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ORIGINAL ARTICLE

Analysis of α-lactalbumin-, β-lactoglobulin-, and casein-specific IgE among children with atopic diseases in a tertiary medical center in northern Taiwan

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KEYWORDS
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Specific IgE

Background: Cow's milk allergy is the first manifestation of the allergic march, because cow's milk proteins are the first foreign proteins consumed in large quantities by infants. The aim of this study was to determine which of these proteins causes the greatest sensitization in Taiwanese individuals, and its correlation with atopic patients.
Methods: We retrospectively reviewed the medical records of atopic patients who were treated at the National Taiwan University Hospital from January 2009 to March 2011. A total of 190 patients were enrolled and tested for isolated cow's milk protein (α-lactalbumin, β-lactoglobulin, and casein) sensitization. These patients were further divided into atopic dermatitis and non-atopic dermatitis subgroups, and also grouped by age. Differences in sensitization to cow's milk proteins between groups were analyzed using the non-parametric Mann–Whitney *U*-test.
Results: The sensitization rate to α-lactalbumin-specific IgE was 60%, followed by β-lactoglobulin (46.84%) and casein (40.53%). The levels of specific IgE antibodies against α-lactalbumin, β-lactoglobulin, and casein were higher in patients with atopic dermatitis (1.8, 1.19, 0.95 vs. 0.77, 0.55, 0.40 kUA/L). The younger age group had higher mean specific IgE antibodies against cow's milk proteins than the older age groups.
Conclusion: There was a different distribution of cow's milk protein sensitization in Taiwanese patients in our study, with α-lactalbumin being most common as compared to casein in Western

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countries. Among the patients with cow's milk sensitization, the atopic dermatitis group had significantly higher cow's milk protein-specific IgE antibodies as compared to the non-atopic dermatitis group.

Introduction

Cow's milk allergy is the most frequent food allergy in childhood. It is the first manifestation of allergic march, because cow's milk proteins are the first foreign proteins consumed in large quantities by infants when breast feeding is not possible. Most cases of IgE-mediated milk allergy appear in young children in the first 6 months of life, and in most cases spontaneously disappear by the third year of life.[1,2] In Western countries, the prevalence of cow's milk allergy is reported to be 0.3–7.5%.[2–7] One report from Taiwan showed the prevalence of food allergy (including egg whites, milk protein, peanuts, and shrimp) at 6, 18, and 36 months old; however, the specific prevalence of cows' milk allergy was not shown.[8]

Cow's milk consists of casein and whey. Casein protein comprises 80% of cow's milk, and four different forms are found (alpha, beta, gamma, and kappa). Whey protein makes up 20% of cow's milk, with β-lactoglobulin (BLG) and α-lactalbumin (ALA) being the most abundant. Minor proteins such as immunoglobulins, bovine serum protein, and lactoferrin make up the remaining components of cow's milk.[9] Studies in Italy, Japan, and Spain have shown that the most common milk protein provoking an allergic reaction is casein.[10–12] However, no documented data has been noted in Taiwan.

The aim of this study was to investigate which milk protein accounts for the highest levels of sensitization in Taiwan. By reviewing the charts of patients who received ImmunoCAP(I) or (III) tests (which include whole cow's milk sensitization) from January 2009 to March 2011 at National Taiwan University Hospital, we further performed ImmunoCAP testing for isolated cow's milk proteins ALA, BLG, and casein sensitization. In addition, we investigated the correlation of milk sensitization to allergic diseases and different ages of the patients.

Patients and methods

Patients

A total of 3430 patients received ImmunoCAP test at National Taiwan University Hospital from January 2009 to March 2011. Among these patients, 262 had whole-milk-specific IgE higher than 0.35 kUA/L as detected by an ImmunoCAP system (Phadia, Uppsala, Sweden). The inclusion criterion was the presence of atopic diseases [including asthma and/or rhinitis and/or atopic dermatitis (AD) and/or urticaria and/or clinically reactive intestinal symptoms].[1] Exclusion criteria were an uncertain diagnosis and incomplete medical records.

