Factors influencing the susceptibility to, and characteristics of kiwi fruit allergy

Final Report TO7025

Jane Lucas, Karen Collins, Kate Grimshaw, John Warner, Jonathan Hourihane
Allergy and Inflammation Sciences, University of Southampton.

Start Date: April 1st 2001
End Date: 31st September 2003

Dr Jane Lucas
Allergy and Inflammation Sciences (MP 803)
University of Southampton
Southampton General Hospital
Tremona Road
Southampton SO16 6YD

jlucas1@soton.ac.uk

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1 Title Page

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2 Executive Summary.

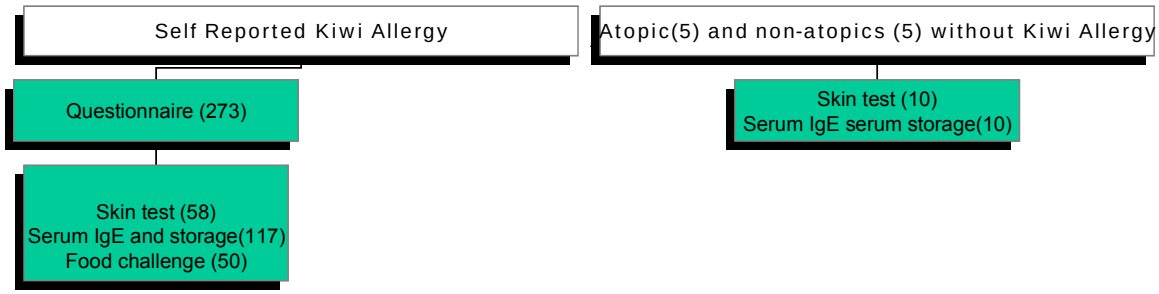
2.1 Aims and objectives:

- To conduct the first study of kiwi fruit allergy in the UK and the largest clinical study of the allergy worldwide.
- To describe the clinical characteristics of kiwi fruit allergy.
- To evaluate methods of clinical investigation of kiwi fruit allergy.
- To assess the importance of other allergies in the susceptibility to kiwi fruit allergy.
- To describe how age influences the onset and severity of kiwi fruit allergy.
- To investigate whether Gold kiwi fruit is an allergen

2.2 Approach:

Subjects with self-reported kiwi fruit allergy were recruited from three sources: from the paediatric and adult allergy clinics at Southampton General Hospital, respondents to an advertisement in Anaphylaxis Campaign Magazine and people who contacted the study following a media release on national radio and in newspapers. All subjects were asked to complete a postal questionnaire which consisted mainly of closed questions about their allergy to kiwi fruit, and associated allergies. Subjects who wished to participate further in the research could have blood taken locally which was sent to the research centre for IgE analysis. People who wished to travel to the research centre were invited to attend for a food challenge, skin testing and a blood test.

Fig 1. Study plan. Number of subjects for each part of study in parentheses.



Five subjects who were allergic to traditional green kiwi fruit also had a food challenge to the newly available Gold fruit (Zespri Gold).

2.3 Key Findings

- Questionnaires from 273 subjects with self-reported kiwi fruit allergy have been analysed.
- The age of respondents at the time of their first reaction ranged from 4 months to 71 years. 33 were under 5 years.

- Respondents reported very little allergy to kiwi fruit in the 1970s, particularly in the now adult population who were children at the time. Reports of allergy were increasingly common in the 1980s, but it was not until the 1990s that kiwi fruit allergy was recognised in children and young infants. During the past 30 years kiwi fruit has increased in popularity, and the increase in allergy to the fruit may be dependent on increased exposure.
- Young children with the allergy usually react on their first known exposure and 40% have severe reactions. Adults often react after numerous uneventful exposures and are less likely to report severe symptoms.
- Respondents report allergies to allergens known to cross-react with kiwi fruit eg. Latex, avocado, pollens. Co-existing allergies to other foods were also commonly reported, especially in young children.
- Fifty percent of 45 subjects with a history suggestive of kiwi allergy, had their allergy confirmed by a double blind placebo controlled food challenge. This is a relatively high rate of positive food challenges in comparison to many studies of food allergy.
- Many of the food challenges were inconclusive. We believe this reflects the technical difficulties of conducting food challenges in subjects with oral allergy syndrome.
- Skin testing using fresh kiwi fruit was a sensitive method of confirming kiwi fruit allergy, but had a high rate of false positive results in this population.
- Skin testing using commercially available skin test solution had very poor sensitivity when compared with food challenge.
- Kiwi Gold produced allergic reactions in some subjects who are allergic to 'traditional' kiwi fruit.

2.4 Technical evaluation

- Kiwi fruit allergy in the UK is not uncommon.
- Reactions may be severe, particularly in young children and infants.
- A new food should not be immediately categorized as having low allergenicity just because the first reactors (usually adults) have mild symptoms: children may react differently
- Current methods of clinical investigation of kiwi fruit allergy are not satisfactory. Work is required to improve food challenge protocols (particularly for oral allergy syndrome), commercially available skin test extracts and the CAP system for kiwi fruit. This finding is in keeping with studies of other fruits involved in oral allergy syndrome.
- Gold kiwi fruit is an allergen. People allergic to 'traditional' kiwi fruit are at risk of allergy to Gold kiwi.
- Post marketing surveillance is required to monitor whether people without allergy to 'traditional green' kiwi fruit develop symptoms to the Gold fruit.

3 Glossary of abbreviations

OAS	Oral Allergy Syndrome
SPT	Skin Prick Test
DBPCFC	Double blind placebo controlled food challenge.
sIgE	Specific IgE

4 Aims and objectives

4.1 Historical Background

Kiwi fruit (Actinidia) is a plant native to the Yangtze Valley of China. Seed was taken to New Zealand in 1904, and almost all kiwi cultivars outside China are descended from the two female and one male plant grown from this single introduction of seed. Commercial plantings began in New Zealand in the late 1930s, and exports to the USA started in 1962. Californian kiwi fruit found their way onto the US market in 1970, and for the past three decades kiwi fruit has been increasingly available worldwide, with producers now in New Zealand, USA, Japan, Italy, Greece, Spain, Australia and Chile.

