [2, 3]. In Africa the prevalence of atopic dermatitis, asthma and allergic rhinitis has also been increasing over the past decades [4]. Whereas birch pollen allergy and concomitant plant food sensitization is well documented in Europe, there are currently no published data about species-specific pollen allergy or associated plant food sensitization in tropical Africa.

Birch pollen allergy is one of the most frequent triggers of respiratory allergy diseases in European patients [5]. About 70% of these patients show IgE-mediated sensiti-

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zation to plant foods such as hazelnut, apple, kiwi, carrot, or soy due to cross-reactivity between the major birch pollen allergen Bet v 1 and homologous plant food allergens [6, 7] such as Cor a 1 [8], Mal d 1 [9], Pru p 1 [10], Api g 1 [7, 11, 12], and Gly m 4 [13]. Typically, the mild clinical symptoms termed oral allergy syndrome are limited to the oral cavity [14].

Bet v 1 is expressed in pollens of taxonomically related trees within the Betulaceae plant family. It belongs to the group I pathogenesis-related proteins, which are engaged in the defence of plants against microbial attack [15]. Bet v 2 belongs to the profilin protein family (Bet v 2 family), which are considerably conserved molecules with a high degree of structural homology between various plant species [16, 17]. Due to its capacity to bind to actin, Bet v 2 participates in the regulation of plant cell division, growth and differentiation [17].

Our study investigated and compared IgE-mediated sensitization between Ugandan and German atopic patients against the recombinant birch pollen allergens Bet v 1 and Bet v 2 as well as potentially cross-reactive plant food allergens.

Patients and Methods

Patients

Ugandan patients attending the Dermatology Department at Mbarara University Hospital during the year 2013 were prospectively selected for this study when they fulfilled at least two of the following criteria: positive family history of atopy and a diagnosis of allergic rhinitis, allergic asthma and atopic dermatitis. Pregnant women and children below the age of 3 years were not evaluated. The study was approved by the Ethics Committee of the University Hospital Mbarara (project No. 09/03-13) and all patients gave informed consent. Data on German atopic patients were retrospectively retrieved from patient files within the time period from 2007 to 2010 of the Allergy Centre at the University Hospital Würzburg when they fulfilled the same criteria as the Ugandan patients. With regard to German patients, at that time IgE screening for birch pollen allergy and pollen-associated plant food sensitization was done as part of routine diagnostic practice and further ethical approval was therefore not required.

IgE Measurement

Venous blood samples of the Ugandan patients were obtained at the time of initial evaluation and sera were stored frozen at –20°C until analysis. Ugandan and German serum samples were all analysed in Germany using the same UniCAP100™ IgE platform according to the procedure described by the manufacturer (Thermo Fischer Scientific; www.phadia.com). The sera were analysed for allergen-specific IgE against hazelnut (Thermo Fischer Scientific code f17), apple (f49), kiwi (f84), pea (f94), peach (f95), cherry (f242), litchi (f348), peanut (f352), and soy (f353) and for the recombinant birch tree pollen allergens rBet v 1 (t215) and rBet v 2 (t216). In clinical practice 0.35 kU/l is used as the cut-off value for positivity of allergen-specific IgE. However, quantitative measuring of IgE ranges from 0.1 to 100 kU/l. Therefore, for improved sensitivity of the assay we, as well as other groups, used 0.1 kU/l as the detection threshold for allergen-specific IgE.