Taken from the original number of cases reviewed, 190 patients who visited the outpatient clinics of the Departments of Pediatrics, Otolaryngology, Dermatology, and Internal Medicine for atopic diseases were enrolled in this study. Most patients were selected from the Pediatrics and Otolaryngology Departments.

The 190 patients were divided into two groups: the AD group, consisting of patients who were diagnosed with isolated AD or AD with other atopic diseases ($n = 44$); and the non-AD group, patients diagnosed with other atopic diseases ($n = 146$). We also grouped the patients based on age: those 3 years old and below; 4 to 18 years old; and above 18 years old.

All of the patients' serum samples were further tested for isolated cow's milk proteins, ALA-, BLG-, and casein-specific IgE antibodies using an ImmunoCAP system (Phadia).

This study was approved by the IRB in Research Ethnic Committee of the National Taiwan University Hospital on July 20, 2011 (IRB number: 201107008RC).

Determination of total IgE and allergen-specific antibodies

We analyzed the stored serum of the 190 patients for ALA-, BLG-, and casein-specific IgE using the ImmunoCAP system. All serum samples were stored at −20°C before being used. Any sensitization was regarded as positive when specific serum IgE levels were noted as being greater than 0.35 kUA/L against the tested isolated cow's milk protein (ALA, BLG, or casein). The total serum IgE level was also measured with the ImmunoCAP system for all patients.

Statistical analysis

The results were shown as mean values. We compared the values of mean levels of IgE against isolated cow's milk proteins between age groups and AD/non-AD groups by using the nonparametric Mann–Whitney *U*-test. The distribution of serum specific IgEs against an individual cow's milk protein and different groups is shown using a box plot. Correlations between specific IgEs against two different proteins were calculated using Pearson's correlation. All analyses were performed using SPSS software version 18.0 (SPSS Inc., Chicago, IL, USA).

Results

Of the 3430 patients who received ImmunoCAP tests at the National Taiwan University Hospital from January 2009 to March 2011, 262 had class 1 or higher whole-milk-specific

IgE (≥0.35 kUA/L). Of these 262 patients, 190 were included in this study (after excluding those whose serum levels were not available and those with incomplete medical records). Serum total IgE was also recorded, with mean level of 831.4 kU/L. There was no significant difference between serum total IgE and cows' milk specific IgE.

Positive sensitization rate to milk allergens and serum milk-allergen-specific IgE levels

Of the 190 people who had class 1 or higher whole cow's milk specific IgE, 114 (60%) had sensitization to ALA, 89 (46.84%) had sensitization to BLG, and 77 (40.53%) had sensitization to casein. The average isolated cow's milk protein specific IgE levels were 1.012 for ALA, 0.694 for BLG, and 0.524 kUA/L for casein. The level of IgE against ALA was significantly higher than those against BLG and casein ($p = 0.0053$ and $p < 0.001$, respectively); however, the level of IgE against BLG was not significantly higher than that against casein (Fig. 1). Thirty-nine (20%) patients had none of the three protein-specific IgEs.

Polysensitization

Most of the patients were sensitized to two or more milk proteins. Of the 190 patients, only 62 (32.6%) were monosensitized. Fifty-five (28.9%) patients were sensitized to both ALA and casein, 64 (33.7%) to both ALA and BLG, and 51 (26.8%) to both BLG and casein. Forty-one (21.6%) patients were sensitized to all three allergens. Sensitivities to casein, ALA, and BLG appeared to be closely related (ALA-BLG, $r = 0.635$, $p < 0.0001$; ALA-casein, $r = 0.398$, $p < 0.0001$; casein-BLG, $r = 0.524$, $p < 0.0001$), whereas ALA-BLG and casein-BLG were moderately correlated, and ALA-casein was mildly correlated (Fig. 2). There were no significant correlations between serum total IgE and cow's milk protein specific IgEs.