Acute allergy to kiwi fruit was first described in 1981[1] and there have since been reports of the allergy presenting with a wide range of symptoms from localised oral allergy syndrome (OAS) to life-threatening anaphylaxis[2] [3-7]. The association of kiwi fruit allergy with allergies to pollen and latex has been widely reported in recent years and cross reactivity has been confirmed by inhibition studies with birch pollen[8-11], timothy pollen[10], avocado[12;13], banana[13], latex[12;13] rye[14] and hazelnuts[14]. Three of the possible major allergens responsible for kiwi allergy have recently been isolated and characterised[9;15;16], but much remains unknown about this increasingly common allergy.

Clinical information about kiwi fruit allergy is mostly based on a handful of case reports and small case series, in addition to extraction of data from scientific papers primarily written to explore cross-reactivity. The first reported case of kiwi fruit allergy was in a 53 year old atopic woman who developed urticaria, wheeze and laryngeal oedema on handling the fruit[1]. Since then there have been a number of reports of kiwi allergy in adults, mostly presenting with oral symptoms [2-7]. In addition some of these individuals have had more generalised reactions including urticaria[3], vomiting[3;6] respiratory compromise[5] and cardiovascular collapse[5]. All case reports bar one[3] involved atopic subjects. Case reports of children remain very limited. A 12 year old atopic boy in Japan developed localised oral symptoms, urticaria and dizziness having eaten the fruit [6], and a hypotensive response to kiwi has been described in a 3 year old boy [17].

There have not been any studies of adequate size to investigate whether children respond differently to adults with kiwi allergy, and there are no data about the natural history of the allergy.

Clinical Investigations

This is the first study to investigate the role of clinical investigations for kiwi fruit allergy in the UK population.

Skin testing with fresh kiwi is the most common clinical investigation reported [18], the main limitation being that skin testing with fresh fruit lacks standardisation[2;5;7;8;11;19]. Some authors have produced an extract of kiwi pulp [1;6]or of fruit skin for skin testing[1], and others have used commercially available skin test solutions[18;20]. Prick to prick with fresh fruit is reportedly highly sensitive (>95% in all studies). Commercial skin test extracts are significantly less sensitive. In a study of 43 subjects with kiwi allergy,

only 40% of subjects had positive skin reactions to one commercial extract, and 28% to another make of skin test solution- all subjects had had positive reactions when tested by prick to prick with fresh kiwi[18].

Although highly sensitive, the specificity of fresh kiwi fruit for skin testing appears poor in subjects allergic to cross-reacting pollens or latex. Gall's study[8] included seven controls allergic to birch pollen but not kiwi fruit, all of whom had positive skin test responses to fresh kiwi fruit. Similarly, two latex allergic individuals with no symptoms on eating kiwi fruit have been reported to have positive skin reactions to fresh kiwi and a commercial skin test extract[21]. Beezhold described 47 latex allergic individuals, eight of whom had positive skin tests with fresh kiwi, but only one had symptoms to kiwi fruit [22]. The converse also occurs- asymptomatic sensitisation to latex may be as high as 86% in fruit allergic patients, but only 11% suffer clinically relevant latex allergy[3]. Screening subjects with fruit allergy by skin test or measuring food-specific IgE levels to other fruits that might share cross-reactive antigens results in an unacceptable number of false positive reactions[23]. However, when combined with a detailed symptom history of oral allergy syndrome, skin prick testing with fresh apple in pollen allergic subjects has showed a good positive predictive value of over 90%[24]. The use of purified fruit allergens in diagnostic tests could improve their specificity, but the major allergens responsible for kiwi fruit remains controversial. The relevance of asymptomatic sensitivity to cross-reacting allergens remains unclear, and continued follow-up of such subjects is required to clarify the significance.

In conflict with Gall's study[8], other groups have found kiwi skin tests to be highly specific, with negative skin tests in all pollen allergic[1;3], mite allergic[3] and 'atopic'[6] subjects. There are therefore discrepancies in the literature concerning the specificity of kiwi fruit skin tests in atopic groups. The negative predictive value in non-atopic controls approaches 100% [1;6;8], [3].

The role of measuring specific IgE to confirm kiwi fruit allergy is less clear. Although positive in some case reports of patients with kiwi allergy [4-6;19], other authors have found it unhelpful[3;20]. In his study of 22 subjects with kiwi fruit allergy[8], Gall found that although he was able to detect specific IgE in all subjects with severe symptoms, the results were negative in subjects with mild local symptoms. It is possible that his subjects with oral allergy syndrome had IgE confined to the oral mucosa with no detectable circulating specific IgE, or were allergic to a labile allergen not present in the specific IgE kit. However, other studies have found that at least some subjects with oral allergy syndrome have detectable circulating IgE to kiwi fruit [10;11;13;25]. Reports of sensitivity of measuring IgE vary between 13%[26] to over 70%[25]. Variation in sensitivity may reflect the different kiwi allergic populations being studied, and the different techniques used to measure specific IgE, with some groups using the CAP system[18] [20] [15], but others using home made allergen discs[8] [25].

The specificity of in vitro tests is also unclear. Using the CAP method to detect specific IgE to kiwi in 136 latex allergic patients, Breler[26] found relatively high specificity (83%). Likewise, Gall[8] found no specific IgE to kiwi fruit in non-kiwi allergic controls with birch pollen allergy, despite having positive skin tests with fresh kiwi fruit. This is in contrast to reports of 4/4 subjects with no symptoms to kiwi, but symptoms of birch pollen allergy, who had detectable IgE to kiwi fruit [25] and a case report of two subjects with latex allergy who had positive RAST to kiwi despite being asymptomatic [21]. It has been suggested that each

individual has a threshold for anti-birch pollen titres to cause oral allergy intolerance with apple [27] and indeed several studies have demonstrated higher specific birch pollen specific IgE or larger skin test reactions in subjects with oral allergy syndrome [28-30].
The present information about the role of clinical investigations in kiwi allergy is therefore confusing and requires further evaluation.

The allergenicity of Zespri Gold

The genus *Actinidia* contains about 60 species, but until recently only one has been eaten regularly in the Western world. Green kiwi fruit, *Actinidia deliciosa,* originated in China, but has been cultivated in New Zealand since 1904. The fruit has green coloured flesh with many small, soft black seeds, and a brown-green skin covered with numerous hairs. *Actinidia chiensis* is a species very similar to *Actinidia deliciosa* and until 20 years ago they were classified in the same species. However, there are distinct differences between the two. *Actinidia chiensis* fruit is almost hairless and the flesh ranges from a lime green colour to bright yellow. The fruit are generally much sweeter than *Actinidia deliciosa. Actinidia chiensis* seeds were collected in China in 1977 and taken to New Zealand. Fruit from a mother plant with yellow flesh was identified as particularly good, and is now grown commercially, and marketed under the name Zespri ™ Gold. Exports were first made to the UK in 2000, and this novel food is increasingly available in North America and Europe.
The allergic potential of all novel foods needs to be assessed, but to date there have been no studies investigating the proteins and IgE binding of Zespri ™ Gold, or its allergenic potential in subjects allergic to green kiwi fruit.

