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Specific IgE-profiles and allergy prediction

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THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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This thesis is dedicated to my beloved friend Ingrid Schiller Rask, the one who always cheered me on and who believed in perusing your dreams.

"Everything is possible"

POPULAR SCIENCE SUMMARY OF THE THESIS

Background

The development and presence of antibodies of type Immunoglobulin E (IgE-ab) in the blood, so called sensitization, is common and more commonly found in individuals with allergic diseases such as asthma, allergic rhinitis, atopic dermatitis and food allergy. The IgE-antibody development is often dynamic in early childhood and while not all individuals with sensitization to food develop a food allergy, some of them seem to be at a higher risk than others.

The overall aim of the thesis was to investigate if certain IgE-profiles can predict future allergic diseases and to identify associated factors.

Methods

Firstly, in study I, we used the Scandinavian study cohort PreventADALL consisting of 2697 women recruited during pregnancy and their children. We analyzed the development of early IgE antibodies in children aged 3 months in relation to maternal and perinatal factors.

Secondly, in study II, we investigated the children in PreventADALL in terms of early peanut sensitization at age 12 months in relation to presence of peanut allergy at 3 years of age.

Thereafter, in study III the Swedish population-based study cohort BAMSE was used, consisting of 4089 children included at birth and followed until 24 years of age, we examined in study III the participants between 4-24 years of age with emphasis on the development of IgE antibodies against peanut in relation to reported allergic symptoms, inflammatory markers and lung function.

Finally, in study IV we studied the development of IgE antibodies against tree nuts in relation to background factors and symptoms of tree nut allergy at 24 years of age in the BAMSE cohort.

Results

We concluded in study I that at 3 months of age 7 % of the children had measurable levels of IgE antibodies, mainly against food, most commonly to milk and egg, but that few of them expressed IgE antibodies correlated with an increased risk of allergic reactions. Maternal food sensitization was associated with child sensitization.

In study II, children aged 12 months often had IgE antibodies against peanut but only few of them were classified as allergic to peanut at 3 years of age. Children that had received early food intervention expressed a different pattern of IgE antibodies, mainly against peanut Ara h 3.

In study III, peanut allergy emerged early in the participants of the BAMSE study cohort and few outgrow their allergy in adulthood, the ones that did all had initially low IgE-levels against peanut Ara h 2. Peanut allergic individuals were found to express higher levels of inflammatory markers and were more prone to severe asthma.

In study IV participants with IgE antibodies against tree nuts seldom reported allergic symptoms, and the majority had IgE antibodies against birch, while those also expressing IgE towards tree nut storage proteins more often had a background with allergic diseases, many of them reported of early atopic dermatitis and an allergy towards egg. They were more often sensitized towards several different tree nuts and described more severe allergic reactions.

Conclusion

IgE antibodies to peanut develop early, and tend to persist, especially in children who experience atopic dermatitis, asthma and egg allergy. However, very early in life few children with IgE antibodies towards storage proteins correlated with increased risk of allergic reactions, indicating that the IgE development process is not complete in this age and ideally still time for a possible tolerance development. Previous studies have indicated that that an early food introduction is preferred over a delayed one, and this research add new insight in this process. It is possible that atopic children in particular might benefit the most in terms of preventing a more severe allergic phenotype if early food introduction would be applied.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Bakgrund

Bildandet av antikroppar av typ immunoglobulin E (IgE) i blodet, den typ av antikropp som oftast förknippas med allergisk sjukdom, är ofta dynamisk tidig i barndomen. Individer med allergisk sjukdom såsom eksem, födoämnesallergi, hösnuva och astma har oftare mätbara IgE antikroppar blodet. Även om inte alla bärare av IgE antikroppar mot ett visst födoämne sedan utvecklar en allergi mot detta, så verkar en del individer löpa större risk för det än andra.

Det övergripande syftet med denna avhandling är att undersöka om vissa IgE-profiler kan förutspå framtida allergisk sjukdom och om möjligt identifiera faktorer som ökar risken.

Metod

För studie I och II använde vi oss av den skandinaviska forskningskohorten PreventADALL som består av 2697 kvinnor som blev rekryterade under graviditet och där sedan deras barn blev inkluderade efter födseln. Barnen lottades till fyra olika interventionsgrupper där en grupp erhöll tidig matintroduktion från 3 månaders ålder, en grupp hudskyddande behandling med fuktighetsbevarande kräm och oljebad flera gånger i veckan, en grupp fick båda interventionerna och en grupp blev kontrollgrupp.

I studie I analyserade vi närvaro av IgE antikroppar i blodet hos barnen vid 3 månaders ålder, och tittade sedan på samband mellan IgE antikroppar hos barnet och olika faktorer i familj och omgivning kring barnets födelse.

Därefter i studie II undersökte vi barnen i PreventADALL gällande IgE antikroppar mot jordnöt vid 12 månaders ålder i relation till utveckling av allergi mot jordnöt vid 3 års ålder.

I studie III och IV använde vi oss av den svenska populationsbaserade forskningsstudien BAMSE, som består av 4089 barn som inkluderades vid födseln och därefter har följts fram till 24 års ålder med avseende på utveckling av allergiska sjukdomar i relation till omgivningsfaktorer.

I studie III tittade vi på deltagare mellan 4–24 års ålder avseende utveckling av antikroppar mot jordnöt i relation till rapporterade allergiska symtom, närvaro av inflammatoriska markörer i blodet samt lungfunktion.

Slutligen tittade vi i studie IV på IgE antikroppar mot trädnötter i relation till bakgrundsfaktorer och symtom på allergi mot trädnötter vid 24 års ålder.

Resultat

Vi fann i studie I att vid 3 månaders ålder hade 7 % av barnen mätbara nivåer av IgE antikroppar, främst mot viss mat, i synnerhet mot mjölk och ägg. Få av dem hade däremot antikroppar av den sort som oftast förknippas med risk för mer allvarlig allergi. Vi fann också

ett samband mellan närvaro av antikroppar mot födoämnen hos modern och närvaro av antikroppar hos barnen.

I studie II, såg vi att barnen vid 12 månader ålder ofta hade IgE antikroppar mot jordnöt men att få av dem sedan blev klassade som allergiska mot jordnöt vid 3 års ålder. Barn som erhöll tidig matintroduktion uttryckte ett annat mönster av antikroppar mot jordnöt än de barn som ej erhöll tidig matintroduktion, de hade främst antikroppar mot proteinet Ara h 3 i jordnöt.

Därefter i studie III fann vi att deltagarna tidigt blev allergiska mot jordnöt och att få av dem sedan blev av med sin allergi när den väl debuterat, detta skedde endast hos ett fåtal där alla hade mycket låga nivåer av IgE antikroppar mot jordnötens Ara h 2. Individer som var allergiska mot jordnöt hade också högre nivåer av inflammatoriska markörer och hade oftare en svår astma än de utan allergi mot jordnöt.

Slutligen i studie IV kunde vi se att deltagare med antikroppar mot trädnötter, i synnerhet mot hasselnöt, sällan rapporterade allergiska symptom, och att majoriteten av dem samtidigt hade antikroppar mot björkpollen. De med IgE antikroppar mot trädnötternas lagringsprotein, som ofta förknippas med risk för mer allvarliga allergiska reaktioner, hade oftare astma, eksem och äggallergi i barndomen. De hade också IgE antikroppar mot ett flertal trädnötter samtidigt och rapporterade kraftigare allergiska symptom vid intag av trädnötter.

Slutsats

IgE-antikroppar mot jordnötens lagringsprotein utvecklas ofta tidigt och växer sällan bort hos barn med jordnötsallergi, i synnerhet hos barn som samtidigt har eksem, astma och allergi mot ägg. Däremot ses dessa antikroppar sällan i blodet hos barn vid 3 månaders ålder, vilket kan indikera att själva processen med utveckling av dessa antikroppar ännu inte är färdigutvecklad och att det fortfarande kan finnas möjlighet till en toleransutveckling. Tidigare studier har visat att tidig snarare än fördröjd matintroduktion är att föredra för att motverka utveckling av jordnötsallergi, och våra forskningsresultat bidrar med fler pusselbitar till att förstå tidig allergiutveckling och toleransutveckling. Det är möjligt att i synnerhet barn med andra allergiska sjukdomar såsom eksem och äggallergi skulle ha allra störst nytt av att tidigt identifieras och introducera mat tidigt för att motverka risken att utveckla framtida allergi.

ABSTRACT

Presence of antibodies of type Immunoglobulin E (IgE-ab), often called sensitization, is common and often precede development of atopic disease, including food allergy. While not all IgE-sensitized individuals develop food allergy, some seem to be at a higher risk. By studying specific IgE-profiles longitudinally in two different birth cohorts we aimed to identify factors associated with atopic disease development and more severe disease.

Firstly, in study I and II, we used the Scandinavian population-based birth cohort PreventADALL, where 2397 infants were randomized into four different groups receiving either preventive skin care with emollient and oil baths from 2 weeks of age, early food introduction (peanut, milk, egg, wheat) from 3 months, both skin/food intervention or control group.

In study I the development of IgE in relation to maternal and perinatal factors were studied in 1110 children aged 3 months. We found that 7 % of the infants were sensitized at 3 months of age, mainly against food allergens, but few of them expressed the corresponding molecular allergens. Any positive maternal food sensitization in mid-pregnancy was associated with infant sensitization at 3 months.

The aim of **study II** was to investigate early sensitization to peanut allergen molecules analyzed in children aged 12 months in relation to peanut allergy at 3 years of age. We found that children aged 12 months often were sensitized against peanut extract but few were classified as allergic to peanut at 3 years of age. Children in the food intervention group expressed a different pattern of peanut allergen molecules, with mainly IgE-levels against Ara h 3.

Secondly, in study III and IV we used the Swedish population based BAMSE cohort, consisting of 4089 participants from 6 different parts of Stockholm followed longitudinally between 0-24 years of age.

In study III participants were examined longitudinally between age 4-24 years with emphasis on peanut IgE development in relation to symptoms and inflammatory markers such as FENO, blood eosinophils and lung function. Peanut allergy seldom appeared after the age of 8 years and few participants outgrew their allergy in adulthood, those who did all had initially low peanut Ara h 2 IgE-levels. Peanut allergic individuals were found to express higher levels of FENO, blood eosinophils and a more severe asthma.

