START: Susceptibility to Food Allergies in a Registry of Twins

© Alizée Dery, MSc Candidate

Department of Experimental Sciences

McGill University, Montreal

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Abstract

Recent studies suggest increased prevalence of food allergy and food- induced anaphylaxis. While genetics is likely to play a significant role in the development of food allergy, this rapid increase in prevalence implies a substantial role for modifiable environmental factors.

I established a twin registry to assess gene- environment interactions in the development of food allergy. I assessed the concordance rate for food allergy in pairs of monozygotic (MZ) and dizygotic (DZ) twins, for the 5 most common food allergies (peanuts, tree nuts, seafood, milk, and egg) and, I evaluated the effect of environmental factors, lifestyle habits and the presence of atopic co-morbidities on the concordance of food allergies. Among 28 and 31 pairs of MZ and DZ twins respectively, concordance rates for peanut, cashew/pistachio, and egg allergy, were greater in DZ twins ((0.59 (%95CI 0.39, 0.77) vs. (0.50 (%95CI 0.26, 0.70), difference of 0.09 (%95 CI 0.05, 0.42), (0.55 (%95CI 0.29, 0.81) vs. (0.5 (%95 CI 0.27, 0.72), difference of 0.05 (%95 CI 0.02, 0.80), (0.51 (%95CI 0.19, 0.74) vs. 0.22 (%95 CI 0.03, 0.59) difference of 0.29 (%95 CI 0.11, 0.78)). respectively, implying that environmental factors contribute more in the development of those allergies.

After controlling for age and sex, asthma was associated with concordance in almond and hazelnut allergy (OR 2.09, 95%CI 1.47, 3.04), (OR 2.11, 95%CI 1.63, 2.74) respectively. Eczema (diagnosed in the first 2 years of life) was associated with concordance in peanut, pecan/walnut, cashew/pistachio, fish, and egg allergy ((OR 1.32, 95%CI 1.05, 1.83), (OR 2.32, 95%CI 1.58, 3.92) (OR 1.81, 95%CI 1.21, 2.68), (OR 2.15, 95%CI 1.96, 2.37), (OR 1.47, 95%CI 1.00, 2.14), respectively.

My results indicate that environmental factors have an influence on cashew/pistachio, egg and peanut allergy, and that eczema (diagnosed in the first 2 years of life) and asthma may be modifiable risk factors in the development of the 5 most common food allergies (peanuts, tree nuts, milk, egg and shellfish). However, wide CI's and small sample size preclude any definitive conclusions.

Résumé

Des résultats d'études récentes concluent que les taux d'anaphylaxies et d'allergies alimentaires sont plus hauts que jamais. Il est dit que la génétique joue un rôle significatif dans le développement d'allergies alimentaires. Par contre, il est important de noter que la croissance soudaine et rapide de la prévalence d'allergies alimentaires implique forcément la contribution de facteurs environnementaux.

J'ai établi un registre de jumeaux identiques et fraternels, pour déterminer la contribution relative de facteurs génétiques et environnementaux envers le développement des allergies alimentaires les plus courantes (arachides, noix, crustacés, lait, œufs). J'ai aussi évalué l'effet qu'on les facteurs environnementaux, les facteurs de style de vies, et la présence de maladies atopiques sur le développement des même allergies. La concordance d'allergies aux arachides, noix de Grenoble et œufs était plus élevée chez les jumeaux fraternels ((0.59 (%95CI 0.39, 0.77) vs. (0.50 (%95CI 0.26, 0.70), avec une différence de 0.09 (%95 CI 0.05, 0.42), (0.55 (%95CI 0.29, 0.81) vs. (0.5 (%95 CI 0.27, 0.72), avec une différence de 0.05 (%95 CI 0.02, 0.80), (0.51 (%95CI 0.19, 0.74) vs. 0.22 (%95 CI 0.03, 0.59) avec une différence de 0.29 (%95 CI 0.11, 0.78)) respectivement. Ceci suggère une contribution de facteurs environnementaux plus importante envers ces allergies.

Une fois que l'âge et le sexe des jumeaux on était contrôlé, une association s'est manifestée entre l'asthme et la concordance d'allergie aux amandes et noisettes (OR 2.09, 95%CI 1.47, 3.04), (OR 2.11, 95%CI 1.63, 2.74) respectivement. Une association s'est aussi présentée entre l'eczéma infantile et la concordance d'allergie aux arachides, pacanes, pistaches, poissons, et œufs ((OR 1.32, 95%CI 1.05, 1.83), (OR 2.32, 95%CI 1.58, 3.92) (OR 1.81, 95%CI 1.21, 2.68), (OR 2.15, 95%CI 1.96, 2.37), (OR 1.47, 95%CI 1.00, 2.14)) respectivement.

Mes résultats suggèrent que les facteurs environnementaux influence les allergies aux pistaches, œufs et arachides. Et que la présence d'asthme et d'eczéma rouent un rôle important dans le développement des allergies alimentaires les plus courantes.

Preface

This thesis aims to bridge two major knowledge gaps related to the pathogenesis of food allergy; the relative contribution of genetic and environmental factors to the development of the 5 most common food allergies (peanut, tree-nut, milk, shellfish and egg) by comparing food allergy concordance in monozygotic and dizygotic twin pairs, and whether eczema, a modifiable risk factor, is associated with food allergy concordance.

First, an introduction highlighting the importance and implications of severe food allergies is given (Section 1)

In order to establish the presence of confirmed food allergy, it is crucial to overview the characteristic clinical symptoms and available confirmatory tests. Sections 1.1 and 1.3 provide an overview of the classifications, clinical presentation and confirmatory tests used to diagnose severe food allergies.

A literature review of the current knowledge regarding food allergies follows (Section 1.2).

To assess the contribution of potential predictors to the development of food allergies, it is important to elucidate genetic, environmental, demographic and lifestyle factors playing a role in the development of food induced anaphylaxis; therefore, the current knowledge regarding these factors is presented in section 1.4

Although findings are inconclusive, one of the strongest factors suggested to have an effect on the development of food allergy is eczema. Section 1.4 also discusses the role that atopic co-morbidities and in particular eczema, play in the development of food allergy.

An outline of the primary and secondary objectives of the thesis follows. (Section 2)

Next, the study methodology (including study design, questionnaire and statistical analyses (Section 3) and the results (Section 4) are presented.

Finally, Sections 5 and 6 discuss future directions and concluding remarks.

Contribution of Authors

The original thesis idea was developed by Dr. Moshe Ben Shoshan. Specific study objectives and design, as well as the study protocol, were developed by Dr. Moshe Ben Shoshan and Gregory Shand The consent form and questionnaire were conceptualized by Dr. Ben Shoshan and Ms. Alizée Dery. Data collection, mainly reviewing participant responses for inconsistencies and re-contacting patients to clarify information was done by Alizée Dery, Jennifer Mill, Sofianne Gabrielli and Gregory Shand.

Alizée Dery oversaw all aspects of data analysis and interpretation ensured quality control, and performed all data entry, cleaning and management. Alizée Dery also performed all statistical analyses and interpretation of data under the supervision of Dr. Ben Shoshan

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Abbreviations

FIA- Food Induced Anaphylaxis

MZ- Monozygotic

DZ- Dizygotic

CI- Confidence Interval

OR- Odds ratio

SPT- Skin Prick Test

FC- Food Challenge

OAS- Oral Allergy Syndrome

PFS- Pollen Food Syndrome

OFC- Open Food Challenge

sIgE- serum-Specific ImmunoGlobulin E

DBPCFC- Double-Blinded Placebo-Controlled Food

1.0 Introduction

In the first nationwide study to assess the prevalence of food allergy, it's been shown that self-reported food allergy affects 7.14 % (95% CI, 5.92%, 8.36%) of Canadian children and that the prevalence of specific food allergies differs between socioeconomic groups and geographic regions.[1;2] Recent research in Canada revealed that up to 20% of Canadian households have at least one member with a food allergy while another 30% consider food allergy when purchasing or preparing foods.[3] Studies indicate that in the last 5 years, [4;11] a substantial increase in the prevalence of food allergy has occurred. A recent meta-analysis conducted spanning the period of January 1, 2002 to January 31, 2012 determined that the prevalence of food allergy world-wide has increased by 0.60% (95% CI, 0.59%, 0.61%).[1;4] Similarly, food-induced anaphylaxis, the most severe manifestation of food allergy,[12] has risen dramatically in recent decades[7-14] with hospital admissions in children up by 350%.[14;15] A cross Canada anaphylaxis registry assembled, revealed that food is the major trigger of anaphylaxis (specifically peanut, tree nut, fish, and shellfish)[1] among children (83.92% of all anaphylaxis cases (95% CI, 77.29%, 88.96%).