4.2 Aims and objectives

The study was designed to investigate kiwi fruit allergy in the UK population. The questionnaire is the basis of a descriptive study of the subjects with kiwi fruit allergy. The population is self-selected. The aims and objectives of the study were:

- **To conduct the first study of kiwi fruit allergy in the UK and the largest clinical study of the allergy worldwide.**
- **To describe the clinical characteristics of kiwi fruit allergy in the UK population.**
- **To evaluate methods of clinical investigation of kiwi fruit allergy** in an attempt to clarify some of the conflicting findings of previous studies. Also, it is important to evaluate the investigations in the UK, since the sensitivity and specificity of clinical investigations varies between populations.
- **To assess the importance of other allergies in the susceptibility to kiwi fruit allergy.** Kiwi fruit has been shown to co-exist with latex, avocado, banana and apple allergies in other populations. This needed to be confirmed in the UK. This is the first study to look at the coexistence of allergy to other allergens not known to cross-react with kiwi fruit.

- **To describe how age influences the onset and severity of kiwi fruit allergy.** This is the first study of sufficient size to investigate the differences between adults and children with the allergy.
- **To investigate the allergic potential of Zespri Gold.**

4.3 Rational of experimental approach

The questionnaire

The questionnaire forms a descriptive study of kiwi fruit allergy in the UK. It was intended to recruit subjects from a variety of sources to obtain an adequate number of subjects. It was also hoped that this was create diversity in the subjects as opposed to only recruiting from one source. The questionnaire was based on one previously used in our department to characterise peanut allergy[31] that consisted predominantly of closed questions. Using the same questions allowed for direct comparisons between the subjects with self-reported kiwi fruit allergy, and those with self-reported peanut allergy. All questionnaires were visually reviewed by a single physician (JSAL), and subjects with symptoms not suggestive of IgE mediated allergy were excluded from further analysis.

This study has limitations. It is not an epidemiological study, and therefore we can make no assessment of the prevalence of the problem. Perhaps the allergy is more common than previously appreciated given the numbers of people who have contacted the study, but an epidemiological study is required to address this. This descriptive study with self-selected subjects provides a financially viable way of obtaining data to indicate but not measure the scale of a problem.

Clinical investigations

Clinical investigations were all conducted according to recognised protocols where they exist. For skin tests to kiwi fruit, we used fresh kiwi fruit using the prick to prick technique, since this has been reported to be the most sensitive method of investigating fruit allergy. Part way through the study we also identified a commercially available skin test extract (Alyostal, France), which was subsequently used for testing in addition to the fresh fruit. The CAP system (Pharmacia) is the most widely reported method of measuring specific IgE, and this was therefore the test used for this study.

There have been no published reports of DBPCFC in kiwi fruit allergy, and we therefore devised our own protocol, using accepted standards where they exist.

5 Experimental Procedures

5.1 Ethics

The Southampton and South West Hants Joint Research Ethics Committee approved the study.

5.2 Subjects

Subjects were recruited from three sources: from the paediatric and adult allergy clinics at Southampton General Hospital, respondents to an advertisement in Anaphylaxis Campaign Magazine and people who contacted the study following a media release on national radio and in newspapers.

5.3 Questionnaire

Four hundred and twenty subjects were sent a self-administered postal questionnaire (Appendix 1), and 291 (69%) completed questionnaires were returned between April 2001 and September 2002. The questionnaire was based on one previously used in this department to characterize peanut allergy which and consisted predominantly of closed questions. It included questions on age of onset of allergy, symptoms, frequency of kiwi ingestion prior to a reaction, treatment received and coexisting allergies and atopic diseases. Symptoms reported in the questionnaire were considered mild if they involved a tingling or sore mouth, or a rash; they were considered moderately severe if they included abdominal pain, a tight throat, breathing difficulties other than wheeze or facial swelling; symptoms were considered severe if they involved wheeze, cyanosis or collapse[31]. All questionnaires were visually reviewed by a single physician (JSAL).

5.4 Skin prick testing

Skin prick testing was performed on the volar aspect of the distal forearm using standard commercially available solutions of kiwi fruit, (Alyostal, France), latex (1 in 10 w:v), birch, house dust mite, egg albumin, hazelnut, and cow's milk allergens (all from ALK-Abelló). Prick to prick testing was performed using fresh flesh of kiwi, apple, banana and avocado. There was no standardization of which part of the pulp the fruit was taken from. A negative control of saline 0.9% and a positive control of histamine 10mg/ml were used. The reaction was regarded as positive if the mean wheal diameter was at least 3mm in the presence of appropriate reactions to the positive and negative controls.

5.5 Total and Specific IgE

Serum was tested for total IgE and specific IgE to kiwi fruit, latex and avocado using the CAP system (Pharmacia, Uppsala, Sweden) in the immunology laboratory, Southampton University NHS Trust Hospital. Remaining sera was stored at -80˚ C for future study.

5.6 Food challenges

A typical kiwi fruit weighs 60g. Double blind food challenges proved difficult to interpret in some subjects, and the recipe and methodology changed in an attempt to overcome this. Adequate blinding was confirmed by non-allergic volunteers.

Recipe 1: Peeled, pureed pulp of kiwi fruit, including seeds, was masked in a sorbet or ice cream vehicle (at room temperature) containing liquid food colouring. 4 placebo doses were randomly dispersed with 9 active doses in incremental doses of kiwi fruit (1mg, 10mg, 50mg, 100mg 500mg 1000mg, 2000mg, 4000mg, 8000mg). The doses were given to the subject at intervals of 15 minutes, whilst the subject wore a nasal clip.

Recipe 2: Peeled, pureed pulp of kiwi fruit, including seeds, was masked in yogurt containing a mix of orange pulp, tea leaves and liquid food colouring. 3 placebo doses were randomly dispersed with 4 active doses in incremental doses (2.5g, 10g, 20g, and 60g). The doses were given to the subject at intervals of 30 minutes.