Finally, in study IV we studied sensitization to tree nut extract and tree nut molecular allergens in relation to background factors and symptoms of tree nut allergy at 24 years of age. The tree nut sensitized participants were often asymptomatic, and the majority were birch sensitized. Individuals with storage protein sensitization often had an atopic background, were polysensitized and described more severe allergic reactions.

Conclusion

Sensitization to peanut develop early, and already at 3 months infants express IgE towards peanut while peanut allergen molecule sensitization increased later on. In adolescence peanut allergy tend to remain, especially in participants with early allergies towards other food as well as childhood asthma and atopic dermatitis. Peanut and tree nut storage protein sensitized participants were found to express higher levels of FENO, blood eosinophils and more prone to severe asthma. As few sensitized infants' express IgE against storage proteins at this age, it could indicate that the IgE development process is not complete at this age, thus still time for a possible tolerance development. Previous studies have indicated that that an early food introduction is preferred over a delayed one, and this research add new insight in this process. It is possible that atopic infants in particular might benefit the most in terms of preventing a more severe allergic phenotype if early food introduction would be applied.

LIST OF SCIENTIFIC PAPERS

I. **Tedner SG**, Söderhäll C, Konradsen JR, Bains KES, Borres MP, Carlsen KH, Carlsen KCL, Färdig M, Gerdin SW, Gudmundsdóttir HK, Haugen G, Hedlin G, Jonassen CM, Kreyberg I, Mägi CO, Nordhagen LS, Rehbinder EM, Rudi K, Skjerven HO, Staff AC, Vettukattil R, van Hage M, Nordlund B, Asarnoj A. Extract and molecular-based early infant sensitization and associated factors-A PreventADALL study. *Allergy*. 2021 Sep;76(9):2730-2739. doi: 10.1111/all.14805. Epub 2021 May 4. PMID: 33751598.

II. **Tedner SG**, Gerdin, SW, Söderhäll C, Konradsen JR, Borres MP, Lie A, Carlsen KCL, Granum B, Haugen G, MD, Hedlin G, Jonassen CM, Mägi CO, Rehbinder EM, Rudi K, Skjerven HO, Staff AC, Vettukattil R, van Hage M, Asarnoj A, Nordlund B. Peanut storage protein sensitization during the first years of life and peanut allergy development– a PreventADALL study. [*manuscript in preparation*]

III. **Tedner SG**, Klevebro S, Bergström A, Kull I, Andersson N, Borres MP, Ballardini N, Westman M, Konradsen JR, van Hage M, Nilsson C, Melen E, Asarnoj A. Development of sensitization to peanut and storage proteins over time and relation to markers of airway and systemic inflammation – a 24 year follow up [*under review*]

IV. Bager J, **Tedner SG**, Andersson N, Ballardini N, Borres MP, Konradsen JR, Nilsson C, Westman M, Kull I, Bergström A, van Hage M, Melen E, Asarnoj A. Prevalence and early-life risk factors for tree nut sensitization and allergy in young adults. *Clin Exp Allergy*. 2021 Nov;51(11):1429-1437. doi: 10.1111/cea.13994. Epub 2021 Aug 6. PMID: 34357659.

CONTENTS

LIST OF ABBREVIATIONS

AD	*Atopic dermatitis*
Ana o3	*Anacardium occidental 3*, cashew nut allergen molecule
Ara h 1, Ara h 2, Ara h 3, Ara h 8, Ara h 9	*Arachis hypogea 1, 2*, *etc., peanut allergen molecules*
Bet v 1	*Betula verrucosa 1, birch allergen molecule*
BMI	*Body Mass Index*
Cor a 1, Cor a 9, Cor a 14	*Corylus avellana 1, 9*, etc., hazelnut allergen molecules
FVC	*Forced vital capacity (mL)*
FEV1	*Forced expiratory volume in 1 second (mL)*
FENO	*Fractional exhaled nitric oxide*
IgE-ab	*Immunoglobulin E antibody*
Jug r 1	*Juglans regia 1*, walnut allergen molecule
IL	*Interleukin*
kU_A/l	*Kilounits of allergen specific sIgE-ab per liter*
LTP	*Lipid transfer protein*
MA	*Molecular allergology*
MMP10	*Matrix metalloproteinase-10*
OAS	*Oral allergy syndrome*
OR	*Odds ratio*
PR-10	*Pathogenesis-related protein class 10*
S-IgE	*specific Immunoglobulin E antibodies*

SPT	*Skin prick test*
TNFRSF	*Tumor necrosis factor receptor superfamily*
TSLP	*Thymic stromal lymphopoietin*
95% CI	*95% Confidence interval*

1 INTRODUCTION

Allergic diseases are often found in predisposed individuals more prone to allergic reactions when exposed to allergens. Allergic diseases are collectively named atopic diseases, usually including atopic dermatitis, food allergy, allergic rhinitis and asthma. Atopic disease is very common and affects around 25-45 % of the population in the western world, thus causing large expenses for health care as well as impacting patients and families in their daily life (1-3).

Sensitization is defined as the presence of elevated allergen-specific Immunoglobulin E antibodies (IgE) in the blood, and can be caused both by food and/or air-borne allergens. Elevated IgE-levels are commonly found in patients with atopic diseases (4-6).

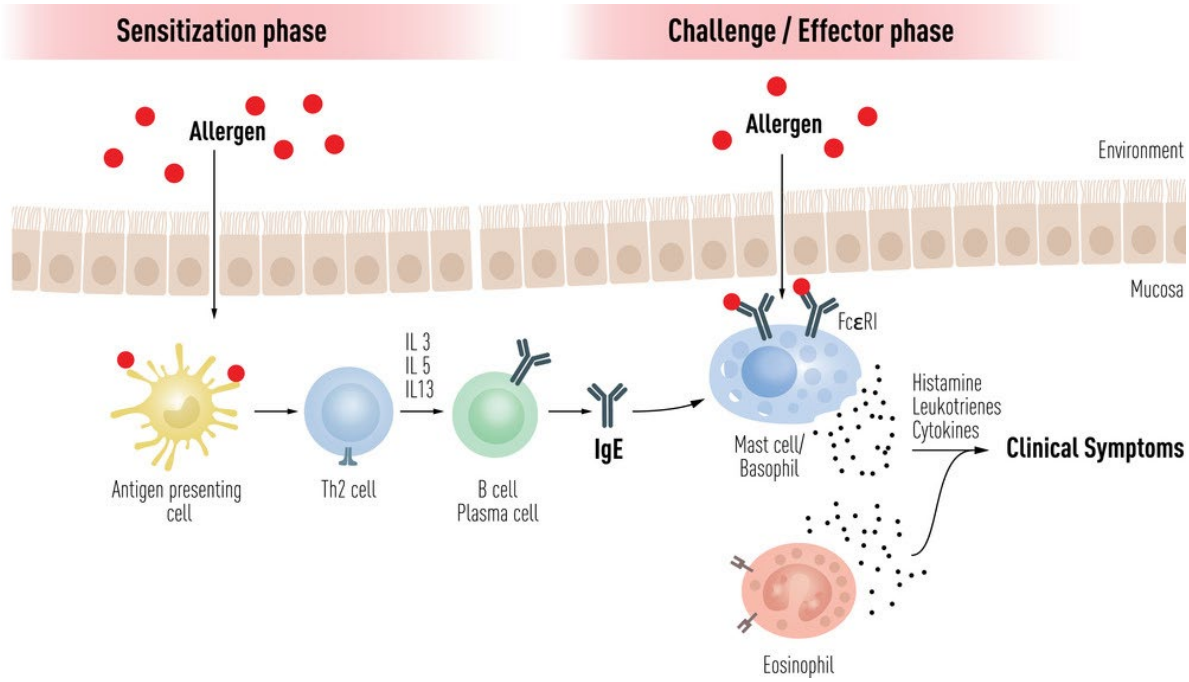
Since sensitization often precedes the development of atopic disease it would be of great importance to identify different IgE patterns in patients at high risk for future disease development in order to improve prediction and recommend potential early interventions to susceptible individuals.

2 LITERATURE REVIEW

2.1 IMMUNOGLOBULIN E AND SENSITIZATION

The development of IgE sensitization is the basis of many hypersensitivity reactions from both food and air-borne allergens (7).

Immunoglobulins are proteins taking part in the human body's immune system to help discover and identify foreign substances or possible threats such as virus, bacteria, parasites and allergens. Immunoglobulin E, the antibody mainly involved in allergic reactions, is composed of two heavy and two light protein chains, and is produced by the plasma cells. It has the potential to bind to receptors on the mast cells, see Figure 1, and the basophilic granulocytes, and by doing so has the ability to trigger an allergic or inflammatory reaction when it meets a corresponding allergen (8).



J Internal Medicine 2022 Mar;291(3):283-302

Figure 1. Role of IgE in the immune systems response to allergen exposure

High levels of IgE are commonly found in patients with atopic diseases. The development of IgE is dynamic throughout childhood and the IgE-profile seems to affect the course, symptoms and the grade of allergic diseases. The exact time point for when the infant starts to develop IgE antibodies is uncertain. Bonnelykke et al found that no production started in utero, and that IgE detected in cord blood were maternal and had vanished before the age of six months. (9).

Studies with IgE data from infants up to 3 months is sparse. Some previous studies have found prevalence rates ranging between 5-13 % for any sensitization (10-13). Corresponding levels at the age of 12 months are found between 16-21 % (14, 15). Among the risk factors for IgE development in children above 12 months is male gender, atopic dermatitis and parental atopy (10, 16-20).

2.2 MOLECULAR ALLERGOLOGY

Sensitization give valuable information of a possible allergy, but many individuals also develop sensitization without symptoms (21). In the last decade new methods have been developed in the area of molecular allergology, enabling identification of specific protein molecules correlated to each allergen. Some are found to be associated with severe reactions while others are more correlated to mild reactions. This has made it possible to more accurately define a true allergy and rule out reactions due to cross sensitivity with other type of allergens, usually due to the similarity of birch bet v 1, fruit and vegetables with the tree nuts and peanut, mostly causing mild oral allergy symptoms such as itching and discrete swelling of the oral mucosa, usually defined as oral allergy syndrome (OAS) (22). An overview of the most important food allergen molecules presented in the thesis is provided in Table 1.