Although an interplay of genetic and environmental factors is likely responsible for the relatively short term increase in the prevalence of food allergy, the specific determinants remain largely speculative.[4;4;16] Thus, assessing the intricate relationships between genetic and environmental factors in order to provide novel insights into the pathogenesis of food allergy is crucial. This work will contribute to the development of effective evidence based primary and secondary prevention strategies that will ultimately improve the health of Canadians. By comparing the concordance for food allergy in pairs of MZ and DZ twins, the relative contribution of genetic and environmental factors to the development of food allergy can be determined.

One of the strongest factors suggested to have an effect on the development of food allergy is eczema. Therefore, understanding the role of skin barrier is of particular interest as this represents a potentially modifiable risk factor. Indeed, recent studies suggest increased risk in children with eczema exposed early in life through their skin to food allergens and that controlling eczema might reduce the risk of food sensitization and potentially allergy.[38;39] The association between eczema and food allergy is demonstrated in several studies. However, the true effect of eczema is not clear given that none of these studies controlled simultaneously for gene environment interactions.

2.0 Literature Review

2.1 Classification

Adverse reactions to foods can be one of two reactions; non-immune mediated or immune mediated. Non-immune mediated reactions are called food intolerances, while immune mediated hypersensitivity reactions can be referred to as food allergies. Food allergies can fall into one of the following categories: IgE mediated, non-IgE mediated, mixed IgE and non-IgE mediated and cell mediated. [17;18] This thesis will focus solely on IgE mediated food allergies.

2.2 Clinical picture

Reactions to allergens usually occur within 120 minutes after exposure and can manifest in various ways, depending on the route of exposure. [68] Generally speaking, exposure to an allergen by inhalation can produce respiratory symptoms such as rhinitis and bronchospasm while ingestion of the same allergen can also lead to respiratory issues as well as diarrhea, urticaria, and/or anaphylaxis. [68]

Anaphylaxis is the most severe clinical manifestation of an IgE mediated food allergy, with symptoms primarily involving: the skin, mainly causing urticaria (80-90% of cases), the respiratory tract (70% of cases), the gastrointestinal tract (30-45% of cases), and the cardiovascular and central nervous system (10-45% of cases, 10-15% of cases respectively), with cardiovascular symptoms ranging from decrease in blood pressure and dizziness, to fainting and loss of consciousness. [21;23]

2.3 Diagnosis

The diagnosis of food allergy is established only by corroborating a suggestive clinical history and positive confirmatory tests such as skin prick tests (SPT), specific IgE (sIgE) levels and food challenges. [22, 24-25] If, within 12 to 15 minutes of placement, the largest diameter of the wheal is at least 3 mm larger than the negative saline control, the SPT is considered positive.[30] Typically, SPT's are the first test used in the evaluation of food allergy, with the majority of health care centers offering measurements of sIgE for a wide variety of foods. [26] Both of these tests evaluate the presence of IgE, determining sensitization, without necessarily correlating with clinical reactivity.[24] In an effort to improve diagnostic accuracy, component testing allows the allergist to examine IgE levels against a particular protein of the culprit food. Examining sensitization profiles to specific food allergen components aims to distinguish between sensitized and truly reactive patients.[24]

Possessing a positive history of any food allergy only has a 50% positive predictive value in establishing allergy diagnosis. [26] Therefore, a single diagnostic test, such as a SPT or peanut-specific IgE level, is not sufficient in establishing a diagnosis in those with no previous peanut exposure, or an uncertain clinical history.[27] One study reported only 31.3% of children who had no known peanut exposure and a positive SPT[28] were truly allergic to peanut. [32] Also, in a recent population based study done in Australia, among 2848 infants, the prevalence of any sensitization (based on positive SPTs) to peanut was found to be 8.9% (95% CI, 7.9-10.0) and sesame, 2.5% (95% CI, 2.0-3.1). In contrast, the prevalence of allergy proven by food challenge was 3.0% (95% CI, 2.4-3.8) and 0.8% (95% CI, 0.5-1.1) respectively. [32]

The DBPCFC (Double Blind Placebo- Controlled Food Challenge) is recognized as the gold standard for the diagnosis of food allergy. [28;33] However, it requires trained personal as well as specialized facilities. It is costly and time consuming, and is rarely employed by physicians outside of an academic context. [32-33] The open food challenge (OFC) is a more viable option for most clinicians, though it is not without its own pitfalls. [35]

2.4 Prevalence

Over the last few decades, studies suggest a significant increase in the prevalence of food allergy and anaphylaxis.[43] IgE-mediated food allergies are the most common during early childhood, with a prevalence ranging between 2.2% and 5.5% in the first year of life, whereas, in adolescents, the prevalence is roughly 2.3%. Cow's milk, eggs, wheat, soy, peanuts, tree nuts, and sesame,

are responsible for the majority of food- induced allergic reactions in young children. Whereas, fish, shellfish, tree nuts, and peanuts are more common food allergens in older children and adults. [193] Another study, looking at anaphylactic cases admitted in the emergency department at the Montreal Children's Hospital reported that food was responsible for 84.5% of the reactions. [240] This is in line with other studies, also looking at specific triggers of anaphylaxis. [191-192]
Although early US and European studies report increased prevalence of food allergy, more recent studies suggest a stabilization in prevalence in the last 5 years in English speaking countries. [45] However, prevalence continues to rise in developing countries. A Chinese study looking at infants randomly coming in for a check-up, performed open challenges on the ones having a positive history, or SPTs, suggesting food allergy. The results showed that positive SPT response was significantly higher in 2009 (18.0 %) than in 1999 (9.90 %) and the prevalence of confirmed food allergy (based on food challenge) increased markedly from 3.50% to 7.70 %. [4]
Based on the increase in prevalence, it is possible that modifiable non genetic factors are implicated in the pathogenesis of food allergy. Identifying such factor will benefit the development of effective primary prevention strategies.

2.4.1 Peanut Allergy

The prevalence of peanut allergy among children in the United Kingdom, North America, and Australia has doubled in 10 years and is approximately 1.8%, 1.4%, and 3.0% respectively. [44;56] A study conducted in 2013 states the most recent estimates of IgE- mediated peanut allergy to affect 1.5% to 3% of the pediatric population. [37] These estimates are confirmed by a study reporting that peanut prevalence has increased in the last 4 years, currently affecting 1.5 % of children. [56] Furthermore, a Canadian study by our group reveals that almost 2% of Canadian children have peanut allergy. [1-3] While it is widely believed that the prevalence of food allergy is on the increase, it's important to keep in mind that most of these estimates are based on self- report which is thought to be inaccurate due to reporting bias. [5;11;46;189] One study reported a prevalence of 1.62% among Montreal school children that stabilized during the last 5 years. However, confirmation of food allergy was based on self- report. [65]
Peanut and tree nut allergies are particularly troublesome as they are infrequently outgrown and are associated with significant morbidity rates. [193] Of the major peanut allergens Ara h 1, [41] Ara h 2 [42] and Ara h 3,[43] Ara h 2 is the most clinically important peanut allergen and is most frequently recognized by IgE and basophils in mast cells in individuals with peanut allergy.[42] The onset of IgE sensitization to peanut usually occurs during infancy. [56] It is usually within the first 2 years of life that peanut allergy manifests itself, and about 75% of reactions occur with the first known peanut

exposure. [44] Diagnosis of peanut allergy is confirmed by having a convincing clinical history as well as the presence of a positive SPT defined by a wheal diameter of at least 3 mm larger than that elicited by the negative control within 10 to 15 minutes of placement, or an IgE level of at least 0.35 KU/L. [35;76]. If exposure to peanuts never occurred, or if clinical history is uncertain, diagnosis is based on having a positive SPT and a peanut- specific IgE above 15 kU/L. For peanut allergy, a SPT greater than 8 mm in those over 2 years of age, and a SPT greater than 4 mm in those under 2 years of age has been reported as highly predictive of peanut allergy. [35]

