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# Food Allergy Without a Safety Net—Molecular Sensitisation Patterns and Family Quality of Life Among Bulgarian Children in a Setting Without Epinephrine Auto-Injectors

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## Abstract

Pediatric food allergy is an increasing public health challenge associated with severe allergic reactions, psychosocial burden, and healthcare inequalities. This study aimed to characterise molecular sensitisation patterns among Bulgarian children with suspected food allergy using component-resolved diagnostics and to evaluate family quality of life in a healthcare setting without access to epinephrine auto-injectors. A total of 1163 pediatric patients underwent molecular allergy testing using the ALEX$^2$ multiplex platform, while 90 caregivers completed structured quality-of-life questionnaires. A sensitisation threshold of >0.10 kUA/L was applied to maximise sensitivity for molecular profiling and co-sensitisation analyses. Tree nut allergens showed the highest sensitisation prevalence, predominantly involving Ana o 3, Pis v 1, Jug r 1, Cor a 9, and Cor a 14. Younger children showed a predominance of stable storage protein sensitisation, whereas older children more frequently demonstrated PR-10-associated cross-reactive profiles. Correlation analyses identified strong co-sensitisation clustering among phylogenetically related allergen families, particularly cashew-pistachio, walnut-hazelnut, mammalian milk, and fish allergens. Questionnaire responses revealed substantial psychosocial burden related to fear of anaphylaxis, chronic hypervigilance, restricted daily activities, and inadequate emergency preparedness. These findings highlight the importance of molecular risk stratification and underscore major unmet needs in pediatric food allergy management in Bulgaria.

**Keywords:** food allergy; component-resolved diagnostics; quality of life; children; epinephrine auto-injector



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## 1. Introduction

Food allergy has become an increasingly important global public health problem, currently affecting approximately 5–10% of children and up to 10% of adults worldwide [1]. Although prevalence rates appear to have stabilised in some high-income Western countries

after decades of steady increase, food allergy incidence continues to rise in many developing and transitional regions [2]. This epidemiological shift is thought to result from a complex interaction between genetic susceptibility, environmental exposures, urbanisation, dietary westernisation, and alterations in the human exposome [3]. Food allergy is considered an important early manifestation of the allergic march and is associated with an increased risk of subsequent allergic rhinitis and asthma, often developing alongside or following atopic dermatitis. Early recognition and accurate diagnosis are therefore essential for long-term clinical management [4].

The clinical significance of pediatric food allergy is further underscored by the increasing incidence of food-induced anaphylaxis [5]. Food allergens are now among the leading causes of anaphylaxis in children, and several international studies have demonstrated substantial increases in hospital admissions for severe allergic reactions over the past two decades [6]. The highest rates of emergency presentations are consistently observed in infants and preschool children, highlighting the particular vulnerability of younger pediatric populations [7,8]. Although fatal outcomes remain relatively uncommon, the unpredictable nature of anaphylaxis and the potential for rapid respiratory or cardiovascular compromise create a constant sense of danger for affected families [9].

Beyond the immediate medical risks, food allergy imposes a major psychosocial burden on both children and caregivers [10]. Daily activities such as school attendance, social gatherings, travelling, and eating outside the home frequently become sources of anxiety and extensive planning. Studies consistently demonstrate impaired health-related quality of life among children with food allergies compared to healthy peers and even compared to children with other chronic diseases [11]. Fear of accidental allergen exposure may lead to social isolation, food-related anxiety, and avoidance behaviours. Adolescents often face additional psychosocial challenges associated with increasing independence and self-management responsibilities, while caregivers commonly report elevated levels of chronic stress, anxiety, sleep disturbances, and emotional exhaustion [12,13]. The school environment may further contribute to these difficulties due to inadequate allergy awareness, insufficient emergency preparedness, and the risk of bullying or social exclusion related to food allergy [14]. Despite growing recognition of these medical and psychosocial challenges, comprehensive molecular sensitisation data from Eastern European pediatric populations remain limited. Moreover, little is known about the psychosocial burden experienced by families living in healthcare systems where epinephrine auto-injectors are not readily available. Addressing these knowledge gaps was a primary objective of the present study.

In recent years, component-resolved diagnostics (CRD) have substantially transformed the evaluation of IgE-mediated food allergy [15]. Traditional diagnostic methods, including skin prick testing and extract-based serum-specific IgE measurements, frequently lack the specificity required to distinguish a clinically relevant allergy from an asymptomatic sensitisation or pollen-related cross-reactivity. As a result, many children may undergo unnecessary dietary restrictions that negatively affect nutrition and quality of life. CRD overcomes some of these limitations by measuring IgE antibodies directed against individual allergenic proteins, enabling detailed molecular sensitisation profiling and more precise clinical risk assessment [16].