Table 1. Clinical data of the Ugandan and German patients

	Uganda (n = 83)	Germany (n = 97)
Age, years		
Mean	29.8	39.1
Range	3–96	14–78
Male/female	27/56	27/70
Atopy characteristics, n (%)		
Family atopy history	41 (49)	52 (54)
Allergic (seasonal) rhinitis	54 (65)	67 (69)
Allergic (seasonal) asthma	9 (11)	41 (42)
Atopic dermatitis	2 (2)	8 (8)
Total serum IgE, n		
Not done	2	60
<100 kU/l	5	14
101–500 kU/l	20	14
501–1,000 kU/l	22	3
1,001–5,000 kU/l	30	5
>5,000 kU/l	4	1

Results

Patients

A total of 83 Ugandan and 97 German atopic patients aged 3–96 years were evaluated. Patients recruited in Uganda were younger than the Germans and showed higher total serum IgE values (table 1). In this context it has to be considered that, unfortunately, in the majority of German patients no total serum IgE values were available retrospectively. Both the Ugandan and German atopic patient groups contained more females than males. With the exception of allergic rhinitis, the prevalence of allergic asthma and atopic dermatitis was higher in the German cohort.

Bet v 1 and Bet v 2 Sensitization

The difference in the serum concentrations of IgE antibodies against Bet v 1 and Bet v 2, expressed as the classical IgE CAP™ classes, is shown in table 2. Compared with the allergen-specific IgE values of German atopic patients Bet v-specific IgE values of Ugandans generally fell within lower classes. As expected, the prevalence of Bet v 1 sensitization was higher in German atopic patients

Table 2. Prevalence of IgE-mediated sensitization against rBet v 1 and rBet v 2 in Ugandan and German atopic patients

Betula verrucosa allergens	Uganda (n = 83)	Germany (n = 97)
rBet v 1 (≥0.1 kU/l)	22 (26%)	68 (70%)
0.10–0.35 kU/l	19	3
0.36–0.70 kU/l	2	3
0.71–3.50 kU/l	1	11
3.50–17.50 kU/l	0	15
17.51–50.00 kU/l	0	23
50.01–100.00 kU/l	0	8
>100.00 kU/l	0	5
rBet v 2 (≥0.1 kU/l)	22 (26%)	23 (24%)
0.10–0.35 kU/l	17	3
0.36–0.70 kU/l	1	4
0.71–3.50 kU/l	3	13
3.50–17.50 kU/l	1	3
17.51–50.00 kU/l	0	0
50.01–100.00 kU/l	0	0
>100.00 kU/l	0	0

Table 3. Age distribution (years) of Ugandan and German atopic patients with allergen-specific IgE against rBet v 1 and rBet v 2

	Age distribution				
Patients	0–19	20–39	40–59	60–79	≥80
Ugandan atopics					
All (n = 83)	29	33	16	2	3
rBet v 1 IgE positive (n = 22)	10	8	4	0	0
rBet v 2 IgE positive (n = 22)	11	5	5	1	0
German atopics					
All (n = 97)	10	43	33	11	0
rBet v 1 IgE positive (n = 68)	6	31	24	7	0
rBet v 2 IgE positive (n = 23)	3	13	5	2	0

(70%) compared with Ugandans (26%). Using the detection threshold of 0.1 kU/l, the prevalence of sensitization against Bet v 2 was quite similar in the Ugandan and German patients (Uganda 26%, Germany 24%). Our study showed again that the prevalence of atopy and allergen-specific IgE sensitization generally decreases with increasing age (table 3).

Sensitization against Specific Plant Food Allergens

The prevalence of sensitization to specific plant food allergens was generally lower among Ugandan atopic patients compared with the Germans (table 4). Surprisingly, we found IgE-mediated sensitization of ≥0.1 kU/l in Ugandan atopic patients to plant food allergens that are neither consumed nor cultivated in Uganda, namely hazelnut (Uganda 54%, Germany 71%), pea (Uganda 33%, Germany 69%), peach (Uganda 53%, Germany 73%), cherry (Uganda 63%, Germany 78%), and litchi (Uganda 51%, Germany 56%). Sensitization of Ugandans against these plant food allergens was also detected in a significant number of patients when a detection threshold of >0.35 kU/l was evaluated (table 4). On the other hand, in Ugandan patients the prevalence of sensitization to peanut (20% with ≥0.1 kU/l and 2% with >0.35 kU/l) and soy (17% with ≥0.1 kU/l and 4% with >0.35 kU/l), which are commonly eaten in Uganda, was lower compared with the values concerning plant food allergens which are definitively not eaten in Uganda. Because two of the authors (L.O. and G.M.) are inhabitants of South-Western Uganda, we had first-hand information about the consumption and cultivation of plant foods within this African region.