2.4.2 Tree Nut Allergy

Tree nuts are one of the most common foods causing acute allergic reactions and almost all tree nuts have been associated with occasional fatal allergic reactions. [55] The term "tree nut" is collectively used to categorize nuts that grow on trees. Tree nuts that can cause an IgE mediated food allergy reaction include almonds, brazil nuts, cashew nuts, hazelnuts, macadamia nuts, pecans, pistachios and walnuts. [48;55] Being one of the major food categories responsible for 90% of food induced allergic reactions, and one of the foods associated with more severe reactions, it's been estimated, by a random-digit dial telephone survey, that approximately 0.5% of the US and North American population is allergic to tree nuts. [194] In Canada alone, recent research has shown 1.59 % of children and 1% of adults have a tree nut allergy. [195] Based on studies looking at anaphylactic triggers, tree nuts are held accountable for a significant portion of anaphylactic cases. [190-192;240] McWilliam and her team were the first to conduct a systemic review to determine the prevalence of tree nut allergy in children and adults from January 1996 to December 2014. Challenge-confirmed IgE-mediated tree nut allergy prevalence was less than 2 %, while probable tree nut allergy prevalence based on self- report, ranged from 0.05 to 4.9 %. [48] Prevalence of individual tree nut allergies varied significantly by region with hazelnut the most common tree nut allergy in Europe, walnut and cashew in the USA and North America and Brazil nut, almond and walnut most commonly reported in the UK. Together, peanut and tree nuts account for 70-90% of reported food-induced anaphylaxis fatalities, with tree nuts alone accounting for 18-40% of them. [47-48]
Most tree nut allergens identified to date are seed storage proteins such as the vicilins, legumins, americ globulins, and 2S albumins. Other tree nut allergens include profilins and hevein- related proteins, known for their contribution to the allergenicity of a wide variety of pollens, nuts, seeds, fruits and vegetables and their propensity for exhibiting a significant degree of IgE- mediated cross-reactivity. [201] Cross-reactivity allergies occur when the antibodies against a specific allergen are also capable of identifying other allergens from other allergen sources and may thus induce an

allergic reaction to those allergens as well. [200] Cross-reactivity among nuts may occur because of minor constituent panallergens such as profilins and lipid transfer proteins, or may involve major nut storage protein allergens, including albumins, legumins, and vicilins. [200-202] One study reported no cross-reactivity between peanut and tree nuts. [202] However, strong cross-reactivity was present among walnuts, pecans, and hazelnuts, and mild cross- reactivity was present among cashews, Brazil nuts, pistachios, and almonds. [202]

Similarly to peanut allergy, diagnosis of tree nut allergy is confirmed by having a convincing clinical history as well as the presence of a positive SPT defined as a wheal diameter at least 3 mm larger than that elicited by the negative control within 10 to 15 minutes of placement, provided by a physician, or an IgE level of at least 0.35 KU/L. [35;76].

2.4.3 Shellfish Allergy

Shellfish can be subdivided into crustaceans (mainly shrimps and crabs) and mollusks (oysters, clams, scallops).[57-60] Allergic reactions to shellfish can generate clinical symptoms ranging from mild urticaria and oral allergy syndrome, to life-threatening anaphylactic reactions. [59-60]

The prevalence of crustacean allergy seems to vary largely between geographical locations, most probably as a result of the availability of seafood. [207] A study conducted in Spain reported that among 355 children, 6.8% of patients reacted to crustaceans by SPT, whereas in Asian countries, where seafood allergy is very common, a study from Singapore looking 227 children with food hypersensitivity confirmed that crustacean and fish are significant sensitizers in about 40% and 13% of children, respectively. [209] A systemic review also reported the prevalence of seafood allergy in Asian countries to range between 9% to 73% depending on the country and the specific allergen. [197]

Very few studies have established the prevalence of fish and shellfish allergy using the gold standard challenge criteria. Where this is used, the prevalence of shellfish allergy ranges between 0.2%-0.9%. [208] Population-based prevalence data among US adults from a Food Safety Survey reported the prevalence of shellfish allergy to be 1.9 and 1.3% among respondents with self-reported food allergy and self-reported doctor-diagnosed food allergy, respectively. [196]

While the consumption of seafood is steadily increasing world-wide, it is generally considered that shellfish and fish are among the four foods most commonly provoking severe food anaphylaxis. In a study looking at anaphylactic cases reported in a Canadian emergency department, shellfish allergy was responsible for 12.9 % (%95 CI 6.1, 24.4) of all 63.3 % (%95 CI 52.9, 72.6) food triggered anaphylactic cases. [193]

The major shellfish allergen responsible for ingestion-related allergic reactions is tropomyosin, although other allergens may play an important part in allergenicity, such as arginine kinase and myosin light chain. Tropomyosin, responsible for molecular and clinical cross-reactivity between crustaceans and molluscs, may also be responsible for cross- reactivity to other inhaled invertebrates such as house dust mites and insects. Crustacean and mollusc allergens do not cross-react with fish allergens and no reactivity between known allergens or homologous proteins has currently been demonstrated. [206-208]

Lastly, diagnosis of shellfish allergy, just like diagnosis of the other food allergies mentioned, is confirmed by a convincing clinical history as well as the presence of a positive SPT or, by an IgE level of at least 0.35 KU/L. [35;76]

2.4.4 Milk Allergy

Milk is the major food allergen in children and is the leading cause of anaphylaxis in infancy in Europe. [200] Between 1.8% and 7.5% of infants are affected by cow's milk allergy, in the first year of life. In Canadian emergency departments, it was reported that milk was responsible for 1.6% (95%CI 0.08,9.8) of all anaphylaxis cases triggered by food. [193] Another study conducted in Quebec, found that milk allergy was responsible for 5.9 % (%95CI 1.0, 21.1) of all food induced anaphylactic cases. [190] Cow's milk contains around 30–35 g of proteins per litre and includes more than 25 different proteins. However, only a small portion of them are known to be allergenic. Through the acidification of raw skim milk, two protein fractions can be obtained: the casein proteins (such as αS1-casein, αS2-casein, β-casein and κ-casein) and the whey proteins (including α-lactalbumin, β-lactoglobulin, bovine serum albumin, and lactoferrin). Together, these fractions account for 80% and 20 % of the total milk proteins respectively. [203-205]

Diagnosis of milk allergy by having a wheal diameter at least 3 mm larger than that elicited by the negative control within 10 to 15 minutes of placement, when a convincing clinical history of an IgE-mediated reaction attributed to milk was provided. [35]

It's important not to confuse cow's milk allergy from other adverse reactions to cow's milk such as lactose intolerance. The latter is not immune mediated and is not considered life threatening. [62]

Cow's milk allergy is classified by the underlying immune mechanism, the timing of presentation and the organ system involvement. [62] Symptoms involving the skin, respiratory tract, gastrointestinal tract and cardiovascular system, vary in severity, ranging from mild ones to rarely life- threatening anaphylaxis. [45]

2.4.5 Egg Allergy

Egg allergy is considered one of the most common food allergies in infants and young children. [67] Affecting about 1-2% of preschool children, egg allergy differs in a number of ways from other common childhood allergies such as cow's milk and peanut allergies in terms of prevalence and tolerance. [4] In a population-based study of 2 year olds in North America, the prevalence of IgE-mediated reactions to eggs was 1.6%.[40] Global data indicate that egg allergy affects 0.5–8.9% of young children. [67] A recent Canadian study revealed that egg allergy was responsible for 1.6% (95%CI 0.08, 9.8) of all food induced anaphylaxis cases. [193]

Allergic reactions to eggs are commonly observed in the first 12 months of life, [69] where low levels of IgE antibodies against egg proteins often present themselves in healthy infants. [69]

Erythema and urticarial, the most common cutaneous symptoms, occur in 90% of 10 month old children, and usually manifest themselves within 60 minutes of exposure. [67-68]

Depending on the route of exposure, symptoms may vary. For example, following application of egg protein to the skin, contact urticaria is a common finding. However, following ingestion of egg, gastrointestinal symptoms such as abdominal discomfort and vomiting occur in 40-50% of cases.[69] A minority of cases may even experience respiratory symptoms. [79]

The chemical composition of hen's egg has been extensively investigated and the clinically relevant egg allergens have been identified in both the albumen (egg white) and the egg yolk fraction. However, studies have documented that the major egg allergens are mainly contained in the egg white. [205] Early studies involving a cohort of 342 patients reported that based on skin tests, the major egg allergens were, lysozyme, ovomucin (α- ovomucin, β- ovomucin), ovalbumin and ovomucoid. Together, ovomucoid and ovalbumin constitute about 11 and 54% of egg white proteins, respectively . [206]

It's suggested that, in those who were never exposed to egg, a SPT of at least 9 mm or an IgE above 15 kU/L as well as the presence of a convincing history, is highly predictive. [205;35]