The clinical utility of molecular diagnostics is particularly evident in distinguishing genuine primary sensitisation from cross-reactive sensitisation patterns. Sensitisation to pathogenesis-related protein family 10 (PR-10) allergens and profilins, such as Ara h 8 or Cor a 1, is commonly associated with pollen-food syndrome and typically predicts milder oral symptoms. In contrast, sensitisation to stable storage proteins, including 2S albumins and seed storage globulins such as Ana o 3, Ara h 2, Cor a 14, and Cor a 9, is strongly associated with persistent allergy and severe systemic reactions. Similarly,

sensitisation to heat- and digestion-resistant proteins such as casein (Bos d 8) and ovomucoid (Gal d 1) may indicate persistent milk and egg allergy phenotypes with reactions even to baked products [16]. Molecular diagnostics therefore provide valuable biomarkers for risk stratification, prognosis assessment, and personalised allergy management [17].

Despite these advances, important gaps remain in food allergy management worldwide, particularly in countries with limited access to emergency anaphylaxis treatment. Immediate intramuscular administration of epinephrine is universally recommended as first-line therapy for anaphylaxis; however, the availability and affordability of epinephrine auto-injectors (EAIs) vary substantially between healthcare systems [18]. In several countries, including Bulgaria, epinephrine auto-injectors are currently unavailable, forcing families and healthcare providers to rely on ampoule-based adrenaline administration during emergencies. This creates significant challenges for timely treatment, especially in schools, remote settings, and situations involving non-medical caregivers. The absence of accessible emergency medication may therefore increase both medical vulnerability and psychosocial burden among affected families.

Within this healthcare context, precise molecular risk stratification becomes particularly important. Identification of children sensitised to high-risk storage proteins may help clinicians prioritise intensive education, targeted avoidance strategies, and emergency preparedness measures for patients at greatest risk of severe reactions.

Therefore, the aim of this study was to characterise molecular sensitisation profiles in Bulgarian children with food allergies using the ALEX$^2$ multiplex platform, which enables simultaneous assessment of a broad spectrum of allergen extracts and molecular components, providing detailed molecular phenotyping and improved identification of clinically relevant sensitisation patterns. In parallel, we evaluated the impact of food allergy on family quality of life within a healthcare system in which epinephrine auto-injectors are currently unavailable.

## 2. Materials and Methods

### *2.1. Study Design and Participants*

This cross-sectional observational study evaluated molecular sensitisation patterns among pediatric patients undergoing molecular allergy testing in Bulgaria between January 2024 and March 2026. The analysis used anonymised, aggregated laboratory and questionnaire data collected from children referred for evaluation of suspected food allergy.

A total of 1163 pediatric patients were included in the molecular sensitisation analysis. A threshold of >0.10 kUA/L was used to maximise sensitivity for molecular sensitisation profiling and correlation analyses, particularly for low-level and cross-reactive allergen components. Demographic variables included age and sex. Age was calculated at the time of analysis.

### *2.2. Molecular Allergy Diagnostics*

Molecular sensitisation profiles were assessed using the ALEX$^2$ (Allergy Explorer 2) multiplex platform (MacroArray Diagnostics, Vienna, Austria), which simultaneously measures total IgE and allergen-specific IgE against a broad panel of allergen extracts and molecular allergen components. The analysis focused on food allergen components relevant to pediatric food allergy, including peanut, tree nuts, milk, egg, soy, seafood, legumes, and wheat-associated molecules. Both genuine sensitisation markers and cross-reactive allergen components were evaluated. Particular attention was given to high-risk storage proteins, including 2S albumins, 7S globulins, and 11S globulins, due to their established association with systemic allergic reactions and anaphylaxis. For statistical processing, values reported below the analytical detection threshold ("<0.10") were treated as negative, while values

exceeding the upper analytical limit (">50") were capped at 50 kUA/L to reduce distortion during quantitative analyses.

The psychosocial impact of pediatric food allergy in Bulgaria was assessed using an online, study-specific, non-validated questionnaire completed by 90 caregivers. Participation was voluntary and anonymous, and no selection based on disease severity or previous anaphylaxis was applied. The questionnaire was designed to evaluate the practical and emotional burden of food allergy management in a healthcare setting without access to EAIs. It included domains commonly addressed in established food allergy quality-of-life questionnaires, such as caregiver anxiety, emergency preparedness, social restrictions, perceived healthcare support, and overall family burden, using both Likert-scale and open-ended questions. The survey explored the following domains:

- Food allergy history and previous anaphylaxis;
- Prior adrenaline administration;
- Inability to obtain epinephrine auto-injectors;
- Distance to emergency medical services;
- Daily anxiety and hypervigilance;
- Fear of severe allergic reactions;
- Lifestyle restrictions;
- School-related concerns;
- Family stress;
- Financial burden;
- Perceived deficiencies in healthcare support.

The questionnaire was developed specifically for this study and included both structured and open-ended qualitative questions. The full English version of the questionnaire is provided in Supplementary Material S1.

### *2.3. Statistical Analysis*

Descriptive statistics were used to summarize the demographic characteristics of the cohort and the prevalence of sensitisation to various allergens. To evaluate gender-specific differences, categorical variables were compared using Fisher's Exact Test. To further quantify the association between gender and sensitisation risk, Odds Ratios with 95% Confidence Intervals were calculated. Co-sensitisation patterns were analysed using Spearman's rank correlation coefficient (rho). To elucidate the strength of these associations, the coefficient of determination ($r^2$) was calculated, representing the proportion of shared variance between allergen pairs. Correlation matrices were generated for major food groups, with non-significant correlations ($p > 0.05$) excluded to ensure data robustness.

All statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA) and Python version 3.11. A $p$-value $< 0.05$ was considered statistically significant for all tests.

Generative artificial intelligence (ChatGPT, OpenAI, GPT-5) was used during manuscript preparation solely to assist with language editing, improvement of scientific writing, text refinement, and figure conceptualisation. The AI tool was not used for study design, data collection, statistical analysis, data interpretation, or generation of scientific results. All AI-generated suggestions were critically reviewed, verified, and edited by the authors, who take full responsibility for the final content of the manuscript.

The study analysed retrospectively collected anonymised laboratory and questionnaire data obtained during routine molecular allergy diagnostics. Participation in the questionnaire component was voluntary and anonymous. The study protocol was approved by the local institutional ethics committee.

## 3. Results

### *3.1. Cohort Characteristics*

A total of 1163 pediatric patients underwent molecular allergy testing using the ALEX$^2$ multiplex platform. Sensitisation was defined as allergen-specific IgE levels ≥ 0.10 kUA/L. The analysed cohort included sensitisation profiles to legumes, tree nuts, milk, egg, and seafood allergens. Among the analysed molecular allergen groups, sensitisation to tree nuts showed the highest prevalence, identified in 803 of 1163 sensitised patients (69.1%) (Figure 1). The mean age of the study population was 7.58 ± 4.41 years (median 6.69 years). Most sensitised patients were school-aged children (6–11 years), who accounted for 40.7% of the cohort, followed by preschool children aged 2–5 years (36.8%). Children younger than 2 years represented 5.9% of the study population, and adolescents aged 12–17 years accounted for 14.4%.

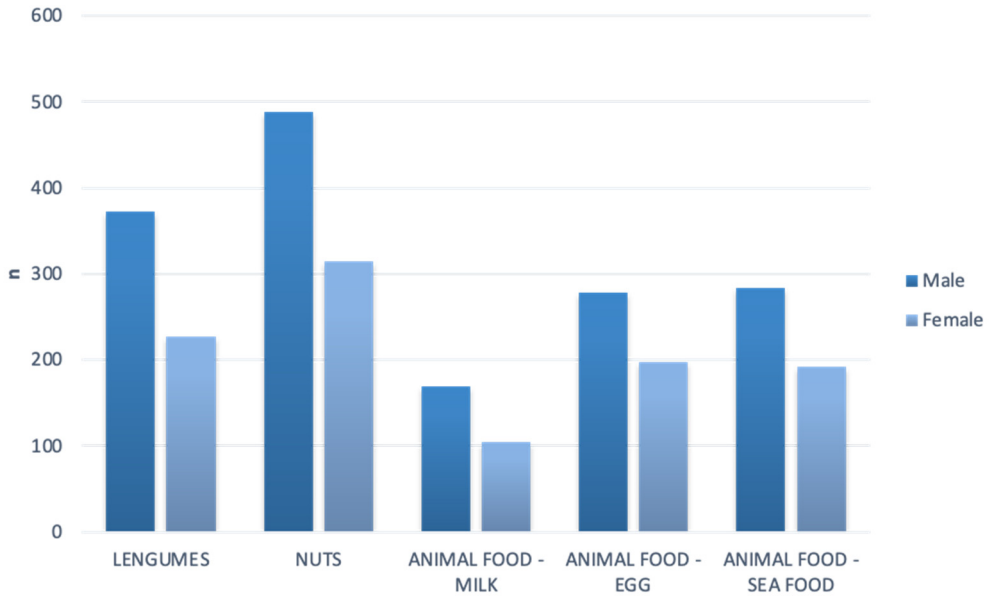


**Figure 1.** Molecular food allergen sensitisation patterns by allergen group and sex.

### *3.2. Sensitisation Prevalence by Food Group*

Cashew allergens constituted the dominant sensitisation profile within this category, with Ana o detected in 30.3% of patients. Other highly prevalent nut allergens were Jug r 1 (walnut) and Pis v 1 (pistachio), detected in 24.1% and 22.6% of patients, respectively (Figure 2).