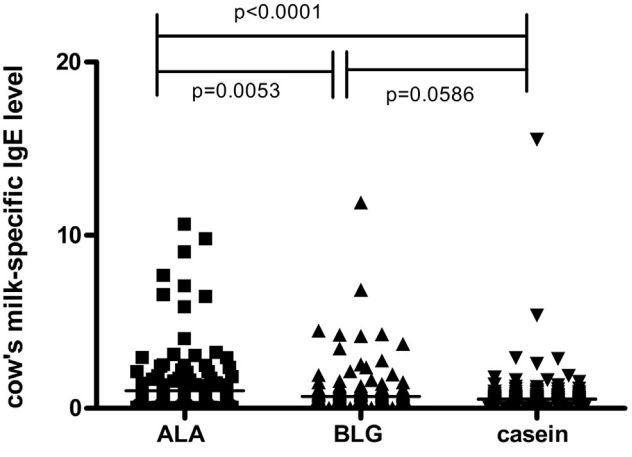

Figure 1. Levels of isolated cow's milk protein specific IgEs in the 190 patients. The mean anti-cow's milk protein specific IgE levels were 1.012, 0.694, and 0.523 kUA/L for ALA, BLG, and casein, respectively. The mean anti-ALA specific IgE level was significantly higher than the anti-BLG and anti-casein specific IgE levels. ALA = α-lactalbumin; BLG = β-lactoglobulin.

Sensitization to other food allergens and Der p/Der f

Patients were also tested for sensitization to other allergens such as soybeans, egg whites, Dermatophagoides pteronyssinus (Der p), and Dermatophagoides farina (Der f). Sensitization to egg white was closely related to all three cow's milk proteins in our study (egg white-ALA: $r = 0.332$, $p < 0.001$; egg white-BLG: $r = 0.448$, $p < 0.001$; egg white-casein: $r = 0.421$, $p < 0.001$). Sensitization to soybean was also related to the three cow's milk proteins, although less closely than with egg whites (soybean-ALA: $r = 0.18$, $p = 0.013$; soybean-BLG: $r = 0.259$, $p < 0.001$; soybean-casein: $r = 0.372$, $p < 0.001$). Sensitization to egg whites and soybeans were also related (egg white-soybean: $r = 0.391$, $p < 0.001$). Neither of the mite allergens (Der p and Der f) was significantly related to any of the three cow's milk proteins.

Distribution of serum milk-specific IgE levels in different age groups

We divided the patients into three age groups: group 1, those 3 years old and below; group 2, those 4 to 18 years old; and group 3, those above 18 years old. There were 49 (25.6%) patients in group 1, 101 (53.2%) patients in group 2, and 40 (21.1%) patients in group 3 (Table 1). The mean levels of isolated cow's milk protein specific IgEs decreased with the increasing age of the patients. The mean cow's milk protein specific IgE levels in the three groups were ALA: 1.72, 0.89, 0.428 kUA/L; BLG 1.24, 0.56, 0.362 kUA/L; and casein: 0.87, 0.43, 0.32 kUA/L (Fig. 3). The mean levels of IgE against ALA in groups 1 and 2 were significantly higher than in group 3 ($p = 0.0042$ and $p = 0.0074$, respectively). The mean levels of IgE against BLG in groups 2 and 3 were significantly lower than in group 1 ($p = 0.0001$ and $p = 0.0007$, respectively). However, there were no significant differences in mean levels of IgE against casein among the three age groups. In addition, there was no significant difference in mean levels of IgE against ALA between groups 1 and 2, and mean levels of IgE against BLG between groups 2 and 3 (Fig. 3).

Distribution of participants according to diagnosis and comparison of serum isolated cow's milk protein specific IgE levels among the two disease groups

The distribution of atopic diseases among the 190 patients is shown in Table 1.