2.5 Pathogenesis

All IgE mediated food allergies share the same common final pathway, regardless of the process leading to sensitization to food allergens. [84;92] The production of food- specific IgE antibodies results from either a failure to develop or a breakdown in oral tolerance. [86] These food-specific antibodies have a high binding affinity for FcεI receptors on both mast cells and basophils and a low binding affinity for FcεII receptors on macrophages, monocytes, lymphocytes, eosinophils, and platelets. Mediators such as histamine, prostaglandins, and leukotrienes are released after the

ingested food allergen reaches the food-specific antibodies on mast cells or basophils. These mediators then promote a series of events including vasodilation, smooth muscle contraction, and mucus secretion, which result in symptoms of immediate hypersensitivity. [91-92] In addition, the release of the various cytokines by activated mast cells is suggested to play a crucial part in the IgE-mediated late-phase response. [91]

2.6 Factors associated with the development of food allergies

Although an interplay of genetic and environmental factors is likely responsible for the relatively short term increase in the prevalence of food allergy, the specific determinants remain largely speculative.[4;4;16] Numerous studies have evaluated the role of genetic and environmental factors, but the results are inconsistent and controversial. [82-86] Only two studies [85 5] assessed simultaneously gene environment interactions. These studies were limited as the first only studies sensitization, while the second only addressed one allergen, and none of them assessed genetic effects specifically in milk and shellfish allergies, the most common food allergies in Canadians. [2;4]
Twins provide an invaluable model for genetic studies by allowing the separation of genetic from environmental factors. Given that numerous environmental factors may contribute to the development of food allergy, this type of study design offers a clear advantage through the natural control of environmental factors in twin pairs sharing the same household, lifestyle habits and geographical location. Studies of concordance among twins allow the determination of genetic influences on complex phenotypes, since twin pairs are generally exposed to a similar environment, allowing the estimation of genetic and environmental causes of familial resemblance. [5;182]
MZ twins have all their genes in common, whereas DZ twins share on average only half of their genes. Assuming: (1) equal environment for both twin types, (2) random mating and (3) no genotype-environment correlation or interaction, a greater similarity between MZ versus DZ twins is attributed to accumulated effects of several genes (additive genetic effects). On the other hand, environmental influences shared by the twins (such as parenting style and eating habits) will contribute to twin similarity in both MZ and DZ twins.
A study done on 58 twin pairs, looking at the concordance rate of peanut allergy among twins concluded that the concordance in MZ twins (64.3%) was significantly higher than that among DZ twins (6.8%). [5] Another study, done on 826 Chinese twin pairs (472 MZ and 354 DZ) aged 12–28 years, examined food sensitization (presence of food- specific antibodies) to common food allergens and found sensitization to peanut and shellfish to be influenced by both genetic and environmental factors. [106] They also revealed that concordance rates and risk of sensitization in one twin given

the presence versus absence of sensitization in the other twin were higher in MZ twins compared to DZ twins. However, a large number of MZ twins were discordant for sensitization to common allergens, implying also non-genetic factors. These results are in line with other studies in the US[4;6] and Europe [88] suggesting a high risk for clinical food allergy and not only sensitization in MZ versus DZ twins

2.6.2 Genetic determinants

Several genes have been reported to contribute to the development of food allergy including genes involved in the function of both innate (e.g. impaired skin barrier [83], pattern recognition signaling [84]), and adaptive immune function (HLA polymorphism[85] and impaired T cell cytokine signalling. [84-85]

Table 1: Genes implicated in the pathogenesis of food allergy

Group	Name of gene	Results	Reference
Barrier genes	Filaggrin	Increased risk of developing allergic sensitization and food allergy independently of the presence of atopic dermatitis (OR: 1.9; 95% CI, 1.4,2.6).	210
Barrier genes	SPINK5	AA/AG genotype displayed a significantly higher prevalence of food allergy (20/91 subjects) than those with the GG genotype (1/26 subjects; AA + AG vs GG, *P* = 0.03).	211
Innate immunity genes	NLRP3: SNPs (rs4612666 and rs10754558)	NLRP3 controls the activity of inflammasomes. NLRP3 SNPs (rs4612666 and rs10754558) were associated with susceptibility to food-induced anaphylaxis (P = .00086 and P = .00068, respectively).	212
Innate immunity genes	TLR	Exclusive breast-feeding duration increased the risk of FS in a dose-responsive manner in children carrying the *TLR9*-rs352140 TT genotype, with ORs of 3.3 (95% CI, 1.0-10.9) and 13.2 (95% CI, 3.0-57.3) for children breast-fed less than 4 months and breast-fed 4 or more months, respectively.	213
Innate immunity genes	CD14	CD14 gene codes a lipopolysaccharides receptor. Patients with food allergy had a 4-fold increased odds of having the TT genotype versus carriers of the C allele compared with control subjects (OR: 3.9; 95% CI , 1.5,10.3).	214
Innate immunity genes	CD4	dysregulation of DNA methylation at MAPK signaling-associated genes during early CD4+ T-cell development may contribute to suboptimal T-lymphocyte responses in early childhood associated with the development of food allergy	215
Innate immunity genes	TSLP	Exclusive breast-feeding duration significantly increased the risk of FS in a dose-responsive manner in children carrying the *TSLP CT/* TT genotype, with ORs of 2.2 (95% CI, 1.3-3.7) and 3.1(1.7,5.8).	213
Adaptive immunity	HLA-II	In children with cow milk allergy, HLA (DR15)-DQB1*0602 haplotype was associated with high levels of beta-lactoglobulin-specific total IgG ($p < 0.001$) and IgG4 ($p < 0.001$) and alpha-casein-specific total IgG ($p = 0.003$) and IgG4 ($p = 0.002$), but not among control subjects. (DR1/10)-DQB1*0501 was associated with lower levels of beta-lactoglobulin-specific total IgG ($p < 0.001$) and IgG4 ($p < 0.001$), ovalbumin-specific total IgG ($p = 0.002$) and IgG4 ($p < 0.001$), particularly in control subjects ($p < 0.001$).	215
Adaptive Immunity	HLA- II	Four class II genotypes (DRB1, DQB1, DPB1) were present at a significantly higher frequency in the study group compared with controls.	86
Adaptive Immunity	HLA-II	An association exists between two allelic groups of the HLA-DQB1 gene (DQB1*02 and DQB1*06:03P) and Peanut allergy	87
Adaptive immunity	HLA-II	No association between 7 DQ and 18 DR allele groups and peanut allergy	216
Adaptive immunity	HLA-II	Latex-fruit allergy is associated with HLA-DQB1 *0201, DRB1 *0301, and *0901, as well as with HLA-DR functional group E.	217

Adaptive immunity	HLA-II	DRB1(*)13 and DQB1(*)06 were both increased in frequency in the nut allergy patients over both the atopic and blood donor controls. However, none of these increased frequencies were significant when corrected for the number of comparisons undertaken.	218
Adaptive immunity	IL12R	Children breast-fed (n = 739), including exclusively breast-fed children, were at a 1.5 (95% CI, 1.1-2.1; P = .019) times higher risk of FS than never breast-fed children (n = 231). This association was significantly modified by rs425648 in the IL-12 receptor β1 gene (IL12RB1; P for interaction = .0007): breast-feeding increased the risk of FS (odds ratio, 2.0; 95% CI, 1.4-3.1; P = .0005) in children carrying the GG genotype but decreased the risk (odds ratio, 0.6; 95% CI, 0.3-1.4; P = .252) in children carrying the GT/TT genotype.	213
Adaptive immunity	STAT6	Key transcription factor involved in both IL-4- and IL-13-mediated biological responses. Dinucleotide repeat polymorphism of the STAT6 exon 1 (13/15-GT repeat heterozygosity and the 15GT repeat homozygosity) was higher in children in the Japanese population with allergic diseases (bronchial asthma, atopic dermatitis and/or food-related anaphylaxis) compared to controls (P = 0.0158).	220
Adaptive immunity	FOXP3	This gene is located on the X-chromosome and encodes a transcription factor that directs T cells toward a regulatory phenotype. Five FOXP3 SNPs (rs5906761, rs2294021, rs2294019, rs6609857 and rs3761548) were significantly associated with sensitization to egg at ages 1 and 2 and with sensitization to indoor allergens at age 2 (P < 0.05. Rs5906761 and rs2294021 were associated with remission of sensitization to food allergens in boys.	221
Adaptive immunity	FOXP3	Abnormalities affecting FOXP3, Tregs and Tr1 cells will lead to autoimmune manifestations and/or allergic reactions	232
Adaptive immunity	FOXP3	Children with food allergy showed lower levels of FOXP3 and IL10 gene expression than healthy children. Children acquiring tolerance to the food show higher levels of the FOXP3 gene expression than children with active food allergy.	234
Adaptive immunity	IL10	IL-10 A-1082G gene polymorphism is associated with food allergy susceptibility (OR: 2.5 ;95% CI, 1.0, 6.4 vs atopic control subjects).	96
Adaptive immunity	IL4	A significant interaction between IL4 gene polymorphism (rs2243250) and vitamin D deficiency (p(interaction) = 0.003, p(FDR) = 0.10) was found: Vitamin D deficiency increased the risk of food sensitivity among children carrying CC/CT genotypes (OR = 1.79, 95%CI: 1.15-2.77).	97
Adaptive immunity	IL9	IL9 best differentiated between children with peanut allergy and children with peanut sensitization. Production of IL-9 and the TH2-specific cytokine IL-5 suggests that the IL-9-producing cells belong to the recently described TH9 subset	233
Adaptive immunity	IL12	Breast-feeding increased the risk of food sensitization (odds ratio, 2.0; 95% CI, 1.4-3.1; P = .0005) in children carrying the GG genotype in IL-12 receptor β1 gene but decreased the risk (odds ratio, 0.6; 95% CI, 0.3-1.4; P = .252) in children carrying the GT/TT genotype.	213