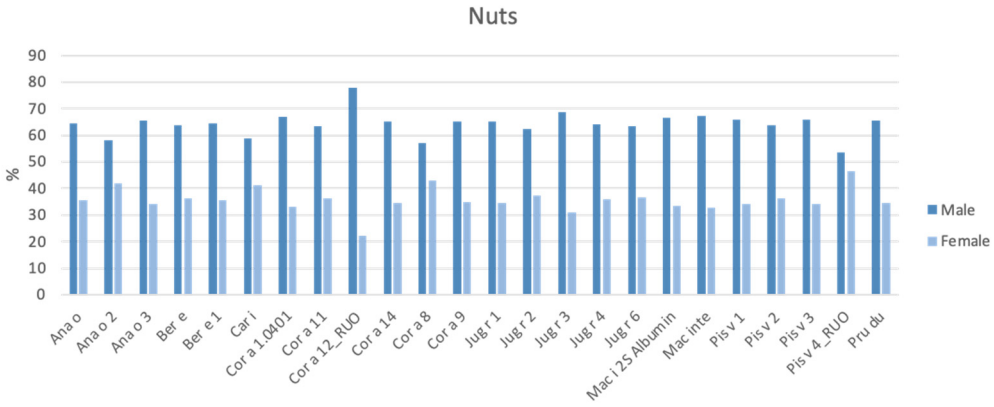


**Figure 2.** Distribution of tree nut molecular sensitisation profiles in the study cohort.

Legume sensitisation was also highly prevalent, affecting 51.6% of the cohort. Among peanut-related molecular components, Ara h 1 and Ara h 8 were the most frequently detected allergens, identified in 15.1% and 13.8% of patients, respectively.

Within animal-derived food allergens, egg and seafood sensitisation showed identical prevalence rates (40.9%), while milk sensitisation was lower (23.5%). Among egg allergens, Gal d 2 (ovalbumin) represented the most prevalent molecular component, detected in 20.5% of sensitised individuals.

### *3.3. Sex-Associated Sensitisation Patterns*

Significant sex-related differences in molecular sensitisation patterns were identified, with an overall predominance among boys across multiple allergen groups (Figure 1).

Within legumes, boys showed a significantly increased sensitisation risk for Ara h 3 (OR 1.98, $p = 0.0028$), Gly m 5 (OR 2.03, $p = 0.0097$), and Ara h 1 (OR 1.44, $p = 0.0367$) (Figure 3). A similar male predominance was observed among tree nut allergens, particularly for Ana o 3 (OR 1.46, $p = 0.0161$), Cor a 1.0401 (OR 1.44, $p = 0.0342$), and Jug r 1 (OR 1.37, $p = 0.0301$).

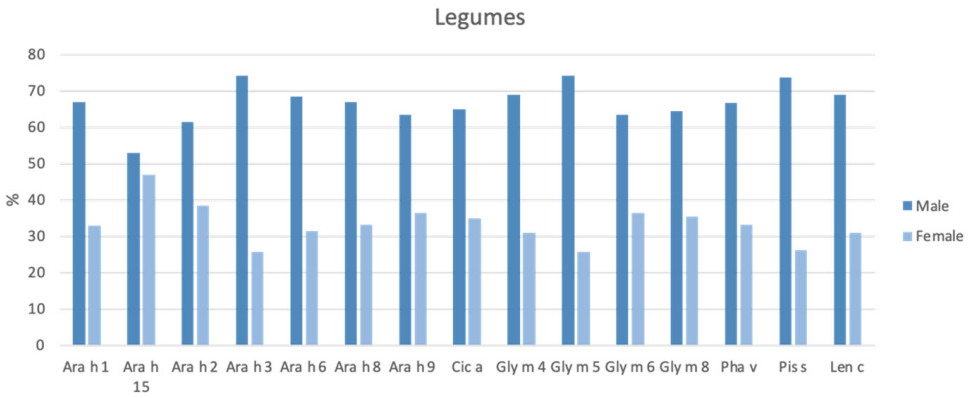


**Figure 3.** Gender-specific sensitisation patterns among legume allergens.

Among seafood allergens, sensitisation patterns showed greater heterogeneity. A significant female predominance was observed for Raj c (thornback ray), with 59.1% of sensitised patients being girls (OR 2.21, $p = 0.0118$). In contrast, sensitisation to Cyp c 1 (carp) showed a strong male predominance, with boys accounting for 74% of sensitised individuals (OR 1.98, $p = 0.0388$).

### *3.4. Storage Protein and PR-10 Profiles*

The molecular sensitisation profile of the cohort was characterised by substantial reactivity to stable storage proteins commonly associated with clinically significant and systemic allergic reactions. High sensitisation frequencies were identified for Ana o 3 (25.5%), Jug r 1 (24.1%), and Pis v 1 (22.6%), supporting the predominance of molecular profiles consistent with genuine primary sensitisation to tree nut storage proteins.

Sensitisation to PR-10-associated allergens was also common. Ara h 8 and Gly m 4 were detected in 13.8% and 11.1% of patients, respectively. Gly m 4 sensitisation also showed a significant male predominance (OR 1.58, $p = 0.0225$). The coexistence of storage protein-dominant and PR-10-associated sensitisation profiles suggests the simultaneous contribution of genuine primary food allergy and pollen-related cross-reactivity within the cohort.

Forest plot analysis further demonstrated a significant male-skewed sensitisation risk for several allergen components, particularly among legumes, tree nuts, and seafood allergens (Figure 4). Boys showed approximately a twofold higher sensitisation risk for Pis s,

Ara h 3, and Gly m 5. Significant male predominance was also identified for Ana o 3 and Jug r 1. Among seafood allergens, the strongest association was observed for Raj c parvalbumin, which showed the highest odds ratio within the cohort, although interpretation is limited by wide confidence intervals and likely smaller subgroup size.