Allergen	Storage protein	Lipid transfer protein (LTP)	PR-10 (Homologue to birch bet v 1)
Cashew	Ana o 3		
Cow's milk	Casein		
Egg	Ovomucoid		
Hazel nut	Cor a 9 Cor a 14	Cor a 8	Cor a 1
Peanut	Ara h 1 Ara h 2 Ara h 3 Ara h 6	Ara h 9	Ara h 8
Walnut	Jug r 1 Jug r 4		
Wheat	Omega-5-gliadin		

Table 1. Overview of the most important molecular food allergens used in this thesis

Peanut

Peanuts, seeds from the plant called Arachis hypogea, are classified as a legume belonging to the pea or bean species, thus technically not a nut, and cultured in tropic and subtropical areas of the world. Allergy towards peanut is common in many countries, and found in both children and adults, with a reported prevalence between 1.8-2.2 % (23-25).

An allergy towards peanut is usually developed early in life and is rarely outgrown (21, 24, 26). It is known to cause severe reactions in certain individuals and can lead to lethal anaphylactic reactions (27-29). Individuals with a coexisting asthma are at risk for severe

reactions, especially if undertreated (27, 30, 31) and can aggravate the severity of the reactions. With molecular allergology, so far 16 peanut molecular allergens have been identified with the potential to induce an IgE-development. Especially the heat resistant storage proteins Ara h 1, Ara h 2, Ara h 3 and Ara h 6 have been found to correlate with severe allergic reactions with the intake of peanut in sensitized patients(32, 33). While Ara h 2 and Ara h 6 share similarities in structure and often are found to occur in the same patients (34), individual differences have been reported (35). Ara h 3 are have been found early in the sensitization process and have been found in both peanut tolerant and peanut allergic participants (33, 34, 36, 37). Regional differences in prevalence have been described (33, 38). Sensitization to the lipid transfer protein (LTP) Ara h 9 has also been found in patients experiencing severe reactions, often the predominant peanut molecular allergen found in patients from the Mediterranean area (39, 40). In contrast, Ara h 8 molecule IgE is found to be correlated to a cross-sensitivity with the birch protein bet v 1, causing mainly OAS (32, 35, 41-43) .

Still, this knowledge about the diagnostic value of molecular allergology is not known to the general public, and many patients live in fear of severe reactions even though they can actually expect mild reactions.

Tree nuts

Among the tree nuts, hazel nut is common, mainly cultured in the southern parts of Europe and western Asia. Hazel nuts are rich of proteins and unsaturated fat and the fruit of the hazel tree. While a popular food item it is a common cause of allergic reactions, both mild and severe (44-46). It is usually the heat resistant storage proteins in the nuts that are the cause of the systemic reactions. For hazel nut the storage proteins sensitization to Cor a 8, Cor a 14, and Cor a 9 have been found to correspond to severe reactions while Cor a1 IgE is correlated to cross-sensitivity reactions related to birch allergy (24, 44-47).

Other tree nuts associated with intake reactions are walnut, pecan nut, almond and cashew (47, 48). For cashew, sensitization to the corresponding storage protein Ana o 3 is associated to more severe reactions (49, 50) and for walnut Jug r 1 and r 4 IgE that are linked to severe reactions (51, 52).

Milk, egg and wheat

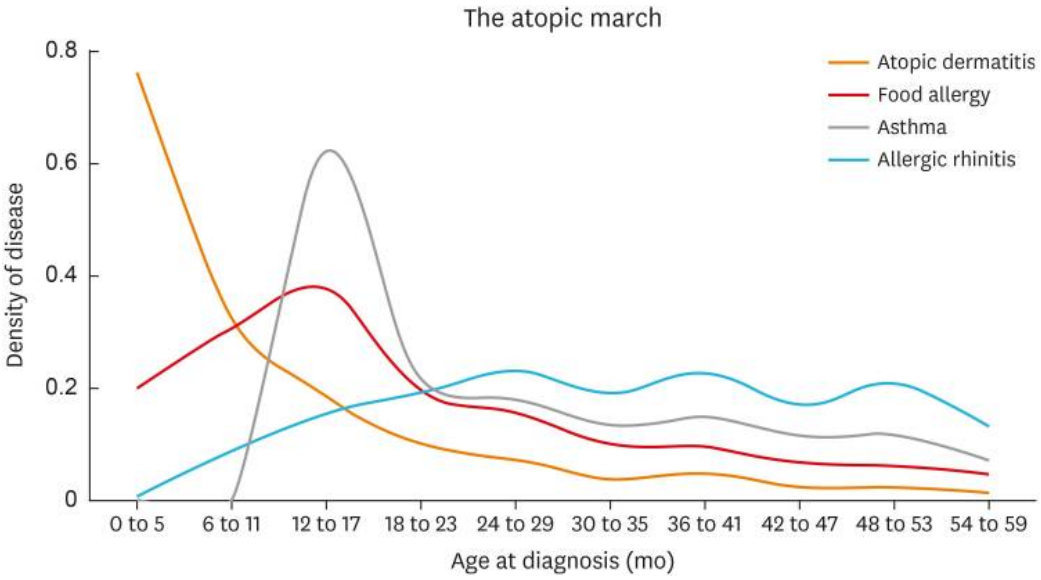
Allergen molecules have also been identified for common food such as wheat with Omega -5-gliadin (53), for milk Casein (54), for egg Ovomucoid (Gal d 1) (55), and is now used together with a medical history and descriptions of previous reactions to clinically evaluate actual allergy or mere sensitization.

The knowledge regarding presence of IgE to food allergen molecules in very small infants is sparse.

2.3 ATOPIC DISEASE

Atopic individuals are usually defined as persons with an underlying genetic susceptibility more prone to respond with the production of high levels of IgE-antibodies when exposed to allergens in the environment and to develop allergic diseases. Among the atopic diseases we generally include atopic dermatitis, food allergy, allergic rhinitis, and asthma. In the industrialized part of the world atopic diseases are among the most common chronic diseases, and affect individuals of all ages (56, 57).

The origin of these diseases is believed to be multifactorial, most likely affected by several a combination of factors, such as genetic susceptibility, perinatal exposures as well as environmental factors. While some individuals are affected by only one atopic disease, others develop several and suffer greatly. This type of development, often referred to as the *atopic march*, see Figure 2, is often beginning with the development of atopic dermatitis in infancy, followed by food allergy in toddlers and then in older children and adolescents by the development of allergic rhinitis and asthma (58, 59).



Ann Allergy Asthma Immunol **2018; 120:131-7**

Figure 2. Age at development of atopic diseases in childhood, (months)

To date, few *primary* prevention strategies are available against atopic disease except avoidance of tobacco smoke (60, 61) and in recent years evidence has emerged that delaying food introduction does not prevent allergy, but rather that early food introduction in infants at risk for peanut allergy is protective (62). Some studies have found the similar for egg (63, 64).

2.3.1 Atopic dermatitis

The human skin is a multilayer organ that protects the body from harmful substances (65). Several factors and diseases are known to affect the skin, one of the most common diseases is atopic dermatitis (AD), defined as an inflammatory process causing an itchy rash, previously

often named eczema. In small children it is most commonly located in the face and extensor side of the extremities, followed by bending folds and cheeks, while in teenagers and adults it is more common on elbows, hands or feet (66-68). It affects approximately 10-20% of the population in the western world and is often of more widespread character in small children than in adults (69, 70).

The skin serves as an important barrier, and a damaged skin surface makes it vulnerable to bacterial infections as well as easier exposed to both food and air-borne allergens (71, 72). One important factor identified to cause a deficient skin barrier is loss-of-function mutations in the filaggrin gene (*FLG*). Filaggrin is a protein vital for the function and structure of the skin's outer layer stratum corneum (73). Filaggrin mutations have been found in several studies to strongly predispose to atopic dermatitis and even to increase the risk of future asthma and rhinitis (74-76).

Skin care is important in treatment of AD, and use of emollients keep the skin moist and help reducing itching due to dry skin, and can be used as the only treatment in milder types of AD. With increasing severity topical corticosteroid creams are usually added to the treatment. Preventive use of emollient has been mentioned as a possible method to decrease development of atopic dermatitis, however as recently shown in two large birth cohorts preventive early skin care did not seem protective (77, 78).

Earlier research by Roduit et al has stated that the phenotypes of individuals with atopic dermatitis differs. Infants with atopic heredity from both parents suffered a five-fold higher risk of developing an early persistent atopic dermatitis compared to those with non-atopic parents (79). Early development of atopic dermatitis was also associated with co-existing food allergy, and an almost at three-fold risk of development of asthma later on. Atopic dermatitis usually precedes other atopic diseases such as asthma and allergic rhinitis (80).

2.3.2 Food allergy

Food allergy is usually defined as an adverse reaction after intake of a food allergen, which can be immediate, often within minutes, or more delayed and presenting after 24 hours. The symptoms range from minor/severe urticaria, light/sever swelling of lips, tongue and throat, itching, respiratory symptoms such as asthma, and in some cases lead to an anaphylactic reaction, a severe state that can be lethal (29).

The prevalence of food allergy varies depending on definition, age, type and part of the world. In earlier material the reported prevalence vary between 5-10 % in adults, often in the higher end higher if self-reported measurements have been used (81). Food allergy often develops early in life, usually in infancy, most commonly towards egg or milk, followed closely by other food allergens such as peanuts, tree nuts, soy, fish and wheat when the children start to introduce solid food in an higher extent (82).

The diagnose of food allergy is usually set when the patient experience symptoms in combination with the finding of elevated corresponding specific immunoglobulin E

antibodies in the blood, so called IgE sensitization, or by a measuring the wheal reaction on the skin after allergen exposure, a so called skin prick test (SPT) (83), as well as by an oral provocation.

While the skin prick test is quickly performed, cheap, minimal invasive and usually provides an answer within 20 minutes, it can be hard to repeat in a standardized way if not performed by skilled personnel (84). Measuring specific IgE in sera has become the most used method, in particular after the introduction of molecular allergology, explained more in detail in section 2.2, making it is possible to measure antibodies to specific parts of the food allergen (85). Research has identified some allergen molecules to be associated with severe reactions, such as Ara h 2 in peanut allergy. While the golden standard method for diagnosing a food allergy is by a double-blind oral provocation (22), this is costly, not always possible and a both time- and resource consuming way, thus the majority of provocations performed in clinical settings are simple unblinded oral provocations. With the use of molecular allergology in addition to specific IgE we can now obtain a more accurate diagnose of a food allergy, especially when oral food challenge is not possible (86).