Discussion

Although birch trees are not domestic in Uganda, our study demonstrated specific IgE sensitization to Bet v 1 and Bet v 2 not only in German but also in Ugandan atopic patients. Moreover, Ugandans showed sensitization to plant food allergens which are neither consumed nor cultivated in Uganda, namely hazelnut, pea, peach, cherry, and litchi. There may be two possible explanations for this observation.

First, in German atopic patients, sensitization against the major birch pollen allergen Bet v 1 caused the well-known Bet v 1-related cross-reactivity with several plant food allergens, as shown in table 5 [18]. For Ugandan atopic patients we hypothesize that sensitization to the profilin Bet v 2, perhaps by pollen of different plant species, may be responsible for serological cross-reactivity to plant foods which are not eaten in Uganda. African finger millet *(Eleusine coracana)* is a major food crop in Uganda and belongs to the botanical Gramineae family [19]. Sensitization against pollen of, for example, this grass may be responsible for profilin-associated plant food sensitization.

Second, we speculate that a related plant of the same botanical family as the birch tree (Betulaceae) caused IgE cross-reactivity with *Betula verrucosa* allergens. *Alnus acuminata* (commonly called alder), which belongs to the Betulaceae family, is native in Argentina, Bolivia, Colombia, Central America, Mexico, and Peru [20, 21]. In South-

Table 4. Prevalence of plant food allergen-specific IgE values (≥0.1 and >0.35 kU/l) in German and Ugandan atopic patients

Popular name	Botanical name	Uganda (n = 83)		Germany (n = 97)	
		≥0.1 kU/l	>0.35 kU/l	≥0.1 kU/l	>0.35 kU/l
Hazelnut	*Corylus avellana*	45	27	69	64
Apple	*Malus domestica*	43	25	66	45
Kiwi	*Actinidia deliciosa*	48	20	48	31
Pea	*Pyrus communis*	27	26	67	54
Peach	*Prunus persica*	44	25	71	57
Cherry	*Prunus avium*	52	29	76	54
Litchi	*Litchi chinensis*	42	31	54	26
Peanut	*Arachis hypogaea*	17	2	67	61
Soy	*Glycine maxima*	14	3	63	56

Table 5. Bet v 1- and Bet v 2-related allergens in specific plant foods

Plant food	Botanical name	Bet v 1-related allergen	Bet v 2-related allergen
Hazelnut	*Corylus avellana*	Cor a 1	Cor a 2
Apple	*Malus domestica*	Mal d 1	Mal d 4
Kiwi	*Actinidia deliciosa*	Act d 8	Act d 9
Pea	*Pyrus communis*	Pyr c 1	Pyr c 4
Peach	*Prunus persica*	Pru p 1	Pru p 4
Cherry	*Prunus avium*	Pru av 1	Pru av 4
Litchi	*Litchi chinensis*	n.d.	Lit c 1
Peanut	*Arachis hypogaea*	Ara h 8	Ara h 5
Soy	*Glycine maxima*	Gly m 4	Gly m 3

n.d. = Not yet determined.

Western Uganda, a study was carried out to examine the effect of the integration of *A. acuminata* on farms on crop yields [22]. Since the Ugandan atopic patients whose sera were analysed in our study were from South-Western Uganda, it is probable that sensitization to pollen of *A. acuminata* caused cross-reactivity with *B. verrucosa* allergens.