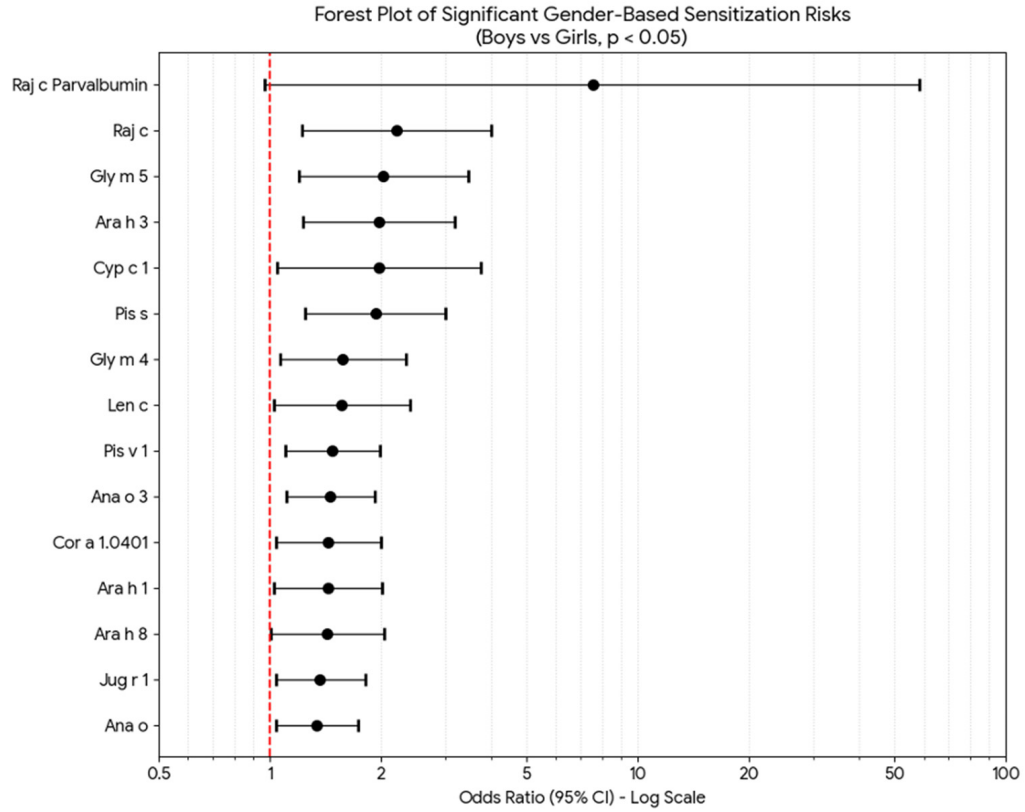


**Figure 4.** Odds ratios for significant gender-associated molecular sensitisation profiles.

The plot shows the Odds Ratios and 95% Confidence Intervals for allergens with significant gender-based differences ($p < 0.05$). Values are plotted on a logarithmic scale. An OR $> 1$ (to the right of the vertical red line) indicates a higher risk of sensitisation in boys compared to girls. Allergens are grouped by biological source (Seafood, Legumes, and Nuts) to highlight group-specific patterns of male-skewed sensitisation. Sex-related differences were less pronounced for milk- and egg-associated sensitisation profiles, which showed a relatively homogeneous distribution between boys and girls.

Among the major storage protein allergens, Ana o 3 exhibited one of the highest mean allergen-specific IgE levels among sensitised individuals, further supporting the predominance of storage protein-driven sensitisation patterns within the cohort. Similarly, sensitisation to Cor a 9 and Cor a 14 indicated a substantial proportion of patients with genuine hazelnut allergy rather than isolated pollen-related cross-reactivity. In contrast, Cor a 1.0401 displayed a markedly different age-related distribution pattern, characterised by increasing prevalence in older children and adolescents, consistent with PR-10-associated pollen–food syndrome profiles.

Additional clinically relevant sensitisation patterns were identified for milk and egg allergens. Sensitisation to Bos d 8 (casein) and Gal d 1 (ovomucoid), both heat- and digestion-resistant allergens associated with persistent allergy phenotypes, showed significant age-related associations.

### *3.5. Co-Sensitisation Correlation Analysis*

Correlation analysis revealed several robust clusters of co-sensitisation across major food allergen groups. The strongest shared sensitisation patterns were observed within homologous storage protein families and phylogenetically related foods.

Within legumes, strong correlations were identified between major peanut storage proteins, particularly Ara h 1, Ara h 3, and Ara h 6. Additional correlations were observed between Pis s and Len c, as well as between Ara h 8 and Gly m 4, reflecting both homologous protein sensitisation and pollen-related cross-reactivity patterns. The strongest correlation within the legume group was between Ara h 8 and Gly m 4 ($r^2 = 0.53$).

The nut heatmap demonstrated extensive co-sensitisation among cashew, pistachio, walnut, and hazelnut components (Figure 5). Particularly strong correlations were observed between Ana o and Ana o 3 ($r^2 = 0.74$), Ana o 3 and Pis v 1 ($r^2 = 0.70$), and Pis v 2 and Pis v 3 ($r^2 = 0.69$), supporting extensive molecular overlap within the Anacardiaceae family. Significant shared variance was also observed between Cor a 9 and Jug r 4, as well as between Cor a 9 and Prunus-related allergens.