One subject who was allergic to the contents of the vehicle for blinding had an open challenge. Subjects who had a negative or inconclusive DBPCFC were offered an open challenge with a whole kiwi. In addition to being seen by a clinician, subjects had facial photographs prior to the challenge, and following the development of visible signs. A dietician and clinician reviewed the challenge signs and symptoms data. Challenges were considered positive if the subject had subjective symptoms which conformed to the pattern of active/ placebo doses, or if they developed an objective sign e.g. urticaria, angioedema, or a fall in peak expiratory flow. The open challenge was only considered positive if the subject developed physical signs indicative of an IgE mediated response.

5.7 Control subjects

Skin tests and specific IgE to kiwi fruit were measured in 5 atopic and 5 non-atopic controls who eat kiwi fruit with no adverse symptoms.

5.8 SDS-PAGE

SDS-PAGE analysis was used to determine the protein profile of an extract of fresh kiwi fruit made using the methodology of Pastorello[10] and a commercially available kiwi skin test extract (Alyostal, France). Protein content of the two extracts was measured by the Lowry method. Samples were run in 12% NuPage gel (Invitrogen) with 40µg, 20µg, 10µg and 5µg of extract per lane. Reference markers with known molecular weight (Mark 12 unstained standard, Invitrogen) were run in the same gel. Following electrophoresis the gel was fixed and then stained with Brilliant Blue G Colloidal concentrate.

5.9 Zespri Gold

We present a case report of one boy with OAS who reported symptoms on eating Zespri Gold, but who eats green kiwi fruit with no problems. He was investigated by skin testing and a DBPCFC using recipe 2. Five subjects, who had positive DBPCFCs with green kiwi fruit, were invited to return for further investigations using Zespri Gold (recipe 2).

5.10 Statistics

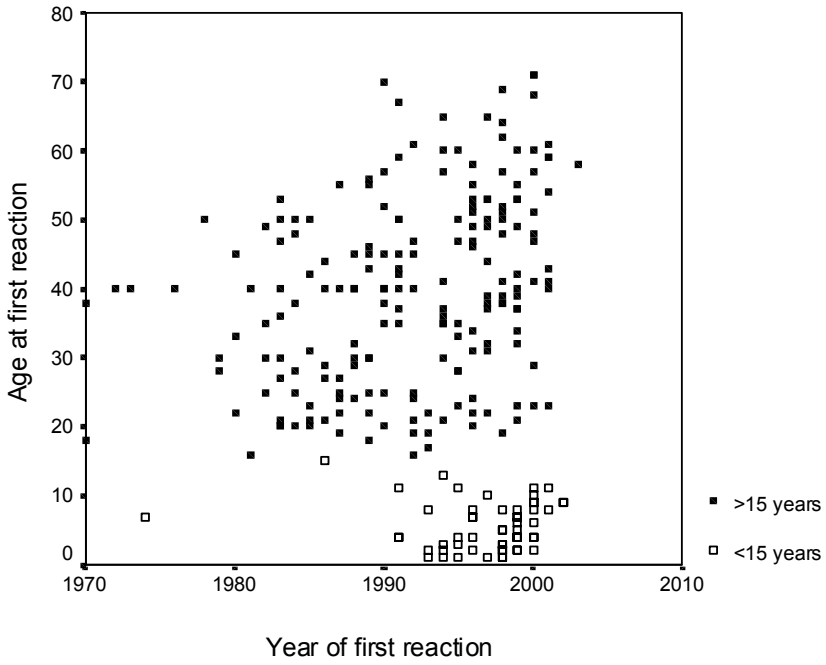
Data were doubly entered in SPSS for Windows (SPSS version 10.0, Chicago, USA), and were analyzed using Pearson's X^2 test and Fisher's exact test where appropriate. All reported p values are two-tailed.

6 Results

6.1 Questionnaire

Two hundred and ninety one questionnaires were returned, but 18 were excluded because the symptoms were not suggestive of IgE mediated kiwi fruit allergy. Two hundred and seventy three valid questionnaires were therefore analyzed, 189 (69%) of which were from female respondents. The age range was 5 months to 86 years (mean 38.8 years, sd 21.1 years). The age of subjects at the time of their first reaction ranged from 4 months-71 years (mean 31.5; sd 18.9). At the time of their first reaction 33 (13%) of the subjects were less than five years of age and 191 (69%) were more than 15 years. Respondents reported very little allergy to kiwi fruit in the 1970s, particularly in the now adult population who were children at the time. Reports of allergy developing were increasingly common in the 1980s, again predominantly in adults. It was not until the 1990s that kiwi fruit allergy was commonly reported by children and young infants (Figure 1).

Figure 1. *The development of allergy to kiwi fruit in adults and children since 1970. Each point represents one subject plotted in the year of their first reaction, and their age at that time.*



Seventy three percent of children of 5 years or less reacted on their first known exposure to kiwi fruit in comparison to only 21% of subjects over the age of 15 years (Pearson's X^2=33.3, P<0.001). 64% subjects reported immediate (< 5 minutes) symptoms on contact with the fruit during their first reaction. 90% of

subjects had reactions within 30 minutes and only 3% reported a delay of more than an hour. All subjects with delayed reactions had mild symptoms.

Associated allergies Kiwi allergy in this UK population was associated with self-reported latex allergy (9%) and allergies to avocado (5%), banana (6%), apple (6%), grass pollen (29%) and tree pollen (23%). Allergies to allergens not known to cross react with kiwi fruit were also common. Commonly reported co-existing allergies included peanuts (14%), tree nuts (17%), milk (6%) and egg (8%). Children under the age of 5 years were particularly likely to have a strong atopic predisposition. 90% had been treated for asthma, eczema or hay fever. 19 of 33 young children (58%) reported peanut allergy, 15 (45%) tree nut allergy, 5(15%) milk allergy, and 10 (30%) egg allergy.