We divided the patients into the AD group and non-AD group as described in the Methods section. There were 44 (23.16%) patients in the AD group and 146 (76.84%) in the non-AD group, and the mean age of the AD group was significantly younger than that in the non-AD group (5.9 vs. 11.8 years, $p = 0.0008$). Patients in the AD group had significantly higher serum total levels of IgE than patients in

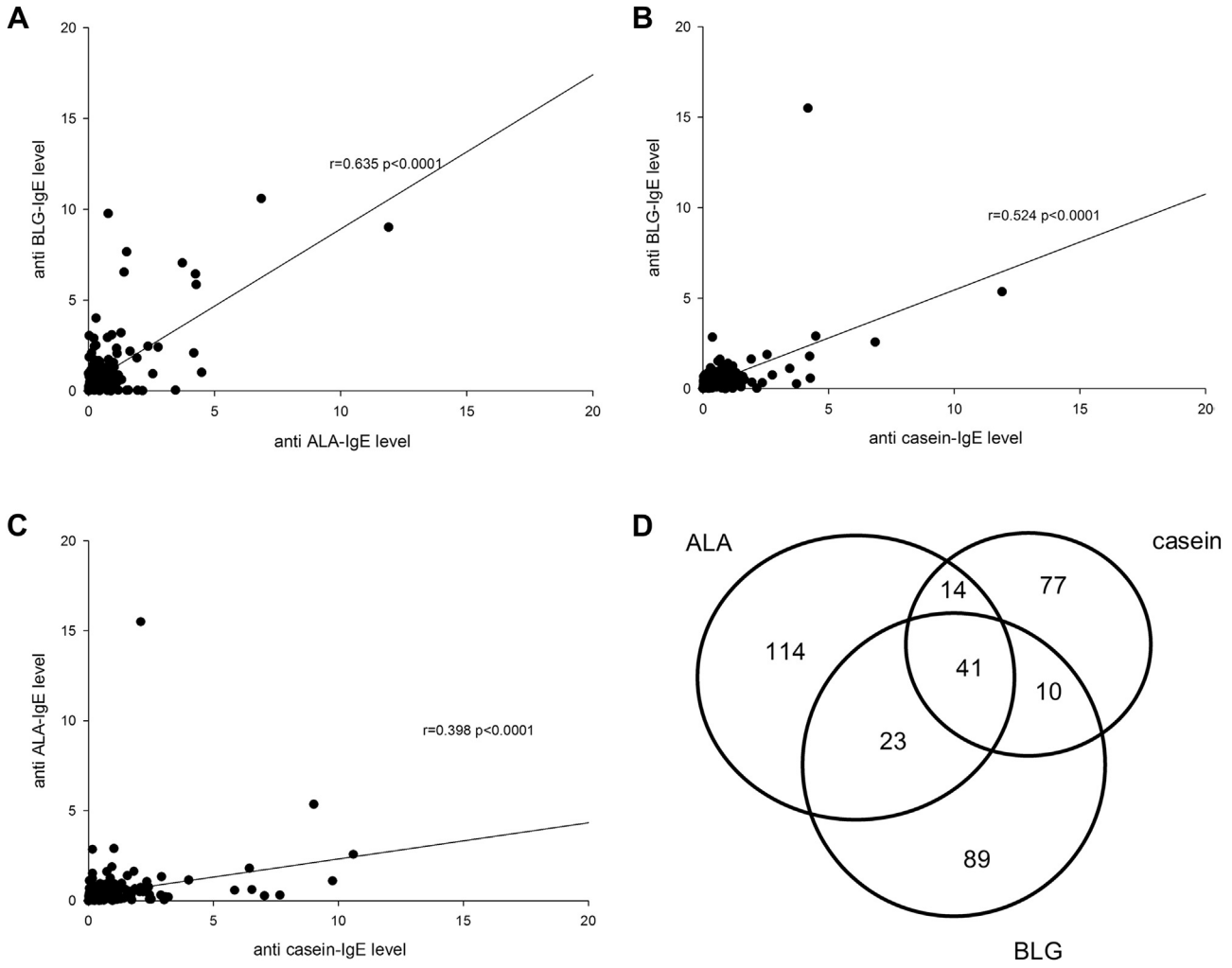

Figure 2. Correlation between isolated cow's milk proteins. (A) Correlation curve between anti-ALA specific IgE level and anti-BLG specific IgE, $r = 0.635$. (B) Correlation curve between anti-casein specific IgE level and anti-BLG specific IgE, $r = 0.524$. (C) Correlation curve between anti-ALA specific IgE level and anti-casein specific IgE, $r = 0.398$. (D) Numbers of patients with sensitization to each of the cow's milk proteins. ALA = α-lactalbumin; BLG = β-lactoglobulin.