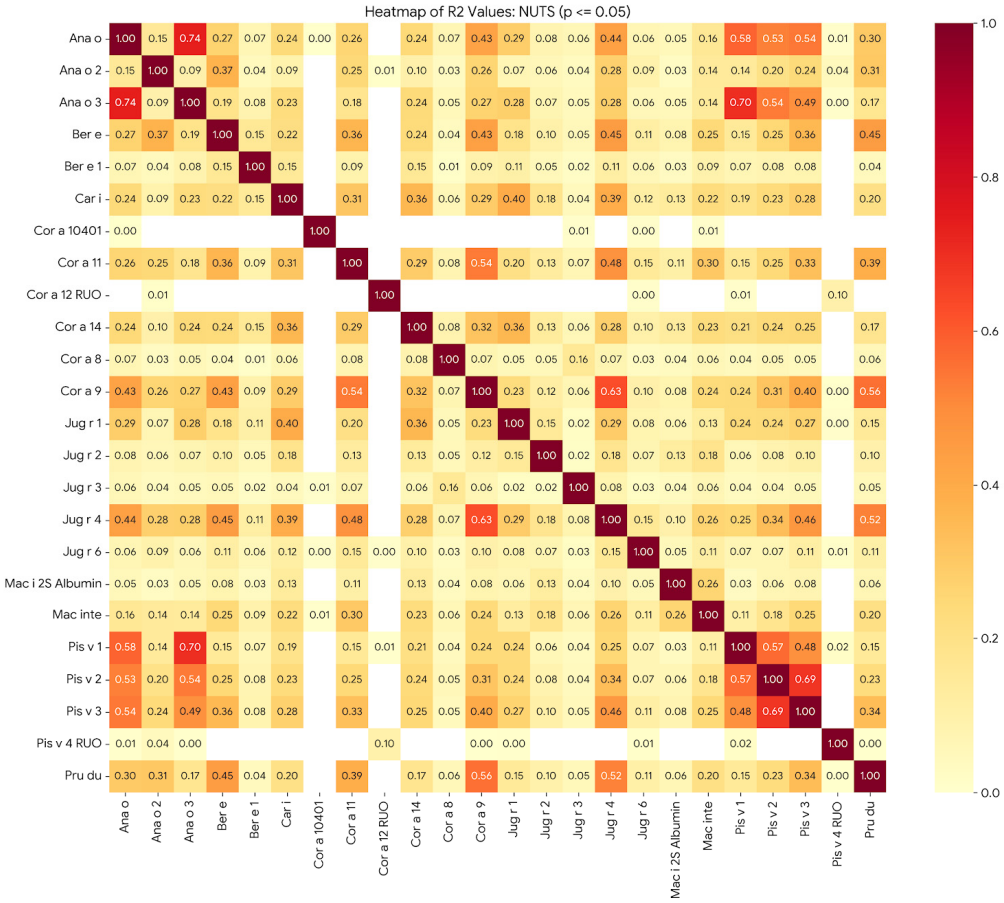


**Figure 5.** Molecular correlation matrix of nuts co-sensitisation patterns.

Heatmap showing significant co-sensitisation patterns between molecular allergen components. Colour intensity represents the coefficient of determination ($r^2$), reflecting the proportion of shared variance between allergen pairs. Non-significant correlations ($p > 0.05$) are omitted.

Milk allergen correlation matrices showed substantial clustering among Bos d 5, Bos d 8, Bos d milk, and milk proteins from other mammalian species. The strongest association within this group was observed between Cap h milk and Ovi a milk ($r^2 = 0.65$), indicating strong cross-species correlations among mammalian milk allergens, particularly

between goat and sheep milk proteins. Bos d 8 showed moderate to strong correlations with several milk-associated proteins, supporting its central role within persistent milk sensitisation profiles.

Within egg allergens, moderate correlations were identified among Gal d 1, Gal d 2, Gal d white, and Gal d yolk. Gal d 1 and Gal d white showed one of the strongest shared sensitisation patterns within this group ($r^2 = 0.35$).

Seafood allergen analysis revealed several distinct sensitisation clusters. Strong correlations were identified among fish parvalbumins and species-related fish allergens, including Sal s 1, Sco s 1, Thu a 1, and Xip g 1. Separate clusters involving crustacean and mollusc allergens were also observed, suggesting biologically distinct seafood sensitisation patterns.

Distinct clustering patterns separated stable storage proteins from PR-10- and profilin-associated sensitisation profiles, supporting the coexistence of both genuine primary food sensitisation and pollen-related cross-reactivity within the cohort.

### *3.6. Questionnaire and Quality-of-Life Findings*

Open-ended responses revealed a substantial psychological and practical burden related to the lack of EAIs. The dominant theme was persistent fear of fatal anaphylaxis and inability to respond adequately during emergencies, particularly when travelling, attending school, or outside major urban areas. Many parents expressed low confidence in administering adrenaline from ampoules, even among those with medical training.