Allergic symptoms The symptoms reported on first and most recent reactions are summarised in Table 1. Localised oral reactions were the most commonly described symptoms. Severe symptoms were reported on the first reaction by 18% of respondents and by 12% of subjects on their most recent reaction. Wheeze was the most common severe symptom described, but severe reactions were no more likely to occur in subjects who had been treated for asthma (Pearson's X^2=2.00; p=0.40). Severe symptoms were significantly more likely to occur in young children (<5 years) than adults over 15years (Pearson's X^2=7.1; p=0.008) (figure 2). If subjects had severe symptoms on their first reaction, the most recent reaction was also likely to be severe. However, more than 30% of those who initially had a mild reaction subsequently had moderate or severe symptoms (Table 2)

Table 1 *Reported symptoms on first and most recent reaction. Subjects could report more than one symptom.*

Symptom	**First reaction N=276 (%)**	**Most recent reaction N=206 (%)**
Tingling, sore mouth	180 (65)	144 (69)
Throat tightening/ difficulty swallowing	125 (45)	103 (50)
Swelling of lips/ tongue	106 (38)	77 (37)
Face swelling	74 (27)	42 (20)
Rash	60 (22)	31 (15)
Breathing difficulty	49 (18)	33 (16)
Vomiting	49 (18)	35 (17)
Abdominal pain	46 (17)	37 (18)
Wheeze	39 (14)	26 (13)
Collapse	13 (5)	7 (3)
Cyanosis	9 (3)	3 (1)

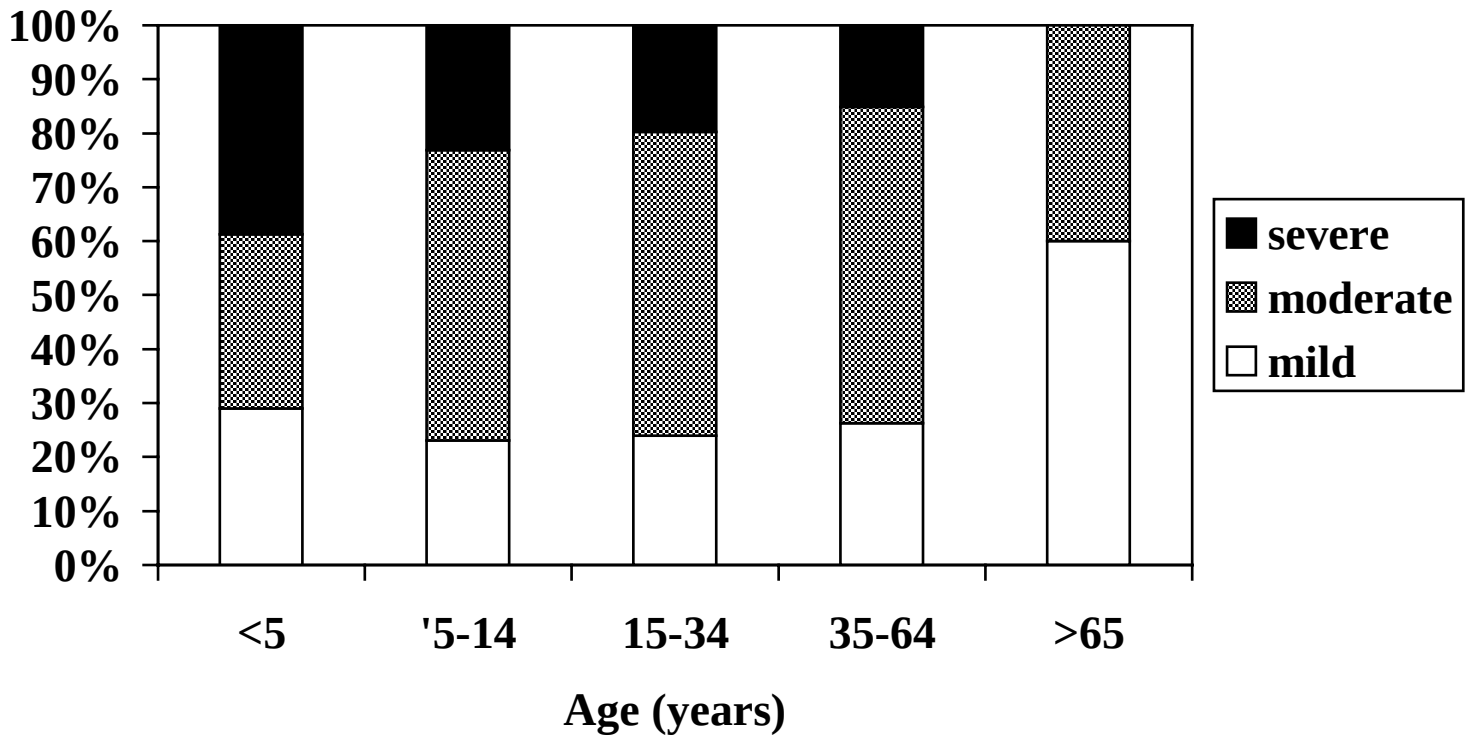
Table 2 *Comparison of first and most recent reactions*

		First reaction		
		Mild	Moderate	Severe
Most recent reaction	Mild	46	7	1
	Moderate	16	86	5
	Severe	4	5	23

Data missing for 13 subjects

Treatment of reactions 15% of respondents had attended hospital on at least one occasion during an acute reaction to kiwi fruit, 3% had been admitted overnight and 3 subjects (1%) had been admitted to an intensive care unit. 31% of subjects had used antihistamines to treat a reaction to kiwi fruit, 7% injected epinephrine, 6% a bronchodilator and 4% steroids.

Figure 2. *Severity of first reactions according to age at the time of first reaction.*



6.2 Food challenges

Fifty subjects attended our centre for food challenge but four either did not start or did not complete the challenge for reasons other than allergy (anxiety x2, challenge recipe not tolerated x2). Therefore oral challenges were completed in 46 subjects with self-reported allergy to kiwi fruit (45 DBPCFC +/- open challenge, 1 open food challenge). Subjects ranged in age from 6 to 64 years (mean 33 years; sd 18 years), and included 12 children under the age of 15 years. 26 (57%) of subjects had a history of symptoms localized to the oral mucosa; the remaining 20 (43%) subjects reported systemic reactions including urticaria, angioedema, and wheeze.

30 subjects received recipe one, 15 recipe two and 1 child had an open challenge. In total, 23 DBPCFCs were positive (Table 3). 12 were negative and 8 challenges were inconclusive. The inconclusive challenges were in subjects with subjective symptoms. They had symptoms that did not strictly correlate with the placebo/ active dose regime, but who we believed were probably positive. The child who only had an open challenge developed swelling of the lips and facial erythema after 2.5g of kiwi (25mg protein). In addition three subjects developed objective clinical signs suggestive of allergy during oral challenge following a negative or inconclusive DBPCFC (Table 3). Therefore 23 subjects (total 50%) had kiwi fruit allergy confirmed by DBPCFC, and a further 4 (59%) developed signs suggestive of IgE mediated food allergy during an open challenge. We had hoped that recipe 2 would eliminate the inconclusive results in subjects with OAS, but this was not the case with 2 of 15 subjects having inconclusive symptoms with this improved recipe.