Adaptive immunity	IL13	The IL-13 promoter -1055 TT genotype, a polymorphism that results in an increase in IL-13 protein production associated with increased odds of food sensitization (OR: of 3.49; 95% CI, 1.52, 8.02).	97
Epigenetic effects (Copy Number Variants)	CTNNA3 and RBFOX1	Knockdown of CTNNA3 resulted in upregulation of CD63 and CD203c in mononuclear cells upon PMA stimulation, suggesting a role in sensitization to allergen. Two plausible genes harboring CNV loci that are enriched in pediatric patients with food allergies. The novel gene candidates discovered in this study by genome-wide CNV analysis are compelling drug and diagnostic targets for food allergy	235
Epigenetic effects (DNA methylation)	Th1 and Th2	Tolerance acquisition in children with IgE-mediated CMA is characterized by a distinct Th1 and Th2 cytokine gene DNA methylation pattern. These results suggest that DNA methylation may be a target for CMA prevention and treatment	236
Epigenetic effects (DNA methylation)	CD4	Dysregulation of DNA methylation at MAPK signaling-associated genes during early CD4+ T-cell development may contribute to suboptimal T-lymphocyte responses in early childhood associated with the development of food allergy	237
Epigenetic effects	STAT3	Food allergies and anaphylaxis were markedly diminished in patients with AD-HIES compared with a cohort of patients with no STAT3 mutation but with similar histories of elevated IgE and atopic dermatitis. Morphine skin prick testing and basophil activation were diminished in patients with AD-HIES, whereas mice carrying an AD-HIES mutation were hyporesponsive to systemic anaphylaxis models. Rapid mast cell STAT3 serine727 phosphorylation was noted after IgE cross-linking, and inhibition of STAT3 signaling in mast cells lead to impaired FcεRI-mediated proximal and distal signaling, as well as reduced degranulation	238
Epigenetic effects	IL4	AA, AG and GG genotypes for IL-4Rα (1652A/G) polymorphisms were statistically different in radioallergosorbent test (RAST) positive versus negative patients. A significant decrease in IL-4 (C-590T) gene expression and increase in IL-4Rα (1652A/G) and IL-10 (C-627A) gene expression between $RAST^{+}$ versus $RAST^{-}$ patients, respectively was observed.	239

2.6.3 Environmental Determinants

During the first few years of life, exposure to various environmental and life style factors is thought crucial for the development of atopy and atopic disease. Studies suggest that exposure to such factors may be responsible for the increase of atopic diseases in the Western world. [94-95;153] Such factors include drugs, microbial exposure, mode of delivery, food consumption (quantity and timing), daycare attendance, pets and farming environment, food processing, omega 3 fatty acids, vitamin D exposure, breastfeeding and atopy. (Table 2)

The following factors have been reported to affect the risk of food allergy.

Season: food allergy is more common in those born in the fall or winter. This could be due to reduced UV-B exposure and subsequent lower levels of Vitamin D exposure during a vulnerable period of immune development. [129]

Drugs: antacid medication may potentially increase the gastric pH and interfere with the digestive function of the stomach, in turn leading to the persistence of labile food protein during gastric transit, which may have a major effect on the development of food allergy. [97] Studies report higher levels of food specific IgE levels in individuals treated with H2-receptor blockers or proton pump inhibitors for 3 months. [97;99]

Microbial Exposure: microbial organisms may be correlated with the development of allergy in several ways: bacteria can enhance the integrity of the intestinal barrier, [99-101] and it is suggested that low-level activation of microbial pattern recognition receptors triggered by low-level pathogen exposure, can result in allergen presentation. [100] However, no clear conclusions have been drawn.

Mode of Delivery: Looking at caesarean delivery, a Norwegian birth cohort study reported that a 7-fold increased risk of parental perceived reactions to eggs, fish, or nuts was associated with birth through a cesarean section. [104] A recent meta-analysis on the relationship between cesarean delivery and atopic outcome [103] found that 6 studies confirmed a mild effect of cesarean delivery, increasing the risk of food allergy or food atopy (OR, 1.32; 95% CI, 1.12-0.55).

One possible explanation is that early colonization of the infant by colonic microflora protects against the development of allergic disease. However, other explanations are possible: mothers who get a cesarean delivery for their firstborns, are more likely to get caesarian deliveries for subsequent siblings, leading to a sibling effect. Another possible explanation is that high maternal age is associated with cesarean sections and this latter factor has been shown to be a possible risk factor for food allergy in a case-control study. [100]

Pets and Farming Environment: the role of pet exposure in the development of allergy remains controversial. An increasing number of studies looking at asthma, including a series of cohort

studies, support the notion of a "protective pet effect" [119-120] Others, however, have found either, an increased risk of sensitization associated with pet exposure [120] or no association between exposure to cat allergen early in life and the occurrence of allergy. [122] Although two studies report an inverse association between having pets and living on a farm and food sensitization [121;123] to date, no study has looked at the potential effect on clinically confirmed food allergy. One study [119] touched upon the topic when it was reported that no clear associations was found between pet exposure in the first year of life and allergy endpoints.

Food Processing: according to several studies [142-144] food processing such as heating or addition of vinegar may either decrease or increase food allergenicity. One study reports that frying or boiling peanuts appears to reduce their allergenicity. [252] Compared with roasted peanuts, the relative amount of Ara h 1 was reduced in the fried and boiled preparations, resulting in a significant reduction of IgE-binding intensity. In addition, there was significantly less IgE binding to Ara h 2 and Ara h 3 in fried and boiled peanuts compared with that in roasted peanuts. [252]

Omega 3 Fatty Acids: studies suggest that over the years, an increase in the consumption of ω-6 polyunsaturated fatty acids (linoleic acid) and similarly, a lower intakes of ω-3 polyunsaturated fatty acids (eicosapentaenoic acid) due to reduced consumption of oily fish is observed. [100-102] Consumption of ω-6 Fatty acids results in increased production of prostaglandin E2 (PGE2), and consumption of v-3 fatty acids inhibits the synthesis of PGE2. PGE2 reduces IFN-g (interferon gamma) production by T lymphocytes, resulting in increased IgE production by B lymphocytes. This proposed mechanism is suggested to increase the prevalence of asthma, eczema and allergic rhinitis.[100;101]

Vitamin D:vitamin D is becoming increasingly recognized regarding its' role in the regulation of immune responses. [130] Studies suggest that vitamin D may have a number of tolerogenic effects on the differentiation of T regulatory cells [130-131] as well as direct effects on B cells to promote IL-10 (interleukin 10) production and decrease IgE production [131] Vitamin D mediates the association between season of birth and childhood food allergy [232] and/or food-induced anaphylaxis [133], as there is inadequate UVB intensity for the synthesis of active vitamin D in winter. The role of vitamin D has also been indirectly reflected by geographic studies, which indicate a higher prevalence of allergic disease occurring in areas further away from the equator.[134-137]

Despite some studies [131,133,232,134-137] stating an inverse relationship between vitamin D and the development of food allergy, others [139-140, 119;141]state the opposite. It was reported that in German farming communities with less vitamin D supplementation in foods, the prevalence of food allergy was lower, [139] due to its' effect on the Th1/Th2 shift to Th2 dominance. [132;133-135;139]