Parents also described chronic hypervigilance, avoidance of social activities, sleep disturbances, and reduced family quality of life. Recurrent concerns included insufficient physician guidance, lack of school protocols, and inadequate institutional preparedness for pediatric anaphylaxis.

The overwhelming majority of respondents identified immediate access to EAIs as the highest priority for improving safety. Additional requests included better education for physicians and caregivers, improved school preparedness, and broader public awareness regarding anaphylaxis management.

## 4. Discussion

This study represents the first large-scale molecular characterisation of food allergen sensitisation patterns in Bulgarian children using multiplex component-resolved diagnostics, combined with a qualitative assessment of family quality of life in a healthcare setting without access to epinephrine auto-injectors. The molecular sensitisation profile was dominated by tree nut storage proteins, particularly Ana o 3, Pis v 1, Jug r 1, Cor a 9, and Cor a 14. Age-related patterns distinguished stable storage protein sensitisation in younger children from PR-10-associated cross-reactive profiles in adolescents. Qualitative data demonstrated a substantial psychosocial burden associated with fear of anaphylaxis, chronic hypervigilance, restricted daily activities, and inadequate emergency preparedness.

The predominance of tree nut storage protein sensitisation in this cohort is consistent with findings from Mediterranean and Southern European pediatric CRD studies, where cashew, pistachio, walnut, and hazelnut allergens are major drivers of clinically significant food allergy and anaphylaxis risk [19,20]. Sensitisation to Ana o 3, Cor a 9, Cor a 14, and Jug r 1 has repeatedly been associated with systemic reactions and persistent allergy phenotypes in pediatric populations [16]. The strong co-sensitisation observed between cashew and pistachio allergens, particularly Ana o 3 and Pis v 1, reflects the known phylogenetic relationship within the Anacardiaceae family and parallels observations from Spanish and French molecular allergy cohorts [21].

In contrast, sensitisation to PR-10-associated allergens such as Cor a 1.0401 and Ara h 8 increased progressively with age, supporting the emergence of pollen-food syndrome

during later childhood and adolescence. Similar age-dependent sensitisation trajectories have been described in Central and Eastern European studies, particularly in regions with substantial birch pollen exposure [22]. The coexistence of storage protein-dominant and PR-10-associated sensitisation profiles within the Bulgarian cohort suggests the simultaneous contribution of genuine primary food allergy and pollen-related cross-reactivity.

Although the basis for the observed male predominance remains uncertain, our findings are consistent with previous studies reporting a higher prevalence of IgE-mediated sensitisation and food allergy in boys during childhood. This sex-related pattern appears to become less pronounced after puberty and, for several allergic diseases, may even shift towards female predominance in adulthood. Current evidence indicates that these differences are likely multifactorial, reflecting complex interactions among genetic susceptibility, immune maturation, sex hormones, epithelial barrier function, environmental exposures and behavioural factors. Experimental and clinical studies further suggest that sex hormones modulate both innate and adaptive immune responses, thereby influencing IgE production, mast cell activation and the clinical expression of allergic disease. As these biological and environmental factors were not evaluated in the present study, no conclusions can be drawn regarding the mechanisms underlying the observed sex-related differences. Further longitudinal studies integrating molecular sensitisation profiles with hormonal, immunological and clinical data are warranted to clarify these age-dependent patterns and their clinical significance [23,24].

The milk and egg sensitisation patterns further support the clinical utility of CRD-based risk stratification. Sensitisation to Bos d 8 (casein) and Gal d 1 (ovomucoid), both heat- and digestion-resistant allergens, has consistently been associated with persistent allergy phenotypes and reduced tolerance to baked products. Similarly, the strong correlations observed between cow's, goat's, and sheep's milk allergens are consistent with previously described cross-reactivity among mammalian milk proteins [25]. Seafood correlation matrices also demonstrated biologically plausible clustering among fish parvalbumins, reflecting conserved allergenic structures shared across multiple fish species [17].

These findings highlight the growing role of precision allergy medicine in pediatric food allergy. Molecular diagnostics provide substantially greater clinical specificity compared with whole-extract testing and may help distinguish clinically relevant primary sensitisation from asymptomatic cross-reactivity. Identification of high-risk storage protein sensitisation profiles may improve risk stratification, optimise patient counselling, and support more individualised emergency preparedness strategies. Recognition of PR-10-associated sensitisation profiles may also help reduce unnecessary dietary restrictions and minimise the nutritional and psychosocial consequences of overdiagnosis [17].

The qualitative findings also demonstrated a significant psychosocial burden among families managing pediatric food allergy. Parents frequently described persistent fear of fatal anaphylaxis, sleep disturbances, avoidance of travel and social activities, and chronic anxiety related to accidental allergen exposure. These findings are highly consistent with international literature showing significantly impaired health-related quality of life among both food-allergic children and their caregivers [10]. Fear of anaphylaxis has repeatedly been identified as one of the strongest determinants of caregiver anxiety and family stress in food allergy management.

Importantly, the psychosocial burden observed in this cohort appears to be substantially amplified by the lack of accessible EAIs in Bulgaria. Parents repeatedly reported low confidence in administering adrenaline from ampoules during emergencies, even among medically trained caregivers. Similar concerns have been reported in healthcare systems where standardised emergency treatment pathways are unavailable or poorly implemented [23].