Table 3*. Clinical manifestations during challenges*

Clinical signs and symptoms	**Number of subjects**
DBPCFC	
Isolated oral symptoms	9
Facial swelling and oral symptoms	4
Urticaria +/- angioedema	3
Drop in peak flow, urticaria and facial oedema	1
Wheeze	1
Urticaria and abdominal pain	3
Erythema and oral symptoms	1
Erythema and abdominal pain	1
Open challenge	
Swelling of the lips and facial erythema	1
Swelling of tongue (observable)	1
Urticaria + angioedema	1
Stridor and dysphonia	1

6.3 Skin tests

Of the 23 subjects who reacted on DBPCFC, only 1 had a negative skin test on prick to prick testing with fresh fruit. However, there was a high rate of false positive skin test results (Table 4). In this population, prick to prick skin tests had a sensitivity of 95% and a specificity of 31%. Skin tests were negative in the 5 atopic and 5 non atopic controls.

19 subjects were also tested with a commercial kiwi skin test extract (Table 4). The commercial solution would appear to be significantly less sensitive, but the number of false positive skin test reactions was reduced.

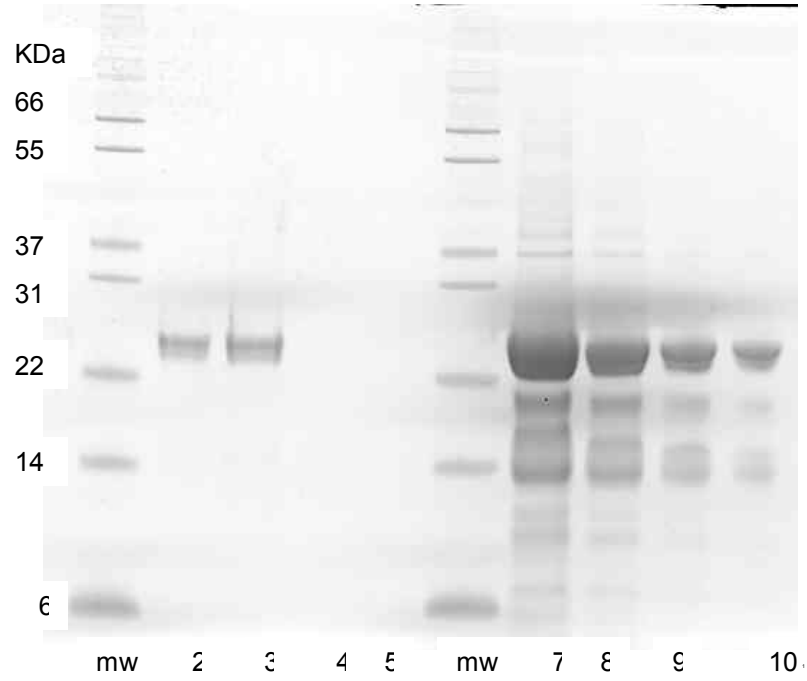
Protein analysis of the skin test solution A gel was run to compare the commercial extract with fresh kiwi fruit. Protein content of the fresh kiwi extract was 0.49 mg protein /mg extract (49%), and the skin test solution contained 50mg protein /ml. The protein profiles of the two extracts are shown in Figure 3. The fresh fruit extract had protein bands at ≈14, 15, 17, 22, 36 and 40 kDa. The skin test solution only had a band at ≈23 kDa. The study was repeated running neat skin test solution with similar results (not shown).

Associated skin sensitization The most common allergens causing positive skin tests in the forty six subjects were birch pollen (14) house dust mite (24), apple (6), banana (4) and avocado (5).

Table 4 *Skin test results with fresh kiwi fruit pulp in 45 subjects (prick to prick) and with a commercial extract in 19 subjects*

		DBPCFC result		
		Positive	**Negative**	**Inconclusive**
Fresh kiwi SPT wheal	**<3mm**	1	4	1
	>=3mm	22	9	8
Commercial kiwi SPT wheal	**<3mm**	3	2	2
	>=3mm	8	1	3

Figure 3. *Kiwi skin test extract (Alyostal, France) lanes 2, 3, 4 & 5; fresh kiwi extract lanes, 7, 8, 9 & 10; molecular markers lanes 1 & 5. Samples were run in 12% SDS-PAGE gel with 40µg, 20µg, 10µg and 5µg of extract per lane*



6.4 Specific IgE

Serum was collected from 118 subjects. Specific IgE was detectable in 42 (36%) of subjects with self reported kiwi allergy. The level of specific IgE did not correlate with reported severity of symptoms or age. In the challenge population the sensitivity of the test was 60% and the specificity was 83%. The results of Specific IgE in 45 subjects who had undergone a DBPCFC challenge are shown in Table 5. One atopic control had a kiwi-specific IgE of 0.7. A challenge was not performed because this control subject reported regular consumption of kiwi fruit with no problem. None of the other 9 control sera had any detectable kiwi sIgE.

Table 5 *Specific kiwi IgE results*

		DBPCFC result		
		Positive	**Negative**	**Inconclusive**
Specific IgE to Kiwi Fruit	**<0.35**	9	10	7
	0.5	3		
	0.6	2	1	
	0.67	1		
	0.9	1		
	1.0		1	
	1.2	1		
	1.8	1		
	1.9	1		
	2.3			1
	2.35	1		
	2.9	1		
	3.6	1		
	6.8	1		
	7.7			1

6.4 *Zespri Gold-case report (positive history and SPT but negative DBPCFC)*

A 13 year old boy complained of symptoms when eating Zespri Gold He complained of severe itching of the oral mucosa within minutes of eating approximately half a fruit. He also reported symptoms of OAS when eating nectarine, strawberry, apple, pear, peach, hazelnut and cherries. Interestingly he frequently eats green kiwi fruit without symptoms. He has positive SPT to birch pollen but does not have hay fever. He had positive SPT to green and gold fruit (Table 6). Serum specific IgE to green kiwi fruit was not detectable (test not available with Gold). He had a negative DBPCFC to Zespri Gold outside the birch pollen season.