Supporting this notion is a study done on infants showing that the ones who had vitamin D supplementation were at increased risk of food allergy. [140] In contrast, others argue that inadequate vitamin D (mainly as a result of inadequate sunlight) is responsible for the increase in asthma and allergies. The argument is supported by studies suggesting higher levels of epinephrine prescriptions in Northern latitudes with less sun exposure.[119;141]

Table 2: Environmental Determinants of Allergy

Factor	Author	Type of study	Results	Reference
Season	Vassallo	Case-control	Food allergy is more common in Boston children born in the fall and winter seasons. children younger than 5 years of age born in the fall or winter had 53% higher odds of food allergy compared with controls.	131
Season	Baeke	Review of cohort studies	Several confounders such as, the season, could affect this relationship where the presence of higher rates of eczema in the winter might be associated with increased risk of food allergies	130
Drugs	Untersmayr	Case- control	IgE synthesis toward novel dietary proteins is promoted, leading to food allergy.	198
Drugs	Scholl	Cohort study	Anti-acid treatment during pregnancy could be responsible for the increasing number of sensitizations against food allergens in young infants	97
Drugs	Scholl	Case- control	Anti-ulcer drugs may lead to the induction of immediate-type food hypersensitivity toward hazelnut	98
Drugs	Pali-Scholl	Case- control	Antacids and dietary supplements influencing the gastric pH increase the risk for sensitization against allergenic food proteins	99
Dietary Fat Consumption	Furuhjelm	Case- control	Maternal omega-3 fatty acid supplementation may decrease the risk of food allergy and IgE-associated eczema during the first year of life in infants with a family history of allergic disease	154
Microbial Exposure	Lack	Review of case-control and cohort studies	Limited support for hygiene hypothesis, eczema, c-sections correlated with food allergy, no evidence that antioxidants, vitamin supplements have an effect on FA	95
Microbial Exposure	Novverr	Review of cohort and case- control	Probiotic and prebiotic strategies be considered for patients coming off of antibiotic therapy	100
Microbial Exposure	Dioun	Cohort study	The presence of a food allergy in children was related to older maternal age at delivery	104
Mode of Delivery	Eggesbo	Cohort study	Cesarean section might increase the risk of development of food allergy	103
Mode of Delivery	Bager	Review of cohort and case- control studies	Delivery by c-section is associated with a moderate risk increase for allergic rhinitis, asthma, hospitalization for asthma, and perhaps food allergy/food atopy	104
Pets and Faming Environment	Waser	Case-control	The inverse relation between current dog contact, asthma and allergy was mostly explained by simultaneously occurring exposure to stable animals or was restricted to farm children	119
Pets and Farming Environment	Riedler	Cross- sectional	Lower prevalence of hay fever, asthma and allergic sensitization in children living on a farm might be the development of immunotolerance or the stimulation of TH1 cells and suppression of TH_2 cells	180
Pets and Farming Environment	Nafstad	Cohort	Early-life exposure to pets or lifestyle factors associated with exposure to pets reduce the risk of developing atopy-related diseases in early childhood	121

Pets and Farming Environment	Almqvist	Cohort	Early exposure to cat seems to increase the risk of sensitization to cat but not of asthma at 4 years of age. Dog ownership, is associated with lowered risk of sensitization to airborne allergens and asthma	123
Pets and Farming Environment	Lau	Cohort	Induction of specific IgE responses and the development of childhood asthma are determined by independent factors	125
Pets and Farming Environment	Platts-Mills	Cross-sectional	Exposure to cat allergen can produce an IgG and IgG4 antibody response without sensitisation or risk of asthma	126
Pets and FarmingEnvironment	Ege	Cross- sectional	Maternal exposure during pregnancy influences atopic sensitization patterns in cord blood. The context of allergen contact modifies risk of atopic sensitization	127
Pets and Farming Environment	Von Hertzen	Cross- sectional	Atopy was several-fold more common in Finland compared with in Russia, and disparities in sensitization rates between the countries have further increased during these generations	128
Vitamin D	Baeke	Review of case-control and cohort studies	1,25(OH)(2)D(3) plays a role in maintenance of immune homeostasis	130
Vitamin D	Unger	Case- control	VD3- and Dex-DC possess tolerogenic features, acting *via* different mechanisms. Both are useful to specifically down-regulate unwanted immune responses and induce immune tolerance	133
Vitamin D	Vassallo	Cohort	Seasonal fluctuations in sunlight and vitamin D involved in pathogenesis of food allergy	135
Vitamin D	Mullins	Cohort	EpiPen prescription rates and anaphylaxis admissions more common in southern regions of Australia. Additional support for a possible role of vitamin D in the pathogenesis of anaphylaxis	137
Vitamin D	Wjst	Case- control	Neither time point of first exposure to certain allergens nor early infections during winter months seems to be a major factor for adult allergy	139
Vitamin D	Millner	Cohort	Early vitamin supplementation is associated with increased risk for asthma in black children and food allergies in exclusively formula-fed children	172
Food processing	Liu	Cohort	Early vitamin supplementation is associated with increased risk for asthma in black children and food allergies in exclusively formula-fed children	142
Food processing	Chung	Case- control	Maturation and curing, with roasting, may be associated with allergenicity, suggesting that these processes may lead to changes in the allergenic properties of peanuts	143
Food processing	Samson	Case- control	16.5-kD protein may be an important allergen that is clinically relevant in both atopic and non atopic patients with adverse reactions to shrimp even if it is not detected	144

2.6.4 Demographic Determinants

Few studies have evaluated the demographic predictors of food allergy. Among those that did, the following factors (found in Table 3) have been associated with presence of food allergy:

Age: world- wide, food allergy is more prevalent in children. This could potentially be due to the high rate of resolution with age of milk and egg allergy and/ or due to a possible cohort effect with higher levels of peanut allergy in more recent cohorts. [4] Having conducted the first national study to assess prevalence of food allergies in Canada, our group found that peanut, tree nut, and sesame allergy were more common in children (OR 2.24, (95% CI, 1.40,3.59), 1.73 OR (95%CI, 1.11,2.68), 5.63 OR (95% CI, 1.39,22.87) respectively) while, fish and shellfish allergy were less common in children (OR 1.17 (95%CI, 0.04,0.72), 0.29 OR (95%CI, 0.14,0.61) respectively) [1]

In a recent US study [153], based on IgE levels, food allergy estimates revealed that the prevalence of clinical food allergy differed significantly and declined with age, the highest being at 1-5 years of age and lowest in adults (> 60 years). Estimates for specific foods also varied by age. These findings are consistent with other studies suggesting that milk, egg, peanut, tree nut, and sesame allergy are more common in children, whereas shellfish and fish allergies are more common in adults. [4]

Gender: anaphylaxis in adults is more common in females than males. However, among children, the risk of anaphylaxis is higher in boys versus girls. [4] The higher frequency of anaphylaxis in adult females could be due to the higher estrogen levels that may lead to enhanced mast cell activation and allergic sensitization and/or progesterone inhibiting histamine release. [154] In two recent studies [1;162], it was shown that tree nut and shellfish allergies were less common in males.

Race/ethnicity: studies have not been consistent on the effect of ethnicity on food allergy. While a few studies report higher risk in non-White race and non- Hispanic Black race, [157] others [137-138], report higher rates in whites. However, it is important to keep in mind that self- identified ethnicity and reported origin of parents and grandparents is imprecise compared to a genetic estimation on individual ancestry. [158] It is also possible that these studies are subjected to information bias because minority children may be under- diagnosed or undertreated for allergic disorders. [139]

Socioeconomic Factors: Several studies suggest an increased rate of food allergy in higher socioeconomic populations. [159-161] In a nested case- control study done in Finland, composed of infants up to 2 years of age, low socioeconomic status, high maternal age, and number of previous deliveries and/ or previous pregnancies were all associated with the risk of cow' milk allergy. [161]

Education: Higher education level is associated with a higher prevalence of food allergy. [1;163] A study looking at adults, reported that within categories of school graduation, the prevalence of SPT increased continuously with level of graduation (high school > university> post graduate).