The risk for food allergy increases if there is a history of atopic disease in the family (87-89). While an allergy towards milk and egg has a good chance to grow away, other types of allergy often tend to remain for the rest of the life, such as peanut allergy (90).

Oral allergen exposure is important for the induction of tolerance development to foods. Studies suggest that early, rather than later introduction of peanut may reduce allergic sensitization as well as symptoms (62, 91).

Recently more evidence have emerged indicating that epicutaneous exposure to food allergens, as in patients with a damaged skin barrier, rather than by the preferred oral intake route, might increase the risk of allergy development (92, 93).

Information about the ideal time for food introduction in order to prevent food allergy development and if this vary depending on the different types of food and in different individuals is sparse. However, delaying food introduction does not protect against allergy development (94-96). Knowledge concerning how often and how large amount of an allergen that needs to be consumed in order to protect from allergy development is still uncertain (97).

2.3.3 Allergic rhinitis

Allergic rhinitis, often mentioned as hay fever, is characterized by inflammation in the nasal mucosa due to allergen exposure, causing itching in the nose and eyes, rhinitis, runny nose and often conjunctivitis. The prevalence vary in the world, from 12-46 % depending on country of origin in the ECRHS study (98), and in Scandinavian countries increasing prevalence are seen in the last decade (99). A gradual disease development is often seen that starts in preschool age, affecting 8-15 % of the children (98), but rhino conjunctivitis can emerge in adults as well.

The major triggers are of allergic rhinitis are air borne allergens such as timothy, birch or mug worth, furry animals, mold and dust mite. Allergic rhinitis is also known to be precursor to allergic asthma (100-102).

2.3.4 Asthma

Asthma is a respiratory disease caused by inflammation in the respiratory tract and an involuntary contraction of the airways exposed to specific triggers and found both in children and adults. Prevalence rates vary depending on used definition, gender, asthma phenotype, age and country of origin, but roughly it varies from 8-12 % in small children and adolescents, to around 4-8 % in adults (103-105) in western countries, tending to be much higher in areas with much air pollution (106).

In small children asthma is most commonly triggered by respiratory infections (107-109), while in older children and adults it's mainly triggered by exercise (110) or allergens such as pollen or furry animals (111, 112). Other trigger factors can be cold temperature (113, 114) or indoor dust (115-117). Allergic asthma triggered by intake of food allergens in food allergic patients can lead to severe reactions, as part of an anaphylactic reaction, in some cases even with lethal outcomes (27, 29).

The risk for asthma increases in for example children exposed early to certain respiratory infections such as the respiratory syncytial virus (RSV) or rhinovirus (107-109, 118). Exposure to cigarette smoke also increases the risk, even with indirect exposure (61, 119-122). Parental atopic history and other atopic traits in the child have been found as the highest risk factors for asthma development in children, as well as for the development of a more persistent asthma phenotype development (123). While the risk for asthma development was approximately 10 % in children from non-atopic parents the accumulative risk has been found as high as 40 % if both parents had an atopic history in the German MAS study (124).

2.4 MARKERS OF AIRWAY AND SYSTEMIC INFLAMMATION

Several inflammatory markers have been identified to be elevated in patients with asthma as well as in patients suffering from IgE-mediated food reactions (112, 125, 126). In allergic reactions mast cells release histamine and other cytokines triggering a cascade in which inflammatory cells attract and build up inflammation and pruritus (127, 128). The damage in the epithelial cells triggers a so-called T helper cell type 2 (Th2) mediated cell response with increased levels of Th2 helper cells.

Among the markers that have been studied so far is measurement of eosinophilic granulocytes in blood (129, 130) and fraction of exhaled nitric oxide, FENO, that are found to be elevated in both asthmatics, in patients with nasal polyposis as well as in patients with peanut allergy (129, 131-133). However, new research is ongoing concerning other potential inflammatory markers that can be useful in the future (134-136).

From at clinical point of view we are often faced with a patient without asthma but with peanut allergy that is expressing elevated FENO (131, 132), posing the question if this is a sign of untreated air way inflammation requiring inhaled corticosteroid treatment or some other mechanism without need of clinical intervention.

2.5 SENSITIZATION PATTERNS AND KNOWLEDGE GAPS

There is a knowledge gap regarding how the different IgE-patterns develop, if they vary depending on type of allergen or what type of patterns that tend to remain vs disappear.

There is also lack of research regarding the very early development of IgE in infants, in relation to maternal IgE, atopic history and perinatal factors, especially regarding previous studies with information on relevant molecular allergens. The effect of early food intervention and or/ skin treatment on IgE development is not sufficiently studied in small children of preschool age, especially in terms of future peanut allergy.

Previous research has found that patients expressing with molecular spreading of IgE, e.g. those who are polysensitized against several corresponding molecular allergens simultaneously, are often found to have a more severe disease phenotype. This is also seen in patients with airborne allergies against grass and birch, leading to earlier time of onset and/or development of allergic asthma, than in those who are merely mono-sensitized (137). The concept of molecular spreading is not sufficiently studied in children with food allergy. Few population-based cohorts have followed patients from birth to adolescence with specific IgE, molecular allergens and information about allergic symptoms.

Furthermore, asthma, lung function and respiratory inflammation during childhood in relation to development and severity of peanut allergy need to be further investigated.

Longitudinal birth cohort studies examining intrauterine, early-life, childhood and adolescent exposures combined with blood sampling for IgE offer novel approaches to study this further.

3 RESEARCH AIMS

Objectives

Main objective: to investigate in longitudinal cohort settings if specific IgE-profiles can predict allergic diseases from birth to adulthood.

Specific objectives:

Study I: to investigate the relationship between maternal and perinatal risk factors and early infant sensitization.

Study II: to describe peanut allergen sensitization during first year of life in relation to food allergy at 36 months of age

Study III: to study if IgE-levels to peanut allergens and to peanut allergen molecules are associated to peanut allergy symptoms, from childhood to adulthood.

Study IV: to evaluate how tree nut sensitization correlates to clinical allergy symptoms at 24 years of age

4 MATERIALS AND METHODS

4.1 STUDY DESIGN AND STUDY POPULATIONS

All four studies included in the thesis were performed as observational studies within two preexisting birth cohorts.

4.1.1 The PreventADALL birth cohort

For study I and II we used the Nordic population based birth cohort PreventADALL (Preventing Atopic Dermatitis and ALLergies in Children) consisting of 2697 pregnant women recruited at the 18-week routine ultrasound examination from December 2014 until October 2016, in Norway (Oslo and Østfold) and Sweden (Stockholm) (138), see Figure 3. Inclusion criteria for enrolment in pregnancy were sufficient maternal language skills in the Scandinavian languages, singleton or twin pregnancy without severe malformations or disease. Enrolment at gestational week 18 included a brief structured interview, and measurements of height, weight and blood pressure measures as well as blood sampling. The included women completed detailed electronic questionnaires both at enrolment and at 34 weeks of pregnancy including information on socio-demographics, atopic heredity, living conditions, smoking, and maternal antenatal health.

Their offspring (N=2394) were included at birth usually within 24 hours, given a gestational age of ≥35 weeks and no severe disease, and randomized to four different equally sized groups, 3 interventions groups and one control group. The interventions consisted of either early skin care (with oil baths at least 4 times a week and facial cream application with emollient cream between 2 weeks and 9 months of age), or early food introduction from 3 months, and a combined skin and food group. The food introduction was given as taste sensation applied on parent's finger or by teaspoon, to be given at least 4 times a week until 6 months of age. First dose of peanut butter was given at time of 3 months follow up visit, then followed by weekly introduction of cow´s milk, wheat (wheat porridge) and egg (scrambled). Birth data was collected from birth charts at inclusion of the new-born as described elsewhere (138).

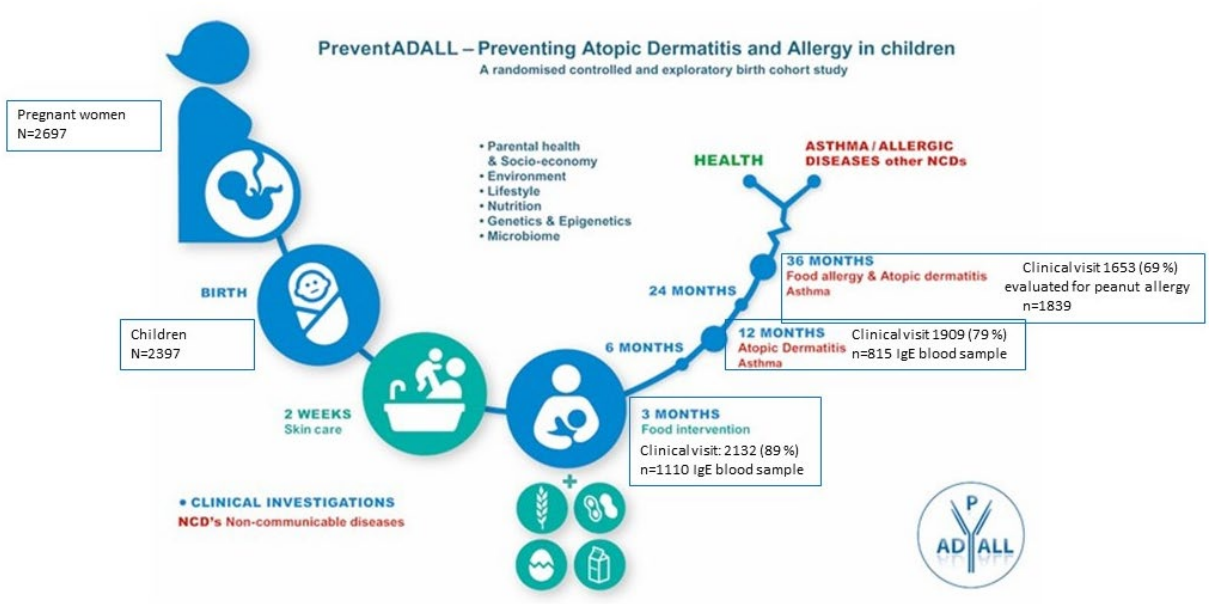


Figure 3. Overview of the PreventADALL cohort and time of clinical investigations.

In study I the study population consisted of 1110 mothers and their infants with data from both clinical visits, background information about atopic history and available sera for IgE measurements.

In study II our study population consisted of 815 children with available data from IgE measurements at 12 months of age and results from the peanut allergy evaluation at 3 years of age.