the non-AD group (1555.5 vs. 588.3 kU/L, $p = 0.0002$). In addition, patients in the AD group had significantly higher levels of isolated cow's milk protein specific IgEs than those in the non-AD group (1.80 vs. 0.77 for ALA, $p = 0.0229$; 1.19 vs. 0.55 for BLG, $p = 0.0065$; and 0.95 vs. 0.40 for casein, $p = 0.002$) (Fig. 4).

Discussion

The prevalence of atopic manifestations is increasing worldwide, especially in children. Symptoms are most common in Western countries, where approximately one-third of children show symptoms.[13,14] Food allergy is known to provoke AD in a subset of affected children.[15] In Greenhawt's[16] review article, food allergens provoked AD in around 35% of patients. Among the food allergens, cow's milk is one of the most common allergens to cause allergy.[3,16] In Western countries, the prevalence of cow's milk allergy has been reported to range from 0.3% to 7.5% in prospective studies.[2–7] No documented data are available in Taiwan, but the prevalence does not seem to be as high as other studies. The prevalence of cow's milk sensitization (class 1 or higher whole cow's milk specific IgE) in patients who received ImmunoCAP testing at our institution from January 2009 to March 2011 was 7.6% (262 out of 3430 individuals). However, patients who have class 1 or higher whole cow's milk specific IgE do not all have symptoms after ingestion of the cow's milk, and the patients who receive ImmunoCAP testing do not represent the normal population. A previous study showed that about one-third of the Taiwanese population is atopic; hence, the estimated prevalence of cow's milk allergy might be lower than 2.5%.[17]

Studies in Italy, Japan, and Spain have shown that the most common isolated cow's milk protein provoking an allergic reaction is casein.[10–12] In our study, ALA was the most common allergen to cause sensitization among the three cow's milk proteins, and the mean level of ALA-specific IgE was significantly higher than the mean levels of casein-specific and BLG-specific IgEs. This indicated that there is a different distribution of isolated cow's milk

Table 1 Demographics and disease characteristics of patients

N	190
Sex	106 M/84 F
Age (y)	11.8 ± 1.05[a]
Group1[b]	49 (25.8%)[c]
Group2	101 (53.2%)
Group3	40 (21.1%)
Diagnosis	
Allergic rhinitis	107 (56.3%)
Atopic dermatitis	22 (11.6%)
Asthma	9 (4.7%)
AR + AD	16 (8.4%)
AD + asthma	3 (1.6%)
AR + asthma	19 (10%)
AR + AD + asthma	3 (1.6%)
Urticaria	9 (4.7%)
Gastrointestinal upset	2 (1.1%)
Total IgE (kU/L)	831.4 ± 98.4[a]

[a] The data ± indicates the mean value with standard deviation.
[b] Group 1 = 3 years old and below; group 2 = above 3 to 18 years old; group 3 = above 18 years old.
[c] Number in brackets indicates the percentage of case number out of 190 patients.
AD = atopic dermatitis; ALA = α-lactalbumin; AR = allergic rhinitis; BLG = β-lactoglobulin.

protein sensitization in Taiwan when compared to Western countries, where casein is the most common isolated cow's milk protein causing sensitization. Regarding those in the same Asian ethnic group, this difference between Taiwan and Japan is probably due to different geographic