Table 6 *Skin tests results of subject reporting symptoms of OAS to Zespri Gold*

TEST SUBSTANCE	REACTION	TEST SUBSTANCE	REACTION
NEGATIVE CONTROL	0	Avocado (fresh)	1
POSITIVE CONTROL	3	House dust mite (ALK)	0
Green kiwi (fresh)	3	Nectarine (fresh)	7
Gold kiwi (fresh)	4	Strawberry (fresh)	4
Green kiwi (commercial solution)	0	Apple (fresh)	3
Birch Pollen	4	Banana (fresh)	0
Tomato (fresh)	2	Hazelnut (ALK)	3

6.5 Zespri Gold- clinical investigations in subjects allergic to green fruit

Four of the five subjects who had confirmed allergy to green kiwi fruit, had a positive DBPCFC to Zespri Gold (Table 7). One child had moderately severe symptoms to Zespri Gold on the first dose (2.5g fruit/ 25mg protein), with lip swelling, abdominal pain and vomiting. The reaction was identical to that experienced at the same dose in a challenge to green fruit. One subject who reacts systemically to green fruit had symptoms of oral pruritus with Zespri Gold. Two subjects with OAS to green kiwi had oral pruritus with Zespri Gold, but both commented that the itching was less intense than that experienced during the DBPCFC with green fruit.

Table 7 *Results of clinical investigations to Zespri Gold in 5 subjects with known allergy to green fruit*

ID	Reported reactions to green	Green SPT (mm)	Gold SPT (mm)	Birch Pollen SPT (mm)	Specific IgE Green	Green challenge	Gold challenge
9 year male	Rash, GI	7	0	0	1.9	Rash and abdo pain	Negative
52 year male	OAS	13	12.5	0	6.8	Oral pruritis	Oral pruritus (itching less intense)
10 year girl	Anaphylaxis	4	0	4	3.6	Lip swelling, urticaria	Oral pruritus
10 year girl	GI, facial and oral swelling	8	8	5	2.38	Lip swelling, abdo pain	Lip swelling, abdo pain
44 year male	OAS	6	2	0	0.5	Oral pruritis	Oral pruritus (itching less intense)

7 Discussion

7.1 *Questionnaire*

Kiwi fruit was introduced into the UK diet in the late 1960s/ early 1970s. The retrospective questionnaire survey shows that the first people to develop allergy to kiwi fruit were adults, and despite an increasing consumption of the fruit throughout the 1970s and 80s, it was not until the mid 1990s that a large increase in the number of children developing kiwi allergy started to occur. The large number of self selecting respondents to this study suggests that kiwi fruit allergy in the UK may be more common than previously recognized by the medical profession. Until now clinical knowledge about kiwi allergy has depended on reports of small groups of patients and scientific information has concentrated on cross-reactivity between birch pollen and latex allergens. Some of the studies investigating cross reactivity have specifically recruited subjects with OAS. This may have led to misconceptions that kiwi fruit allergy is almost exclusively a 'mild' allergy presenting as a result of cross-reactivity, with symptoms of oral allergy syndrome. The reasons why children did not start developing kiwi fruit allergy until some 30 years after its introduction into the diet will require further investigation. It is possible that kiwi fruit has only been consumed by children in the UK in recent years. Children in this study were reported to react differently to kiwi than the adults in the study. Children were more likely to react on their first known exposure to kiwi fruit, whilst adults commonly reported multiple exposures before developing symptoms. Children who reacted to kiwi fruit were likely to be strongly atopic, with 90% reporting atopic disease, and 58% reporting allergy to peanuts. Children were also more likely to have severe reactions. The youngest subject in our study had a severe anaphylactic reaction at 4 months of age, requiring resuscitation with epinephrine and oxygen, having eaten kiwi fruit prepared using a recipe provided by a Health Visitor. The severity of reactions experienced by young children may simply reflect that they represent a population with a strong and increasing allergic predisposition, and as such are more likely to have severe reactions. Alternatively they may be recognizing different IgE binding proteins, children and adults may consume different preparations of the fruit or perhaps early life sensitization predisposes to more severe reactions. Kiwi fruit has only been easily available in the UK for the past 20-30 years, and the adult population will therefore not have had exposure to the allergen in early life. The striking clinical differences between adults and children with kiwi fruit allergy require further evaluation.

The finding of severe reactions occurring more frequently in younger children is unlike that which exists in peanut allergy, where the deaths predominantly occur in later adolescence and early adulthood, and more severe symptoms are reported in the older age groups[31] One could speculate that if we had looked at the range of severity of reaction to peanuts only 30 years after it had been introduced into the UK diet, then we might have seen the same pattern seen in this study. If there is a cohort effect, reflecting a critical age of first exposure to a food allergen, then in 20 years time the most severe reactors to kiwi fruit will be in adolescence and early adulthood, possibly leading to fatalities due to kiwi allergy, which have not been seen to date. This has significant implications for the way in which one monitors the development of

allergy to a new food. It would be wrong to assume that the food is not particularly allergenic just because all the first set of reactors have mild reactions.

The clinical association of pollinosis with allergy to fresh fruit including kiwi is well-recognised[32]. Initial respiratory sensitisation results in IgE antibodies to pollen proteins which are homologous to those found in some fruits or vegetables. For example, antigens in birch pollen and apples share allergenic epitopes leading to cross reactivity that may cause clinical symptoms of OAS when a birch pollen allergic subject eats an apple[27]. Many allergens in kiwi fruit are readily digested by simulated gastric fluid[33], and per oral sensitisation to these unstable allergens is unlikely. However, pre-sensitisation by inhaling birch pollen allergens could predispose to allergic symptoms to kiwi fruit. The lability of the kiwi allergens may explain why allergic reactions are restricted to the oral cavity (OAS) in some patients with pollen allergy. However, in our study population, subjects with seasonal rhinitis, or reporting allergy to pollens, were no less likely than the rest of the study population to have systemic symptoms. Further work is required to determine why some subjects react with symptoms localised to the oral mucosa, while others have severe systemic reactions. Perhaps those with oral allergy syndrome are indeed reacting to labile allergens that cross-react with allergens in pollens, whilst the systemic reactors are atopic individuals whose pollen allergy coexists with allergy to stable kiwi allergens.