4.1.2 The BAMSE birth cohort

For study III and IV we used the BAMSE-cohort which is a Swedish population-based birth cohort consisting of 4089 children born 1994-1996 in Stockholm (139). The participants have been followed from birth (median age 2 months) and at 1, 2, 4, 8, 12, 16 and 24 years of age. Background data was collected at time of inclusion (baseline). At follow-up, history of disease as well as extensive data of different exposures was collected, Figure 4.

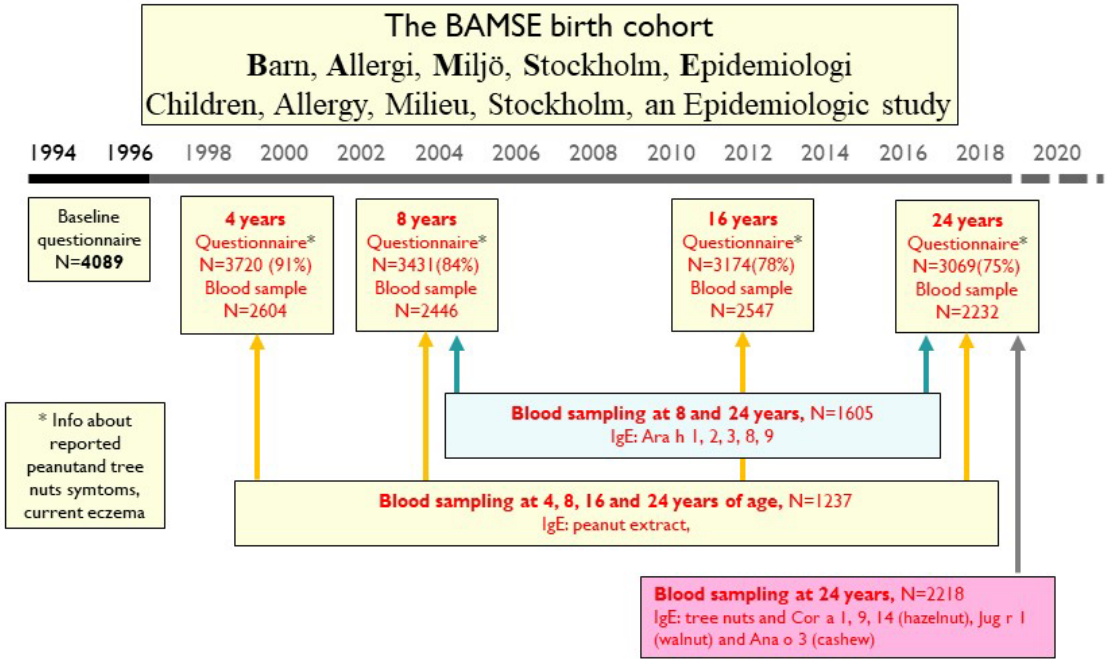


Figure 4. The BAMSE cohort: questionnaire and IgE blood sampling timepoints.

In study III our study population consisted of 1167 participants with available background data regarding atopic history, reported allergic symptoms, measurements of lung functions and blood samples for IgE measurements at 4, 8, 16 and 24 years of age.

In study IV the study population consisted of 2217 participants with available background data, history of atopic disease, reported allergic symptoms and blood sampling for IgE analysis of IgE towards tree nuts at 24 years of age.

4.2 DATA COLLECTION AND DEFINITIONS

In PreventADALL the children were followed-up clinically at visits at three, 6, 12, 24 and 36 months of age including anthropometric measurements, clinical examination as well as blood sampling, all done by trained study personnel. Week 2-26 short electronic diaries were filled in by the parents and during the first year every 3rd month a more extensive electronic questionnaire, then given twice annually. At the 1 year follow up 79 % of the original cohort attended the clinical examination, and blood samples were provided by half of them. At the age of 36 months 69 % attended the examinations, and blood samples were provided by 56 % of them, see figure 3.

In BAMSE clinical investigations with measures of lung function and IgE measurements were performed at 4, 8, 16 and 24 years of age, figure 4. At the 24- year follow-up the questionnaire response frequency was 75% of the original cohort and 56 % of the original cohort participated in the clinical investigation. The 24-year follow-up included clinical

examination with blood sampling and lung function tests such as spirometry, NO and information about asthma.

4.2.1.1 Sensitization

In PreventADALL the pregnant women provided sera at inclusion around 18 weeks gestational age, and were analyzed for allergen-specific IgE levels using ImmunoCAP (Thermo Fischer Scientific, Uppsala, Sweden): with the Phadiatop® (for birch, cat, dog, horse, grass, mugwort, house dust mites (Dermatophagoides pteronyssinus), and Cladosporium herbarum) and with Fx5 (for cow's milk, egg white, wheat, peanut, cod). If a sample scored positive IgE ≥0.35 kU_A/L to one of the mixes, further analyses of specific IgE towards allergens included in the mixes were performed. Allergic sensitization in women was defined as IgE levels ≥0.35 kU_A/L.

Blood samples were collected from the children at the three months and 12 months visit, and analyzed for specific (s-) IgE to food and inhalant allergens by using ImmunoCAP Phadiatop Infant® (birch, cat, dog, grass, cow's milk, egg white, peanut). In case of positive Phadiatop Infant (≥0.1 kU_A/L), s-IgE to each allergen in the mix was further analyzed. Additionally, s-IgE to wheat extract was analyzed in all infants with available sera. In infants that scored positive to whole extract (IgE ≥0.1 kU_A/L), we further analyzed relevant allergen components within the food allergens; for egg ovomucoid (Gal d 1), for milk casein, for peanut Ara h 1, Ara h 2 and Ara h 3, and for wheat omega-5-gliadin. Infant sensitization was defined as an allergen-specific IgE level of ≥0.1 kU_A/l.

In BAMSE IgE measurement was done for food allergens with Fx5-mix (cow's milk, egg white, wheat, peanut, cod) and to air- borne allergens with Phadiatop® mix (birch, cat, dog, horse, grass, mugwort, house dust mites (Dermatophagoides pteronyssinus), and Cladosporium herbarum) in sera with ImmunoCAP (Thermo Fisher, Uppsala) at 4, 8, 16 and 24 years of age. If the mix was positive (cut off ≥0.35 kU_A/L), IgE to the included allergens where analyzed. Sensitization was defined as specific IgE ≥0.35 kU_A/l.

ImmunoCAP was also used for IgE measurement of peanut allergens Ara h 1, Ara h 2, Ara h 3, Ara h 8 and Ara h 9 at 8 and 24 years of age, and performed if specific IgE to peanut were ≥0.35 kU_A/l. Sensitization to peanut allergen molecules was defined as IgE ≥0.1 kU_A/l.

Among participants at 24 years of age IgE to tree nuts were analysed in sera with ImmunoCAP (Thermo Fisher, Uppsala) using fx1® mix (peanut, hazel nut, coconut, brazil nut and almond) and fx22® mix (pecan, cashew, pistachio and walnut). If the mix was positive (cut off ≥0.35 kU_A/L), IgE to the included allergens were analyzed. Sensitization was defined as specific IgE ≥0.35 kU_A/l and if specific IgE to the extract ≥0.35 kU_A/l, IgE towards the corresponding tree nut allergen molecules Cor a 1, Cor a 9, Cor a 14, Jug r 1 and Ana o 3 were analyzed.

4.2.1.2 Atopic dermatitis

In PreventADALL at time of clinical visits at 3 months, 6 months, 12 months and 36 months the children were examined by trained study personnel for signs of clinical observation of eczematous lesions using evaluation forms based on the criteria set by both Hanifin & Rajka (140) and the UK Working Party criteria (141). As small children rarely fulfill these criteria even though they could be experiencing extensive atopic dermatitis in study II we defined *Atopic dermatitis by 24 months* as clinical observation of eczematous lesions by trained physicians, excluding common differential diagnoses for atopic dermatitis, such as seborrheic dermatitis and irritative contact dermatitis, observed at any of the clinical investigations by 24 months of age.

In BAMSE the diagnose atopic dermatitis (AD) at 24 years was based on clinical examination fulfilling UK Working Party criteria also called William's criteria (141) and defined as reporting an itchy rash in the last 12 months prior to the questionnaire at 24 years of age in combination with 3 out of 4 of the following criteria:

1) dry skin last 12 months prior to questionnaire 24
2) atopic dermatitis onset below age 2 years (based on questionnaire data)
3) History of flexural atopic dermatitis (at any follow-up)
4) Personal history of asthma and/or rhinitis (at any BAMSE follow-up from age 4 years).

4.2.1.3 Peanut allergy and peanut allergy symptoms

In PreventADALL *peanut allergy at 3 years* of age were evaluated with a diagnostic classification system based on the algorithm used in the BEEP study (142). A diagnose of food allergy was defined based on parental reported symptoms of suspected allergic reactions (rash/erythema on face or body, urticaria, aggravated atopic dermatitis, angioedema, vomiting/stomach pain, red eyes, itching of mouth/lips, sneezing, cough/hoarseness, difficulty breathing, somnolence/unconsciousness, red eyes) AND either a positive skin prick test larger than 3mm/ or peanut extract IgE $\geq$0.1 kU_A/L, or confirmed by a positive oral food provocation (91). Unclear cases underwent judgement of an expert panel.

In BAMSE *peanut allergy symptoms at 24 years* were defined as having specified avoidance of peanut at 24 years and reported specified symptoms at 16 years and/or 24 years, such as unconsciousness, asthma, hoarseness, swelling of face, lips and eyes, general urticaria, partial urticaria, GI-symptoms (vomiting, stomach pain), rhino conjunctivitis, oral symptoms consistent with oral allergy syndrome (OAS) or other.

4.2.1.4 Allergy towards egg/milk/wheat

In PreventADALL *Egg/Milk/Wheat allergy at 3 years* was defined by judgement of the expert panel (based on parental reported symptoms of suspected allergic reactions (rash/erythema on face or body, urticaria, aggravated atopic dermatitis, angioedema, vomiting/stomach pain, red eyes, itching of mouth/lips, sneezing, cough/hoarseness, difficulty breathing, somnolence/unconsciousness, red eyes) AND either a positive skin prick

test above 3mm/ egg/milk/wheat extract IgE ≥0.1 kU_A/L, or confirmed by a positive oral food provocation).