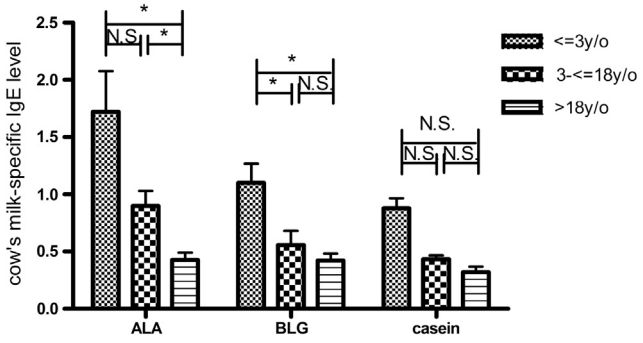

Figure 3. Levels of IgEs against isolated cow's milk proteins by age group. The mean anti-ALA specific IgE level was significantly higher in the ≤3-year-old group than the above 18-year-old group ($p = 0.0042$). The mean anti-ALA specific IgE level was significantly higher in the 3–18-year-old group than the above 18-year-old group ($p = 0.0074$). The mean anti-BLG specific IgE level was significantly higher in the ≤3-year-old group than the 3- to 18-year-old group and the above 18 year-old groups ($p = 0.0001$ and $p = 0.0007$). The mean anti-casein specific IgE levels were not significantly different between the three age groups. *$p < 0.05$. ALA = α-lactalbumin; BLG = β-lactoglobulin; N.S. = not significant.

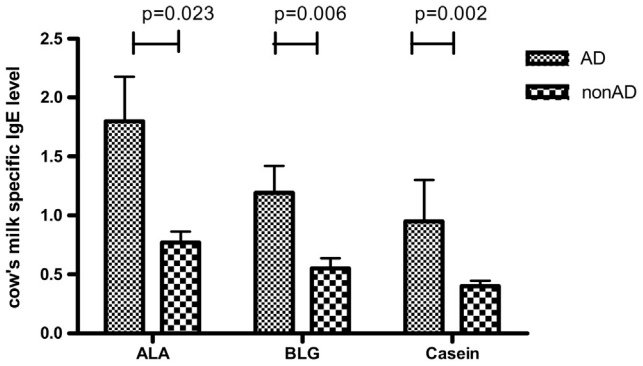

Figure 4. The levels of IgEs against isolated cow's milk proteins in the AD and non-AD groups. Anti-ALA, anti-BLG, and anti-casein specific IgEs were significantly higher in the AD group than in the non-AD group ($p = 0.0229$, $p = 0.0065$, and $p = 0.0020$, respectively). ALA = α-lactalbumin; BLG = β-lactoglobulin.

constitutions or life style discrepancies. But at this time the cause of this discrepancy requires further study to be identified. Thirty-nine (20%) of the 190 patients in our study had none of the three cow's milk protein specific IgEs, which may indicate that they were sensitized to other cow's milk proteins such as lactoferrin, immunoglobulin, or bovine serum albumin.[9]

Most children outgrow their milk allergy by the age of 3.[1,2] Bishop et al[1] prospectively followed 100 children with challenge-proven milk allergy, and tolerance was achieved in 78% of them by the age of 6. However, in a study by Skripak et al,[18] resolution was considerably delayed, and tolerance was achieved in 79% of the children in a specialty practice by 16 years of age. It is believed that the prognosis is worse in cases where cow's protein allergy persisted until 5 years of age.[1,2] In our study, the average level of allergen-specific IgE decreased with increasing age, with those 3 years old and below having the highest mean levels of IgEs against ALA, BLG, and casein. As this is a retrospective study, longitudinal follow-up of the patients' tolerance could not be performed. By utilizing cross-sectional observation, the younger groups were noted as having significantly higher mean anti-ALA- and anti-BLG-specific IgEs than the older groups (Fig. 3). This might indicate that as the patients grow older, the levels of anti-cow's milk protein-specific IgEs decrease. This might support the idea that oral tolerance could be reached at an older age.

Although anti-ALA-specific IgE was not significantly different between group 1 (3 years old and below) and group 2 (3–18 years old), these younger groups had significantly higher anti-ALA-specific IgE than the oldest group (above 18 years old). This might indicate that children have a homogeneous sensitization to ALA before they are 18 years old, and they might outgrow their ALA allergy at a later age compared to BLG. As shown in this study, ALA was the most common milk protein to cause sensitization, and this might indicate that Taiwanese outgrow their milk allergy at a later age. However, the case numbers of the study itself are relatively small, and it is limited to one medical center in Taiwan. As a result, it is difficult to apply these results to all Taiwanese.