Similar results were obtained when testing this association on the level of single allergens. They concluded that allergic sensitization to common aeroallergens in adults followed a significant and linear association with school education. [165] A more recent study also concluded that the trend for most food allergies seemed to be more prevalent in the more educated. More educated parents have higher health literacy and may be more likely to consult a physician on a regular basis. Therefore, the actual prevalence may not be higher, but may appear as increased due to the greater likelihood of seeking a diagnosis. [1]

Maternal age: very few studies address the issue of maternal age being a predictor of food allergy. One study reports an association between high maternal age and increased risk of cow's milk allergy.[79] Another study reports higher odds for food allergy with increased maternal age, potentially due to higher genetic predisposition for developing food allergy in children with older mothers. However results remain inconsistent. [4]

Household size & birth order: studies report an inverse association between birth order and the risk of respiratory allergic disease. [166] One study indicates that having an older brother delays the onset of IgE sensitization but may not prevent IgE sensitization per se. [167] The protective association of multiple pregnancies and older siblings could be due to increased exposure to infections in early childhood, resulting from close contact with siblings.[164] Alternatively, these associations may have their origin in the prenatal period and may be due to hormonal and immunologic conditions that differ in the uterus between pregnancies.[4]

Immigrants: the relationship between being an immigrant and the presence of food allergy has been looked at by a few studies. One study reported a trend for food allergies to be less prevalent in immigrants. [1] Studies suggest an increased prevalence of allergic diseases (mainly asthma) that seems to correlate with the length of stay in Westernized countries regardless of age of arrival, sex, or atopic status [4;168;94] The observed reduced risk of food allergy in immigrants could be due to genetic differences as well as environmental influences. Certain Western dietary habits and lifestyles may play a role in the development of food allergies including omega-3 fatty acids deficiency, decreased consumption of fresh fruits and vegetables. It is also possible that immigrants are less likely to consult a physician for a suspected allergy due to lower health literacy and/ or lack of a family doctor. [1]

Geography: based on epinephrine auto- injector distribution data, recent studies suggest higher rates of anaphylaxis in northern areas. [146;205;4] Speculations infer that vitamin D status may be responsible for this north- south gradient. [138] Studies suggest a lower prevalence of allergic diseases in general (according to self- report) in rural versus urban environments, [170] although no

studies have assessed that association for specific food allergies. Recent studies suggest that food allergy may differ not only according to north-south gradients, but in different countries located in the same latitudes. Different dietary habits may be a potential explanation for these differences. [137-139] Thus, while in the US, the predominate allergies in children are to milk, egg and peanut,[11;;156] in Asia, shellfish allergy is reported to be the leading cause of food allergies [168] Lastly, based on a few studies, obesity, family/individual history of diabetes, sleep duration, alcohol consumption and air pollution were all found to contribute to the increased prevalence of allergic diseases. [4]

Table 3: Demographic Determinants of Allergy

Factor	Author	Type of study	Results	Reference
Age	Ben shoshan	Review of cohort and case control studies	Numerous environmental, demographic factors, gene-environment interactions account for in prevalence, but further studies are required to tease out their relative contribution	4
Age	Liu	Cohort	Population-based serologic data on 4 foods indicate an estimated 2.5% of the US population has FA, and increased risk was found for black subjects, male subjects, and children	153
Age	Ben Shoshan	Review of cohort and case control studies	Place of residence, socioeconomic status, and birth place may influence the development of food allergy. Tree nut and shellfish allergies were less common in males (OR 0.55 (95%CI, 0.36, 0.83), OR 0.63 (95%CI, 0.43,0.91) respectively	1
Gender	Ben Shoshan	Review of cohort and case control studies	Numerous environmental, demographic factors, gene-environment interactions may account for this increase in prevalence, but further studies are required to tease out their relative contribution	4
Gender	Ben shoshan	Review of cohort and case control studies	Place of residence, socioeconomic status, and birth place may influence the development of food allergy	1
Gender	Ben Shoshan	Case- control	eczema in the first 2 years of life is associated with food allergies males and older individuals were less likely to have food allergy (OR 0.7 (95%CI 0.6-0.9), OR 0.99 (95%CI 0.99-1.00) respectively)	154
Race/ Ethnicity	Hong	Review of cohort and case control studies	identification of key factors and their contributions to FA will allow us to gain insight into the biological mechanisms by which environmental exposures and genetic susceptibility affect the risk of FA, provide information to develop more effective new paradigms in the diagnosis, prevention, and management of FA	118
Race/ Ethnicity	Sicherer	Cohort	Affected individuals report recurrent and severe reactions, indicating that seafood allergy represents a significant health concern	153
Race/ Ethnicity	Yaeger	Cohort	Genetic classifications of ancestry provide a more objective and accurate method of defining homogenous populations for the investigation of specific population-disease associations	157
Race/ Ethnicity	Dias	Cohort	Ethnic minorities are over-represented in terms of the number of children with food allergy and number of food allergies per child, present at an earlier age with food allergy, and have a greater variety of food allergies compared with Caucasians	158
Socioeconomic status	Kotz	Cohort	lower prevalence in peanut allergy than has been found. Explained by under recording of peanut allergy in general practice. Further research is needed to assess the true frequency of peanut allergy in the population and whether there has been a true increase in recent years	159
Socioeconomic status	Mullins	Cohort	Among young children, hypoallergenic formula prescription rates are more common in the southern and eastern regions of Australia	137
Socioeconomic	Lannero	Cohort	educational level influences risk factors for development of atopic disease in childhood and	161

status			indicates a need of deeper understanding of life style in different socioeconomic groups	
Education	Ben Shoshan	Review of cohort and case control studies	In addition to age, sex, place of residence, socioeconomic status and birth place may influence the development of food allergy	1
Education	Soost	Cross- sectional	women are at greater risk of having symptoms of food allergy and also at greater risk of having DBPCFC-confirmed symptomatic food allergy	163
Education	Luccioli	Cohort	Food-related problems occurred at a high frequency in the first year of life. Understanding of the demographics, family history, disease manifestations, and diagnoses provide insight into public health efforts to minimize food allergies in infancy and to help differentiate food-allergic problems from nonallergic food problems in this age group	164
Education	Schafer	Case- control	Allergic sensitization to common aeroallergens in adults follows a significant and linear association with school education	165
Maternal Age	Ben Shoshan	Review of cohort and case control studies	In addition to age, sex, place of residence, socioeconomic status and birth place may influence the development of food allergy	1
Maternal Age	Hong	Review of cohort and case control studies	identification of these key factors and contributions to FA will allow us to gain insight into the biological mechanisms by which environmental exposures and genetic susceptibility affect the risk of FA provide essential information to develop more effective new paradigms in the diagnosis, prevention, and management of FA	79
Household size & birth order	Upchurch	Case- control	Despite the strong associations between birth order and atopy, reductions in family size account for little of the increase in atopy	166
Household size & birth order	Turner	Case- control	having an older brother delays the onset of IgE sensitization but may not prevent IgE sensitization per se. The apparent protective effect of older siblings on allergic diseases reported elsewhere might involve delaying the onset of IgE sensitization	167
Immigrants	Ben Shoshan	Review of cohort and case control studies	Numerous environmental and demographic factors as well as gene-environment interactions may account for this increase in prevalence, but further studies are required to tease out their relative contribution	4
Immigrants	Ben shoshan	Review of cohort and case control studies	Place of residence, socioeconomic status and birth place may influence the development of food allergy	1
Immigrants	Leung	Cohort study	Adverse food reaction is an atopic disorder in Hong Kong pre-school children	168
Geography	Vassallo	Cohort	seasonal fluctuations in sunlight and vitamin D involved in the pathogenesis of food allergy	135
Geography	Thong	Cohort	The pattern of food allergy in Singapore differs from Caucasian populations, likely to be because of different regional dietary patterns and methods of food preparation	169

2.6.5 Lifestyle Determinants

Epidemiologic studies so far have proposed a number of lifestyle factors (found in Table 4) that may influence the risk of food allergy, such as timing of food introduction, breastfeeding and daycare attendance. But none have yielded definitive results. [4;20]

Food Consumption (Quantity and Timing): low-dose cutaneous sensitization may lead to allergic sensitization to food, while early consumption of food protein induces oral tolerance.[106] Low- dose exposure to environmental foods penetrates the skin barrier, is taken up by Langerhan's cells, leading to Th2 responses and production of IgE by B-cells. Therefore, the timing and balance of cutaneous and oral exposure may determine whether a child develops an allergy or tolerance.[163164] While previous consensus statements suggested that early food exposure leads to food allergy, recent studies seem to argue this point. In 2000, the American Academy of Pediatrics [109] withdrew their recommendations regarding food avoidance during pregnancy and early infancy, and replaced them with comments regarding the lack of current evidence related to delaying the timing of the introduction of complementary foods beyond 4 to 6 months of age in preventing the occurrence of atopic disease. A study of mother- child pairs from a US pre-birth cohort assessed maternal intake of common childhood food allergens during pregnancy. Then, in mid-childhood, they assessed food allergy, asthma, allergic rhinitis and atopic dermatitis. Their results show that higher maternal peanut intake during the first trimester was associated with 47% reduced odds of peanut allergic reaction. [110] A similar study investigated the association between egg allergy in 12- month old infants and lifestyle factors such as timing of introduction of egg and solids, as well as the duration of breastfeeding and found that early introduction of egg appears to protect against egg allergy. The study showed that introducing egg into the diet at either 10-12 months (or post 12 months) was associated with higher risks of egg allergy compared with introduction at 4-6 months. [80]