In BAMSE allergy against egg /milk at 4 years of age was defined as specified symptoms such as unconsciousness, asthma, hoarseness, swelling of face, lips and eyes, general urticaria, partial urticaria, GI-symptoms (vomiting, stomach pain), rhino conjunctivitis, oral symptoms to egg/milk at the four-year follow up questionnaire in combination with sensitisation to egg white extract/milk extract at four years of age (≥0.35 kU_A/L).

4.2.1.5 Inflammatory markers in BAMSE

Expression of 92 inflammation-related proteins in plasma were analyzed using the Proseek Multiplex Inflammation Panel (version 95302) from Olink Biosciences, Uppsala, Sweden, as previously reported (143). Protein levels are expressed as Normalized Protein Expression (NPX) units, a relative quantification unit logarithmically related to protein concentration.

The participants also left blood samples where levels of blood eosinophils were measured.

4.2.1.6 Lung Function Tests in BAMSE

Spirometry was performed using the Jaeger MasterScreen-IOS system (Carefusion Technologies, San Diego, CA, USA)(144). Highest values of forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) were used, and the FEV_1/FVC ratios were expressed as percentages. Standard deviation scores for FEV_1, FVC and FEV_1/FVC were computed taking age, sex, height and ethnicity into account as previously described(145). *Reversibility* was defined as positive if FEV_1/FVC was above 12 %. *Low FEV_1/FVC* was defined as a ratio of FEV_1/FVC <0.7.

Exhaled Nitric oxide (FENO) was measured using Eco Medic instrument system (Eco Medics, Duernten, Switzerland) and the single-breath technique was used according to the American Thoracic Society and European Respiratory Society guidelines (146).

High FENO at 24 years was defined as FENO >20 parts per billion (ppb).

4.2.1.7 Asthma in BAMSE

Asthma at 4 years was defined as: More than 3 episodes of wheeze in the last 12 months prior to the date of the 4-year questionnaire and/or at least one episode of wheeze in the last 12 months prior to the date of 4-year questionnaire, combined with prescription of inhaled steroids for symptoms of asthma.

Asthma at 24 years used for both study III and IV was defined as ever having a doctor's diagnosis of asthma together with symptoms of breathing difficulties in the last 12 months prior to the date of questionnaire at 24 years of age or used asthma medicine occasionally or regularly last 12 months.

Severe asthma at 24 years was defined as fulfilling the *asthma at 24 years* definition above in combination with at least 2 months usage of both inhaled corticosteroids and inhaled long acting beta2 agonist in the last 12 months and either of the following in the last 12 months due to asthma symptoms: Use of oral cortisone tablets or acute visits to emergency room or perceived impaired daily life or more than 12 episodes of breathing difficulties.

4.2.1.8 Rhinitis

In BAMSE *Rhinitis at 24 years* was defined as prolonged sneezing or a runny or blocked nose without common cold in the last 12 months prior to the date of questionnaire at 24 years of age.

4.3 DATA ANALYSIS

Statistical analysis in all four studies was made using STATA Statistical Software (15.0 or later).

In study I prevalence rates were expressed as numbers and proportions. The Chi square test was used for comparison of dichotomous variables between groups. The Fisher exact test was used if one comparison group consisted of 5 observations or less. Group IgE levels were expressed as median values and interquartile ranges. Two-tailed t-test was used on log transformed values for group comparisons of IgE levels. P-values of less than 0.05 were considered significant. Odds ratios (OR) with 95% confidence intervals (CIs) were calculated using logistic regression for the association of sensitiszation in relation to background factors. All estimates with a p-value of 0.2 or below were then in a second multivariate analysis included in the adjusted model.

For study II the results were expressed as numbers and proportions (as percentages) as well as median values. Categorical variables are presented as numbers and percentage and examined through Chi-squared tests. P-values were regarded as statistically significant if <0.05. Odds ratios (OR) with 95% confidence intervals (CIs) were calculated with logistic regression for the analysis of associations between peanut extract and peanut allergen molecules sensitization in relation to peanut allergy at three years of age, followed by interaction analysis and stratification for intervention group.

In study III the prevalence rates were presented as numbers and proportions. Chi (2)-test was used for comparison of dichotomous outcomes and t-test used to account for group differences in normally distributed continuous outcomes, for example log transformed IgE-levels. P values of <0.05 allowing rejection of the null hypothesis of no significant difference were considered as significant. For comparison of median levels of continuous variables, non-parametric median test was used. Mann Whitney U test was used to analyze difference in levels of inflammation-related proteins based on storage protein sensitization and additionally stratified by asthma. Significance in the protein analyses were based on false discovery rate (FDR) of 5% using the Benjamini–Hochberg procedure (147) but nominal p-values <0.05 are reported.

Finally, in study IV the prevalence rates were presented as numbers and proportions, median values and interquartile ranges (IQR) were reported for continuous data. Sensitivity, specificity, PPV and NPV was calculated. Two-tailed t-tests on log transformed values were used to account for group differences in continuous values with skewed distribution, i.e. the sIgE-ab-levels. P-values of <0.05 were considered statistically significant. Odds ratios (OR) with 95 % confidence intervals (CIs) were calculated using logistic regression for tree nut sensitisation at 24 years in relation to early risk factors such as eczema, asthma, other food allergies, sex and parental allergy. OR estimates with 95% CI were presented from both univariate and multiple logistic regression. Logistic regression was also used for estimation of reported allergy in relation to sIgE-ab-levels to the different tree nut allergen molecules. Probability calculations for the likelihood of tree nut symptoms were performed based on the results from the logistic regression.

5 ETHICAL CONSIDERATIONS

No research should be done without careful ethical consideration, especially involving children. However, without research much knowledge would not be available, such as better medications or remedies as well as underlying mechanism that can be prevented. The impact of the possible research results on future health and future disease avoidance must always be weighed against the risk for harm in the participants, both physical and emotional. Ethical considerations are therefore very important and needed for register-based as well as cohort studies. Following the Helsinki declaration research on underage individuals need special considerations. For research on under age children parental consent is mandatory. The parents of participants in both the BAMSE project and PreventADALL project were well informed and highly motivated to participate in the study. We have aimed at making the follow-up visits as agreeable as possible for the participants both regarding investigations and sampling, for example by the usage of EMLA cream (a local anaesthesia) that were applied before blood sampling were done. In the PreventADALL study with very young children no more than two attempts to draw blood were made. No further testing or investigations were done if it was not judged appropriate at each specific visit.

Food provocations were done if skin prick tests, IgE-results and symptoms were inconclusive. This is a process that can cause harmful as well as painful reactions, both minor and severe, and it could be discussed if this was in the best interest of the children. But an uncertain food allergy diagnosis also leads to a lot of anxiety for both child and parents, as well as restrictions in daily life. Food challenges are in general regarded as a positive experience by the families even if the child reacts because they know they do not avoid the food unnecessarily. If following a proper safety protocol this can be done in a secure way (148), which was used in PreventADALL (no food challenges were performed in the BAMSE-studies included in this thesis).

All four included studies had ethical approval, and overview of the permissions is shown in Table 2.

Ethical approval for the PreventADALL study was obtained from the Swedish Ethical Review Authority, Sweden, and the Regional Committee for Medical and Health Research Ethics in South-Eastern Norway and signed informed consent were collected from the women and from both parents of the infants. PreventADALL is also registered in ClinicalTrials.gov; identifier: NCT02449850.

Ethical approval for the BAMSE study was obtained from the Swedish Ethical Review Authority (Etikprövningsmyndigheten). Written informed consent were collected from parents and from the participants when entering the 24-year follow-up.

Study	Cohort	Ethical approvals
I	PreventADALL	2014/2242-31/4 (Swe) & (2014/518) (No)
II	PreventADALL	2014/2242-31/4 with amendment 2018/1437-32 (Swe) & (2014/518) (No)
III	BAMSE	93:189; 98–175; 02–420; 2007/1634–31; 2010/1474–31/3; 2016/1380–31/2
IV	BAMSE	93:189; 98–175; 02–420; 2007/1634–31; 2010/1474–31/3; 2016/1380–31/2

Table 2. Overview of ethical permissions

6 RESULTS

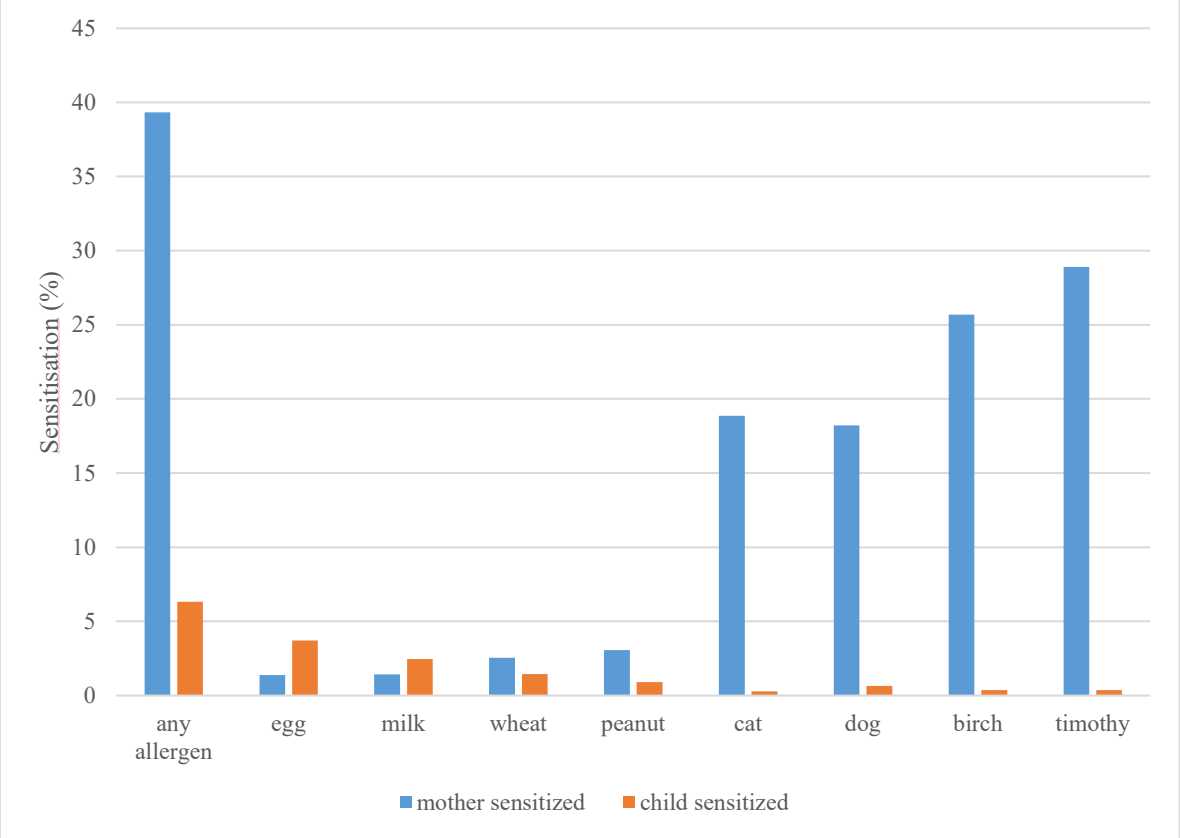
Main findings:

6.1 EARLY INFANT SENSITIZATION AT 3 MONTHS OF AGE

In study I we investigated 1110 mother-child pairs in PreventADALL where the infant had provided sera for IgE analysis and had available background data as well as data on maternal sensitization.