7.2 Skin Tests

Allergy to kiwi fruit is increasingly commonly reported, but the role of clinical investigations has received little critical evaluation. Skin testing with fresh fruit is the most common clinical investigation reported in case reports and series[2;5;7;8;11;19] the main limitation being that skin testing with fresh fruit lacks standardisation. Previous reports suggest that prick to prick with fresh kiwi or skin testing with home made kiwi extract is extremely sensitive in subjects in whom kiwi allergy was suspected. We too have found prick to prick testing with fresh kiwi fruit to be sensitive with only one of 23 subjects having a negative skin test but positive challenge. Although highly sensitive, some studies have described poor specificity, with skin test reactivity in subjects without symptoms of allergy to kiwi fruit, but clinical reactivity to pollens[8] or latex[21;22]. In this study sixty nine percent of subjects who had a negative DBPCFC had a positive skin test. However, given the problems of confirming whether oral challenge was positive or negative in some of our subjects, the specificity in this study is a worse case scenario. Skin tests were negative in all our atopic and non-atopic controls.

Commercial skin test extracts are significantly less sensitive. In a study from Spain of 43 subjects with kiwi allergy, only 40% of subjects had positive skin reactions to one commercial extract, and 28% to another make of skin test solution- all subjects had had positive reactions when tested by prick to prick with fresh kiwi[18]. We have also found that a commercially available extract was less sensitive than fresh fruit, and that the wheals tended to be smaller. Having run a gel to compare the proteins in the commercial extract and an extract of fresh kiwi fruit, it is evident that most protein bands are absent from the commercial extract, presumably reflecting the lability of kiwi fruit proteins.

7.3 Specific IgE

Reports to date are contradictory about the role of measuring specific IgE to confirm kiwi fruit allergy. Although positive in some case reports of patients with kiwi allergy [4-6;19], other authors have found sIgE measurement unhelpful[3;20]. We found the test to have poor sensitivity. Thirty nine percent of subjects who reacted on DBPCFC had a negative specific IgE. It might well be that the CAP system has a similar problem with the lability of allergens as we identified in the skin test solution.

7.4 Food Challenges

Double blind food challenges (DBPCFC) are the 'gold standard' for confirming food allergy [34], but blinded food challenges have rarely been used in the context of oral allergy syndrome. Our recipes were designed to challenge all subjects with reported allergy to kiwi fruit, both subjects with oral allergy syndrome, and those with more generalised reactions. We started challenges at a low dose, reflecting the amount of kiwi some subjects had indicated as the minimum dose that causes them to react. Some subjects with OAS complained of severe oral symptoms that did not correlate with the dosing schedule. These subjects may well not be allergic to kiwi fruit, but our subjective feeling as clinicians is that some of the subjects with oral allergy syndrome were experiencing tolerance, persistence of symptoms from one dose beyond the next or fluctuating severity of symptoms, that did not necessarily coincide with the doses. These subjects were labelled negative or inconclusive in the absence of objective signs, but the subjects were insistent that they experienced symptoms similar to their "usual" symptoms both during the closed and open challenge. We changed the protocol to increase the dose increments and prolong the intervals between doses to half an hour. We also changed the vehicle because subjects complained of feeling nauseated by the large volumes of sorbet. Despite these changes we continued to have inconclusive results, particularly in subjects with oral allergy syndrome. This highlights a need to tailor challenges to the individual. Many of the people with systemic reactions, particularly young children, reacted at low doses. However, in order to maximise the safety of test for systemic reactors, subjects with OAS also started at low doses with relatively small increments. We now believe that this group would have been characterised better using even larger increments with longer intervals (e.g. an hour) between doses.

7.5 Zespri Gold

Green kiwi fruit, *Actinidia deliciosa,* has been widely available throughout the world for several decades, and allergic reactions to this fruit are not uncommon. *Actinidia chiensis* has only been exported from New Zealand since 1998, and imported to the UK since 2000. To date there have not been any published reports of allergy to the gold fruit. Zespri Gold was not submitted to the regulatory authorities for a safety assessment, despite being a new species of fruit. In the absence of a post marketing surveillance strategy for new foods it will prove difficult to monitor reactions to newly introduced food types. Our case report subject was referred to us by the Anaphylaxis Campaign. We are also aware of another person who has reported a reaction to Zespri Gold in the UK. The individual contacted Tesco (who sold the fruit), and in

the absence of a UK surveillance system, Tesco reported the case to Zespri in New Zealand. Despite attempts to receive information from Zespri we have been unable to get any clinical information. In a response (30.10.2001) to Tesco, Zespri replied stating that the case was the first report of an allergic reaction to Zespri Gold.

We have confirmed that Zespri Gold is an allergenic fruit, and subjects allergic to green kiwi fruit are at risk of reacting to Zespri Gold. The symptoms in some subjects were milder to Zespri Gold than the green fruit. Our preliminary work looking at in vitro immunogenicity of Zespri Gold (T07038) suggests that one of the major allergens from green kiwi is missing in Zespri Gold. It might be that removing a major allergen will successfully reduce, but not eliminate the allergenicity of a food. Indeed, one of our subjects reacted moderately severely to both fruits at the same low dose. The EU-funded project SAFE (QLK1-CT-2000-01394) is currently identifying apple varieties with intrinsically low allergen levels (currently Mal d 1 and Mal d 3) in an attempt to provide breeders with guidelines to select new cultivars with low allergen levels.

7.6 Limitations

This was not an epidemiological study, and therefore we can make no assessment of the prevalence of the allergy to green or gold kiwi. Perhaps the allergy is more common than appreciated given the numbers of people who have contacted the study, but an epidemiological study is required to address this. The volunteers are self selected, which may explain the greater number of adult females, as well as a fairly high percentage of subjects with severe symptoms. As with all questionnaire studies, there is likely to be recall bias. However, this report highlights important features of kiwi fruit allergy which further our clinical progress in the field.

7.7 Summary

This study has clearly shown that allergy to kiwi fruit is an important problem, with most severe reactions occurring in young children. A significant number of subjects have required resuscitation with epinephrine, and 3 adult subjects had been admitted to intensive care following ingestion of kiwi fruit. Although kiwi allergy is known to occur as a consequence of cross reactions with pollens and latex, half of our population was only allergic to kiwi fruit. These findings emphasize the need for further studies to explain the apparent increasing prevalence of this allergy, and to explain the differences between reactions in children and adults.

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9 Appendix- Questionnaire