We expected to find a higher prevalence of sensitization against peanut and soy in Ugandan atopic patients since both are commonly consumed and cultivated in Uganda. Surprisingly, our results showed a lower prevalence of sensitization against these foods (peanut 20% with ≥0.1 kU/l and 2% with >0.35 kU/l, soy 17% with ≥0.1 kU/l and 4% with >0.35 kU/l) compared with foods not eaten there such as, for example, hazelnut (54% with ≥0.1 kU/l and 32% with >0.35 kU/l) or cherry (63% with ≥0.1 kU/l and 35% with >0.35 kU/l). It is tempting to speculate that these results support the hypothesis that regular oral exposure may play a role in the suppression of IgE-mediated food sensitization by triggering gastrointestinal immune tolerance mechanisms [23].

To the best of our knowledge, the present study is the first to compare serological Bet v 1 and Bet v 2 reactivity in patients from two countries on different continents. Germany enjoys a moderate climate within Europe characterized by moderate annual temperature fluctuations, whereas Uganda in tropical Africa has a tropical climate all year long. We demonstrated Bet v 1 reactivity in 70% of German atopic patients and in only 26% of the Ugandans. Unexpectedly, we found a striking similarity in the general prevalence of IgE sensitization (≥0.1 kU/l) to Bet v 2 among Ugandan (26%) and German (24%) atopic patients. Previous studies showed regional variations within specific IgE profiles among birch pollen-sensitive European individuals. Moverare et al. [24] examined 242 patients with seasonal rhinitis and/or asthma from Finland, Sweden, Austria, France, Switzerland, and Italy. Finnish, Swedish and Austrian sera contained the highest percentage of IgE positivity for Bet v 1 (≥98%). Bet v 1-specific IgE was found in 90% of the French sera and in 65 and 62% of the sera from Switzerland and Italy, respectively. Only 2% of the Finnish and 12% of the Swedish patients had IgE positivity to Bet v 2, while Bet v 2 reactivity was more common in the other populations (20–43%).

Our study showed that the prevalence of allergen-specific IgE sensitization decreases with increasing age of the patients. This finding is in agreement with results of several other studies. Sekerkova and Polackova [3] stated that the prevalence of Bet v 1-, Bet v 2- and Bet v 4-spe-

cific IgE in birch pollen-allergic individuals is dependent not only on the region in which they live but also on their age. Mediaty and Neuber [25] found that total and allergen-specific serum IgE decreases with age in patients with allergic rhinitis and asthma but not in patients with atopic dermatitis. Within the framework of the SAPALDIA survey (Swiss Study on Air Pollution and Lung Diseases in Adults), it was also shown that atopy prevalence decreases with increasing age [26]. However, rates of IgE sensitization are on the increase worldwide and therefore it is not surprising that the prevalence of atopy and allergen-specific IgE sensitization generally decreases with increasing age.

In the interpretation of our data, the detection threshold of allergen-specific IgE of 0.1 kU/l is an important issue. Principally, the ImmunoCAP method can quantitatively detect allergen-specific IgE down to a threshold of 0.1 kU/l [27]. In comparison with the conventional 0.35 kU/l, a cut-off value of 0.1 kU/l increases the sensitivity of allergen-specific IgE measurement. Importantly, the cut-off value of 0.1 kU/l is obviously not accompanied by a decreased specificity of the assay, as shown, for example, by the small numbers of German patients with rBet v 1- and rBet v 2-specfic IgE values between 0.1 and 0.35 kU/l. Moreover, the rather unexpected sensitization of Ugandans against certain plant food allergens was also detected when a cut-off value of >0.35 kU/l was evaluated (table 4).

We found that the prevalence of asthma and atopic dermatitis was higher in German atopic patients than in Ugandan atopics. This is in line with observations from the ISAAC study (International Study of Asthma and Allergies in Childhood) showing that there was clearly a lower prevalence of allergic diseases in developing countries [28]. The predominantly rural settlement of patients living in South-Western Uganda appears to be protective against the development of certain atopy diseases, supporting the hygiene hypothesis by which early exposure to environmental allergens and infectious agents leads to the development of immunological tolerance [29].

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