One of the most important public health implications of this study is the absence of accessible EAIs in Bulgaria. Current international guidelines consistently recommend immediate intramuscular epinephrine administration as first-line treatment for anaphylaxis [17]. However, Bulgarian families currently lack access to the internationally accepted standard of emergency allergy care. This creates major barriers to guideline adherence and forces caregivers to rely on ampoule-based adrenaline administration, which is associated with delayed treatment, dosing uncertainty, and increased caregiver stress.

The findings also reveal substantial inequality in allergy care compared with most European countries, where EAIs are a routine component of pediatric anaphylaxis management [17]. In addition, parents frequently reported insufficient school preparedness, lack of institutional protocols, and reluctance among school personnel to administer adrenaline during emergencies. These deficiencies indicate a broader systemic gap in pediatric anaphylaxis preparedness extending beyond medication availability. Collectively, these findings suggest that improving access to EAIs should be a major public health priority in Bulgaria. Wider implementation of school anaphylaxis protocols, physician education programmes, caregiver training, and standardised emergency preparedness strategies may substantially improve both patient safety and family quality of life.

Our psychosocial findings broadly align with previous studies showing that pediatric food allergy substantially impairs family quality of life through persistent anxiety, hypervigilance, social restrictions, and fear of accidental allergen exposure [5,10,11]. Prior research has consistently found that caregivers of children at risk of anaphylaxis experience considerable emotional burden and reduced quality of life, particularly when confidence in emergency management is limited [5,10,26]. Although direct quantitative comparison with these studies was not appropriate, as our study used a study-specific questionnaire focusing on the unique challenges associated with the lack of epinephrine auto-injectors, the overall pattern of caregiver burden was remarkably similar. Notably, our findings extend previous observations by suggesting that the absence of accessible epinephrine auto-injectors may represent an additional source of psychological distress, reinforcing anxiety related to emergency preparedness and further restricting daily family activities.

### *4.1. Strengths*

This study is the first large-scale Bulgarian pediatric cohort evaluating molecular food allergen sensitisation using CRD. A major strength is the combination of detailed molecular profiling with qualitative quality-of-life assessment, providing both biological and real-world clinical perspectives. The study also includes extensive co-sensitisation analyses across multiple allergen groups in routine clinical practice. The relatively low analytical threshold may have increased sensitivity for detecting low-level sensitisation and cross-reactive profiles, although not all detected sensitisation patterns necessarily reflect clinically relevant food allergy.

### *4.2. Limitations*

Several limitations should be acknowledged. This was a real-world observational study, which may limit generalisability and introduce referral bias. Some allergen subgroups included relatively small numbers of sensitised patients. Quality-of-life data were obtained using a study-specific, non-validated questionnaire completed by caregivers and may therefore be subject to reporting and response bias. Because questionnaire responses were collected anonymously and were not linked to individual molecular or clinical data, it was not possible to perform correlations between psychosocial burden, sensitisation profiles, and reaction severity. Finally, our molecular analysis was based on allergen-specific IgE sensitisation profiles, which do not necessarily indicate a clinically relevant food allergy.

Because oral food challenges were not systematically performed, molecular sensitisation could not always be confirmed as a clinical allergy and should therefore be interpreted together with the patient's clinical history and appropriate diagnostic evaluation rather than as a direct predictor of clinical severity or anaphylaxis risk.

## 5. Conclusions

This study presents the first large-scale molecular characterisation of pediatric food allergen sensitisation patterns in Bulgaria, using component-resolved diagnostics in a real-world clinical setting. Tree nut and legume storage proteins predominated in the cohort, while age-dependent differences distinguished genuine primary food sensitisation from pollen-related cross-reactivity profiles. The identified co-sensitisation clusters further demonstrated the clinical value of molecular diagnostics for precise risk stratification and personalised allergy management.

Alongside the molecular findings, the study revealed a substantial psychosocial burden among families of children with food allergies. Persistent fear of anaphylaxis, chronic hypervigilance, social restriction, and reduced quality of life were consistently reported by caregivers. These difficulties appeared to be significantly exacerbated by the lack of accessible EAIs in Bulgaria.

Overall, the findings highlight the urgent need for improved anaphylaxis preparedness, wider access to epinephrine auto-injectors, standardised school protocols, and enhanced allergy education in Bulgaria. They also support the growing role of component-resolved diagnostics as an important tool for improving diagnostic precision, guiding risk assessment, and optimising clinical management in pediatric food allergy.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/allergies6030031/s1, Supplementary Material S1: Questionnaire for Parents of Children with Food Allergy in the Context of Lack of Access to Epinephrine Auto-Injectors (English Version).

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## Abbreviations

| | |
|---|---|
| CRD | Component-Resolved Diagnostics |
| EAI | Epinephrine Auto-Injector |
| IgE | Immunoglobulin E |
| OR | Odds Ratio |
| PR-10 | Pathogenesis-Related Protein 10 |
| QoL | Quality of Life |
| ALEX$^2$ | Allergy Explorer 2 |

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