Most of the patients in our study were sensitized to two or more cow's milk proteins. Among the 190 patients, only 62 (32.6%) were sensitized to one milk protein. Forty-seven patients (24.7%) were sensitized to two of the three cow's milk proteins, and 41 patients (21.5%) were sensitized to all three milk proteins. Sensitivities to casein, ALA, and BLG appeared to be closely related (Fig. 2) The results with regard to polysensitization and cross-reactivity were similar to Wal et al's study. Polysensitization was noted in a study by Wal et al,[9] where only 26% of the patients were monosensitized. A correlation between ALA, BLG, and casein was also mentioned. Other milk proteins such as lactoferrin, immunoglobulin, and bovine serum albumin were not examined in our study; however, theseproteins showed less of a correlation to ALA, BLG, and casein than that reported by Wal et al.[9]

Specific IgEs for other food allergens, such as egg whites and soybeans, and mite allergens Der p/Der f were also assessed in our study. The food proteins of cow's milk, egg whites, and soybeans were closely related to each other, cow's milk and egg whites were moderately related, and cow's milk was mildly correlated to soybeans (data not shown). However, airborne allergens Der p and Der f were rather irrelevant. This might suggest that cross-reactivity is prominent within the same allergen category, for example, food allergens such as cow's milk, egg whites, and soybeans. In a previous study, most of the children who were sensitized to milk were also sensitized to eggs.[19] In a study of fungi-sensitized patients, 92.5% of the patients were also positively sensitized to other airborne allergens, including mite allergens Der p and Der f.[20] However, the data on other allergens are lacking.

Although allergic rhinitis accounted for most of the atopic diseases in our study (107/190, 56.3%), this may have been caused by selection bias as the most common problem for which patients attended our outpatient department for ImmunoCAP testing is allergic rhinitis. After grouping the patients into those with AD and those with other atopic diseases in our study, the mean levels of IgE of all three cow's milk proteins were significantly different, with the AD patients having higher levels of cow's milk protein specific IgEs. The mean level of serum total IgE was significantly higher in the AD group compared to the other atopic diseases group. A previous study showed that the mean total serum IgE levels in children with cow's milk allergy were significantly higher than in children without cow's milk allergy.[21] In our study, we found that cow's milk allergy patients diagnosed with AD had higher mean levels of isolated cow's milk protein specific IgEs than patients with other atopic diseases. Although it is believed that total IgE is higher in AD patients in comparison with patients with other atopic diseases, there were no significant correlation between serum total IgE and cow's milk protein specific IgEs in our study. Thus, the confounding effect due to the contribution of total serum IgE in the AD patients to the level of cow's milk specific IgE could be neglected.

Possible approaches to prevent food reactive symptoms include maternal dietary restriction during pregnancy and breastfeeding, the use of hypoallergenic formulas, and delays in the introduction of certain foods into the infants' diet. The American Academy of Pediatrics suggest that solids be delayed until 6 months of age, cow's milk until 1 year of age, eggs until 2 years of age, and peanuts, tree nuts, and fish until 3 years of age.[22] Evidence is limited about the timing of introducing solid foods to infants; however, the KOALA birth cohort study found that delaying the introduction of cow's milk or other food products may not be favorable in preventing the development of atopy.[23] Furthermore, a recent prospective study concluded that the addition of baked milk into the diets of children able to tolerate baked milk products appeared to accelerate the development of unheated milk tolerance compared with strict avoidance.[24]

In conclusion, cow's milk allergy is an important cause of atopic diseases. We found that ALA was the most common cow's milk protein causing sensitization in Taiwanese patients in our study. Patients with AD had more severe sensitization to cow's milk proteins than patients with other atopic diseases. The younger patients had higher mean levels of cow's milk protein specific IgEs than the older patients, which indicates that oral tolerance may be achieved at an older age.

Conflicts of interest

All authors declare that they have no conflicts of interest related to the material discussed in this article.

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