Already at three months 79/1086 (7.3 %) of the infants presented with IgE levels, most often towards food allergens. The majority were sensitized against egg or milk. Corresponding molecular allergens could be found in 1/3 of the analyzed samples. Only 1 % had detectable IgE towards airborne allergens.

While infants mainly expressed IgE towards food the majority of the sensitized women presented with specific IgE towards air borne allergens, most commonly grass, Figure 5.



Allergy. 2021 Sep;76(9):2730-2739

Figure 5. Specific IgE sensitization prevalence among mothers and infants at 3 months of age, N=1110, (%) (>0.1 kU/L).

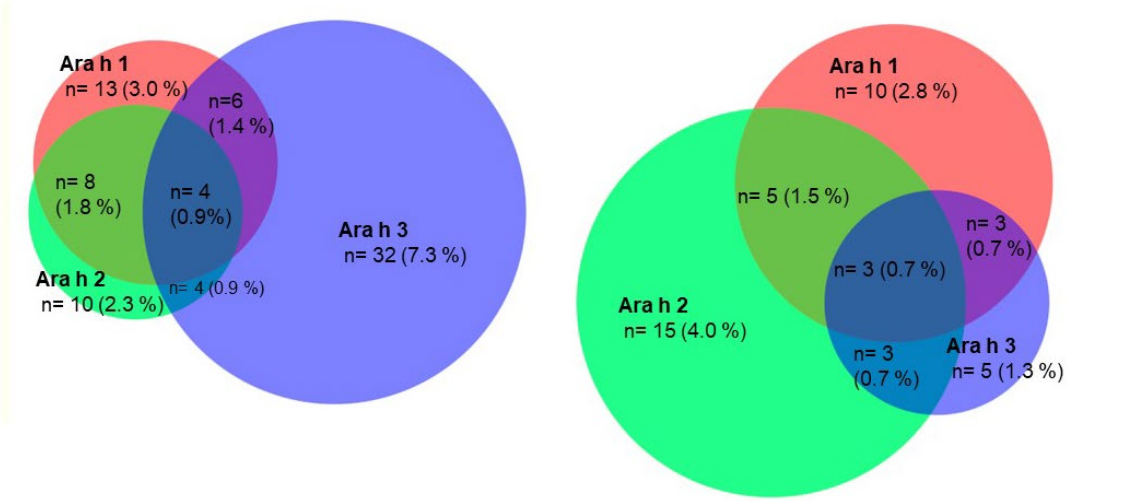
When studying potentially associated maternal and perinatal factors using logistic regression we identified an association between maternal food sensitization and offspring sensitization,

and this association remained significant even after adjusting for other potential confounders, OR 3.64 (95 % CI, 1.53-8.68).

6.2 ARA H 3 MORE COMMON IN FOOD INTERVENTION GROUP

In study II we included 815 children in PreventADALL that provided sera for IgE analysis at the 12 months follow up and had been evaluated for peanut allergy at age 36 months.

120/808 (14.8 %) children that left blood sample for IgE analysis at 12 months of age were sensitized against peanut extract, but only 15/120 (12.5 %) of them were defined as allergic to peanut at 3 years of age. When further analyzing the sera for IgE towards peanut molecular allergens we found Ara h 3 to be the most commonly detected, found in 37/795 (4.6 %), and especially among children randomized to early food introduction, see Figure 6 A-B.



Unpublished data from study 2

Figure 6A. Peanut allergen sensitization at 12 months among food intervention group, N=435

6B. Peanut allergen sensitization at 12 months among non-food intervention group, N=380

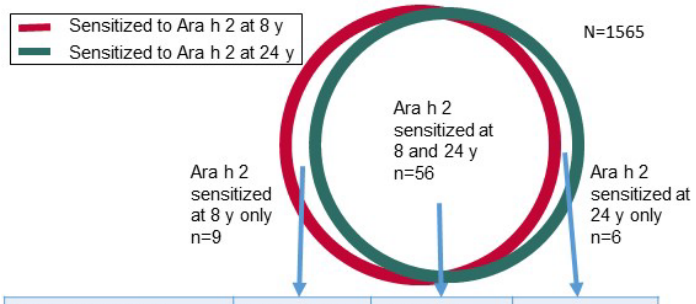
Most of the sensitized children were later defined as not allergic to peanut at the age of 36 months. On the contrary to participants with Ara h 2 sensitization that most often were judged peanut allergic, and also more often had atopic dermatitis and egg allergy.

In this age polysensitization to the peanut allergen molecules was not common, the majority of children were mono-sensitized to one allergen molecule, 48/795 (6.0 %).

6.3 ARA H 2 SENSITIZATION RARELY EMERGES AFTER 8 YEARS AND DO NOT DISAPPEAR

In study III we analyzed BAMSE participants that left blood sample for peanut extract at age 4, 8, 16 and 24 years of age as well as reported peanut intake symptoms to study how peanut allergy and peanut sensitization developed over time. We concluded that genuine peanut

allergy with Ara h 2 sensitization rarely emerges after 8 years of age and seldom disappear, other than in individuals with initial low Ara h 2 IgE levels, Figure 7.



Ara h 2 IgE-level 8y, median (range) kU_A/L	0.4 (0.1-2.0) kU_A/L	39.5 (0.41->100) kU_A/L	-
Ara h 2 IgE-level 24y median/ind. (IQR) kU_A/L	-	15.5 (<0.35->100) kU_A/L	0.1, 0.11, 0.12, 1.1, 1.7, 6.2* kU_A/L
Peanut symptoms 8y, n/N (%)	2/9 (22%)	48/56 (85%)	3/6 (50%)
Peanut symptoms 24y, n/N (%)	4/9 (44%)	54/56 (96%)	4/6 (67%)
Asthma at 24y, n/N (%)	5/9 (56%)	25/56 (45%)	4/6 (67%)
Birch sensitization at 24y, n/N	6/9 (67%)	34/56 (61%)	6/6 (100%)

Unpublished data from study III, manuscript submitted to journal

Figure 7. Characteristics of participants with transient, persistent and de novo Ara h 2 sensitization. N=1565, cut off (>0.1 kU/L), *= individual Ara h 2 IgE levels

6.4 ELEVATED SYSTEMIC INFLAMMATORY MARKERS IN PEANUT SENSITIZED PARTICIPANTS EVEN WITHOUT ASTHMA

In study III we also aimed to investigate if elevated FENO was correlated to peanut and tree nut storage protein sensitization and if this would be a sign of a generalized systemic inflammation process. We therefore analyzed a panel of 92 potentially relevant inflammatory plasma proteins in the 2217 participants that at 24 years of age left blood samples for IgE as well as measurements of FENO-levels and blood eosinophils.

We found that the storage protein sensitized individuals had higher levels of FENO and blood eosinophil cell counts, as compared to individuals sensitized to inhalant or food allergen extract but without being sensitized to storage proteins. The levels of FENO were also higher among non-asthmatics sensitized to storage protein compared to asthmatics sensitized to other allergens but not any of the storage proteins.

6.5 MANY TREE NUTS SENSITIZED ARE ASYMPTOMATIC

In study IV the study population consisted of 2215/4089 participants in the BAMSE study that provided sera for IgE analysis of tree nuts at the 24 year follow up. Sensitization towards tree nuts extract, especially towards hazelnut, was common without reporting symptoms,

especially in patients with simultaneous sensitization to birch. Among the risk factors for persistent tree nut storage protein sensitization at 24 years was allergy towards egg and atopic dermatitis in preschool age and this associations remained in adjusted analysis, see Table 3.

	Tree nut allergen storage molecule sensitization combined with reported tree nut symptoms at 24 years, n=46	
	Crude Odds Ratios	Adjusted Odds Ratios*
	OR (95% CI)	OR (95% CI)
Wheeze at 1-2 years	**2.16 (1.19-3.89)**	0.76 (0.34-1.72)
Asthma at 4 years	**7.00 (3.63-13.5)**	**5.59 (2.35-13.3)**
Egg allergy¤ at 4 years	**24.6 (9.28-65.2)**	**8.50 (2.15-33.6)**
Milk allergy¤ at 4 years	**30.1 (8.12-111)**	1.55 (0.26-9.28)
Eczema (doctor's diagnose) at 1-2 years	**5.24 (2.90-9.46)**	**2.53 (1.21-5.32)**
Male sex	1.65 (0.92-2.98)	1.22 (0.60-2.48)
Parental allergy	**1.89 (1.05-3.40)**	1.19 (0.59-2.42)

¤allergy defined as both specific symptoms (including OAS) and specific food extract sensitization
*adjusted for all included early-life factors in the univariate (crude) analyses

Table 3. Odds ratio for tree nut allergen storage molecule sensitization combined with reported tree nut symptoms at 24 years of age in relation to early life factors. Sensitization (≥0.1 KU_A/L) to Cor a 9 and Cor a 14 (hazelnut), Ana o 3 (cashew) or Jug r 1 (walnut) at 24 years of age.

7 DISCUSSION

7.1 MAIN FINDINGS

We aimed to identify sensitization patterns that could help predict peanut allergy development and possibly to identify other patterns that could be less persistent.

Few infants expressed sensitization at 3 months of age, and only a small fraction of them had developed antibodies to the corresponding allergen molecules, which to our knowledge has not been reported before.

When following participants in BAMSE longitudinally we found that participants with atopic dermatitis and egg allergy in preschool age were at risk for more severe allergy phenotype, as well as tree nut storage protein sensitization and they were also more often polysensitized to several peanut molecular allergens and tree nut storage proteins. Storage protein sensitized individuals were also found to have elevated levels of inflammatory markers in both airways and blood, even without being diagnosed with asthma.

7.2 PREVIOUS RESEARCH IN RELATION TO OUR RESULTS

Few cohorts have measured IgE on an allergen molecule level in very small infants, many used skin prick tests or total IgE, which is why comparison between other cohorts is not always possible. Our prevalence of 7 % for any sensitization prevalence at 3 months in the PreventADALL cohort were lower than the Danish DARC study, who found 12.5 %, but in contrast our prevalence of egg and milk sensitization at 3 months of age were almost twice as high (10), thus more in line with the Australian BEAT study (which in turn used SPT and included infants at high risk for atopic disease) (64). One explanation could possibly be the high rates of included atopic mothers in PreventADALL, thus including more children at risk for future allergic disease.

At 12 months of age 14.9 % of the children in PreventADALL were sensitized against peanut, which is higher than the prevalence reported at the same age by the two British studies FAIR and the Isle of Wight (0.4 % vs 0.65 % had positive SPT for peanut (149, 150)) as well as in a small Swedish study where 5.6 % were sensitized to peanut extract at 12 months of age (151) and a Danish study who found 1.2 % peanut IgE positive (152). In contrast, the LEAP study found over 30 % of their participants to be sensitized to peanut extract IgE at time of inclusion in their cohort of children age 4-11 months at high risk of allergic disease (36).

The 1.9 % prevalence of peanut allergy at 3 years of age (and food allergy in general) tended to be a little lower in PreventADALL than in BAMSE and other similar cohorts such as HealthNuts (3.1 % at 6 years) but similar to FAIR cohort and Isle of Wight who reported 0.5 % and 1.2 % peanut allergy at 3-4 years of age (149, 150). This could possibly be explained by the fact that there is almost 20 years' time difference between onset of the BAMSE study and the PreventADALL cohort. Also, PreventADALL was an interventional study where

many parents had an interest in potentially decreasing peanut allergy, thus more motivated to introduce peanut in time than in the general population even if not in the food intervention group. Also, since included children were followed close by pediatricians/dermatologists/ allergologists, they were more likely to get proper advice on how to continue with food allergens after a suspicious allergic reaction once food allergy was ruled out with an oral food provocation, a method which is not always easily available for the general population. Last, but not least, oral food challenges were performed in the PreventADALL study in children with inconclusive food allergy status. In BAMSE, no food challenges were performed.

Our finding of elevated Ara h 3 IgE at age 12 months in PreventADALL have been seen in other studies, such as Hemmings et al and Zambrano et al.(37, 153), however both these studies were made in small populations of selected individuals with an existing nut allergy rather than in the general population. While few studies have measured peanut allergen molecules before and after peanut introduction this was done in the LEAP study, who included children age 4-11 months at high risk for allergic disease who were randomized to avoidance or introduction of peanut (36). They found at 12 months of age a difference in Ara h 3 levels between the peanut avoidance group compared to the consuming group, were the consuming group presented with higher levels ($p<0.01$). Although this was a smaller sample from a high-risk cohort it strengthens the conclusion that introduction of peanut alters the pattern of molecular allergens in this age.

We also confirmed in both PreventADALL and BAMSE previous research results with Ara h 2 being the most common allergen molecule found in peanut allergic children (35, 154, 155).

While previous studies have found elevated exhaled nitric oxide (FENO) in peanut allergic individuals (129, 131), our results of higher levels of FENO in non-asthmatic peanut allergic participants has not been reported before,

We also confirmed in the BAMSE cohort that early childhood asthma, atopic dermatitis and egg allergy were risk factors for persisting peanut allergy in adolescence, as seen in other studies (90, 156), and also with sensitization to tree nut storage proteins, in particular in polysensitized participants. We found that the majority of individuals sensitized to hazelnut reported no allergic symptoms with hazelnut intake, supporting earlier research that storage proteins better predict true hazelnut allergy then the extract itself (48, 157).

7.3 POTENTIAL UNDERLYING MECHANISMS

While the exact mechanisms are not determined regarding how sensitization is developed early in infancy it is most likely a combination of factors. An underlying genetic predisposition, environmental exposure and oral introduction as well as existing atopic dermatitis facilitating exposure of allergen through the skin is all likely to play important roles, as well as the timepoint for introduction of food in infants.

Oral introduction of solids affects the development of both tolerance and sensitization to allergens, but why and in which individuals this leads to a tolerance development is still

unclear. We could detect the development of antibodies to both peanut extract as well as peanut allergen molecules, in particular to Ara h 3, in a higher extent among children who were introduced to peanut early. Thus, the introduction itself seemed to induce a process in the immune system leading to the development of antibodies to peanut extract and most likely to Ara h 3 in these non-peanut allergic children, and at the same time another pattern of antibodies to peanut allergen molecules were seen in children who were not introduced to peanut at the same timepoint. The majority were not sensitized to several peanut allergen molecules in this age, a factor found in BAMSE participants to be at risk for a more severe allergic phenotype, also supporting the conclusion that the allergen pattern at this age could be potentially altered later in childhood.

Storage protein sensitization was found to be more often associated with persistent allergy and more severe reactions, as well as other atopic manifestations. The characteristics of the storage proteins make them heat resistant and more resilient to gastric digestion, making their effect more potent and long-lasting which could be one reason. Another likely important factor for peanut allergy development is the atopic underlying vulnerability making certain individuals more prone to develop severe allergic phenotypes.

In light of the high prevalence of allergic rhinitis due to tree pollen in northern Europe, our finding of sensitization to the Bet v 1 homologues Ara h 8 and Cor a 1 in the majority of tree nut extract and peanut extract sensitized participants is probably not surprising. Given their vulnerability to heat and digestion in the gastric canal they are less likely to cause symptoms which could explain why so many are asymptomatic in the questionnaires.

7.4 STRENGTHS AND LIMITATIONS

Both study populations (PreventADALL and BAMSE) are population-based cohorts followed prospectively, have a large sample size and a high follow up rate. They have been followed longitudinally with both frequent questionnaires, physical examinations, blood samples as well as lung function measurements. Our selected study sample from the study cohorts did not differ significantly from the original cohort regarding background characteristics.

The PreventADALL cohort also included extensive maternal sampling, and background data from both parents, also concerning atopic heredity. Sera for IgE sampling were collected repeatedly also in young age, which is unique. Food allergy diagnosis were defined by a strict protocol and confirmed with oral provocations in cases who were indecisive.

Both cohorts tended to have included offspring with parents of a high educational level, from more atopic heredity than the average population, with a high rate of breast-feeding, also seen in other cohorts from the western world. The extensive collection of background data made it possible to adjust for several potential confounders relevant for research within allergic diseases.

For BAMSE study, the older participants participating in later follow ups tended to be more females, a fact shared by many other cohorts. It was also not possible to verify the reported food allergic individuals by oral food provocations, which otherwise is known as the golden standard, but though costly and time consuming, as well as potentially harmful for the patient thus not always ethical. There were also no analyses available to tree nut storage proteins in other ages than 24 years, making trajectories harder to study. Among the non-included families and actively excluded families in the original cohort parental smoking was more common, but there was no difference in background characteristics between participants tested for IgE vs those who were not.

In PreventADALL only half of the children left blood samples at the follow-ups, both at 3 months and 12 months and in the 3 months infants lack of enough sera for all intended analyses caused some missing data, especially in the analysis of the allergen molecules. This could lead to both an over-, or an underestimation of the prevalence. Most likely underestimation since in children who had experienced reactions parents would be more interested in determine if the child was allergic. Also, few children were found to be allergic to peanut at 3 years, possible due to the interventional design, but limiting further analysis due to low sample size.

8 CONCLUSIONS

Already at 3 months of age infants express specific IgE, but few sensitized infants express IgE to allergen molecules, indicating that IgE development process is not complete in this age and still time for a possible tolerance development. Sensitization to peanut extract is common at 1 year of age but few of them were judged peanut allergic at 3 years. Early food introduction alters the pattern of developed antibodies to allergen molecules during the first year of life. Previous studies have indicated that an early food introduction is preferred over a delayed one, and this thesis adds new insight in this process.

Peanut storage protein sensitization starts early in life and is rarely outgrown. Storage protein sensitization (both to peanuts and tree nuts) is associated with signs of respiratory and systemic inflammation, and both preschool dermatitis and egg allergy were found to be risk factors for peanut and tree nut storage protein sensitization.

Many tree nut sensitized individuals report no symptoms, especially if sensitized to birch pollen cross reacting hazel nut extract.

9 POINTS OF PERSPECTIVE

9.1 IMPLICATIONS FOR CLINICAL PRACTICE

Our finding of few infants with storage proteins sensitization at 3 months of age could be indication that the developmental process for food allergy had not yet been established in this age, and that a future tolerance development could still be possible. Especially infants with an allergy to egg and atopic dermatitis seem to be at risk for a more severe allergic disease phenotype. This, indicates that this group of infants might benefit the most from advice on early food introduction and should be targeted for early parental guidance. More of these patients would benefit from early contact with an allergologist.

Patients expressing elevated systemic inflammatory markers as well as elevated FENO could be at risk for more severe allergic reactions and until further knowledge is collected regarding this risk these patients should be closely monitored, especially if respiratory reactions have been reported after oral intake of peanut or tree nut.

8.2 FUTURE RESEARCH

Ideally, future studies would study other potential food allergens, since it is not likely the window of tolerance will be equal for the different food allergens. Knowledge is also sparse regarding how often, the quantity and for how long duration a food allergen must be consumed in order for the tolerance to be maintained.

Additionally, more research targeting infants with eczema and egg allergy before the age of 12 months with large sample size would be useful, especially if they also have parents with a food allergy.

Our finding of elevated FENO in non-asthmatic storage protein sensitized individuals points to important questions for clinicians responsible for these patients. Should we recommend treatment with inhaled corticosteroids, especially for patients with respiratory symptoms at peanut intake? More research is much needed to clarify this further.

More research would also be useful in further exploring inflammatory markers to help better diagnose patients at risk for severe reactions and targeted for intensified treatment advice,

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