Lastly, a very recent randomized controlled trial study published in 2015 by George DuToit concluded that early introduction of peanuts significantly decreased the frequency of the development of peanut allergy among children at high risk. [109] Among the 98 participants who initially had positive test results, the prevalence of peanut allergy was 35.3% in the avoidance group and 10.6% in the consumption group. Given that "total" allergen avoidance is quite impossible to achieve, and that current evidence does not completely justify the theory supporting allergen avoidance for allergy prevention, the issue at hand is whether to deviate further from current consensus of "equipoise" and shift to a position of "deliberate exposure" [108]

Day Care Attendance: respiratory tract infections are more commonly seen in children attending daycare. [111] However, the association between daycare attendance and the development of allergic diseases later in life remains, a topic of great ambiguity. [112] Studies state the presence of an inverse association between daycare attendance and development of allergic diseases (daycare having a protective effect). [111-112] Other studies state the opposite, reporting a positive association between daycare attendance and development of food allergy (daycare considered a risk factor)[113]
Results remain largely inconsistent, and it is important to keep in mind that major published studies have neither described daycare group size, nor time exposure in a daycare. [115]

Breastfeeding: numerous studies have looked at breastfeeding as a means of protection against the development of allergic diseases. In a cohort of infants with likely milk or egg allergy, maternal peanut consumption during pregnancy, as well as sIgE to milk and peanut, were linked to an increased risk of having peanut sIgE. Similar findings were reported for tree nut and sesame seeds. However, it's important to keep in mind that sensitization rather than reactivity was evaluated. [34] Another study concluded that increasing the duration and degree (exclusivity) of breastfeeding, decreased the risk of gastrointestinal tract infection and atopic eczema in the first year of life. [253]

Table 4: Lifestyle Determinants of Allergy

Factor	Author	Type of study	Results	Reference
Food Consumption (Quantity & timing)	Du Toit	Case- control	Early introduction of peanut decreased the frequency of peanut allergy in children at high risk for that allergy. Among 530 infants with negative SPT, prevalence of peanut allergy at 60 months was 13.7% in avoidance group, 1.9% in consumption group. Among 98 infants with positive SPT, prevalence of peanut allergy at 60 months was 35.3% in avoidance group versus 10.6% in consumption group	106
Food Consumption (Quantity & timing)	Fox	Case- control	High levels of environmental exposure to peanut during infancy appear to promote sensitization, low levels may be protective in atopic children.	108
Food Consumption (Quantity & timing)	Mullins	Review of cohort and case-control studies	Among young children, hypoallergenic formula prescription rates are more common in the southern and eastern regions of Australia	137
Food Consumption (Quantity & timing)	Hong	Review of cohort and case-control studies	Non-genetic factors may influence the risk of FA, such as timing of food introduction and feeding pattern, diet/nutrition, exposure to environmental tobacco smoking, prematurity and low birth weight, microbial exposure, and race/ethnicity	79
Food Consumption (Quantity & timing)	Bunyavanich	Case- control	Higher maternal intake of peanut, milk and wheat during early pregnancy was associated with reduced odds of mid- childhood allergy and asthma. Higher maternal peanut intake during the first trimester was associated with 47% reduced odds of peanut allergic reaction (0.53 OR; 95%CI, 0.30-0.94), Higher milk intake during first trimester associated with reduced asthma (0.83 OR, 95%CI, 0.69-0.99), and allergic rhinitis (0.85 OR, 95%CI, 0.74-0.97), Higher maternal wheat intake during second trimester associated with reduced atopic dermatitis (0.64, 95%CI, 0.46-0.90)	110
Food Consumption (Quantity & timing)	Tan	Review of cohort and case-control studies	Current state of knowledge regarding genetic and environmental risk factors for food allergy, and considers the potential for furthering our understanding of food allergy aetiology by examining the role of epigenetic variation. Introducing egg into the diet at either 10-12 months or after 12 months was associated with higher risks of egg allergy 1.6 OR (95% CI, 1.0-2.6), compared with introduction at 4-6 months	80
Daycare	Sun	Case- control study	Daycare attendance increases the risk for respiratory tract infections and "allergic" symptoms in children.	110
Daycare	Hurwitz	Case- control	Increased risk of respiratory illness was associated with attending day care	112
Daycare	Kramer	Cross- sectional	Early infection may protect against allergies in later life	114
Daycare	Ball	Cross- sectional	Exposure of young children to older children at home or to other children at day care protects against the development of asthma and frequent wheezing later in childhood	115
Daycare	Svanes	Case- control	Subjects exposed to many children at home or in day care experienced less hay fever and more asthma in adulthood	116

Daycare	Hagerhed-Engman	Cohort	Day care attendance associated with increased risk of symptoms related to airway infection, as well with eczema and allergic reactions to food	141
Breastfeeding	Sanchez	Cohort	Caesarean delivery is demonstrated as being a risk factor for IgE-mediated CMA, but it does not increase the risk of AM in these infants	145
Breastfeeding	Mandhane	Case- control	The influence of breast-feeding on development of atopy and asthma differs by sex and maternal and paternal atopy baseline risk	94
Breastfeeding	Lack	Review of case-control and cohort studies	Limited support for hygiene hypothesis, eczema, c-sections correlated with food allergy, no evidence that antioxidants, vitamin supplements have an effect on FA	96
Breastfeeding	Hong	Cohort	The effect of breast-feeding on FS was modified by SNPs in the IL12RB1, TLR9, and TSLP genes both individually and jointly	79
Breastfeeding	Sears	Cohort	Breastfeeding does not protect children against atopy and asthma and may even increase the risk	151

2.6.6 Atopic co- morbidities

Defined as the presence of atopic dermatitis, allergic rhinitis, asthma, or food allergy, atopic co-morbidities (found in Table 5) are relatively common in subjects who experience anaphylaxis. [152] The extracellular cytokine environment associated with atopic diseases may contribute to the increased risk of an anaphylaxis reaction. The majority of studies report a strong association between food allergy and atopy.[4;152-153] In addition, a history of atopy in family members is also considered a known risk factor for the development of food allergy.

Various hypotheses encompassing several non-genetic factors have been proposed in the medical literature to account for the rapidly increasing food allergy prevalence. Lack et al.[95] coined the term **"dual- allergen- exposure hypothesis"**, which posits that food sensitization can occur through low- dose cutaneous exposure and that early consumption of food protein induces oral tolerance. [106] For a long time, it was assumed that the first step in "the atopic march" was the development of IgE responses directed to food proteins, and that eczema followed shortly afterwards. [4] However, recent cohort studies have challenged this assumption, revealing that eczema and cutaneous exposure to food allergens both precede the development of food allergy.[34-35] It is now proposed that low-dose cutaneous exposure to an allergen, due to impaired skin barrier, may promote allergic sensitization, whereas early oral consumption induces tolerance.[4]

The relationship between diet and the development of allergies has caught the attention of many researchers. It's been suggested that, due to the marked changes in diet in the past 3 decades, differences in macronutrient and micronutrient dietary content may potentially explain the geographic differences seen in the prevalence food allergy in different parts of the world and the increase in allergies. [96] Studies report that higher intakes of ω-6 and lower intakes of ω-3 fatty acids lead to increased production of prostaglandin E2 (PGE2), and thus the development of food allergy.[97-99] It has also been postulated that western diet is deficient in anti-oxidants that have anti-inflammatory effects. [98] However, a common limitation to all studies is that none simultaneously controlled for genetic and environmental factors regarding the major food allergens. Only studies that control for genetic environmental interactions affecting the development of eczema, food allergy and their interaction would have the power to assess the true effect of eczema on food allergy.

Eczema: Although findings are inconclusive, one of the strongest factors suggested to have an effect on the development of food allergy is eczema. Similar to food allergy, interactions between genetic and environmental factors contribute to the development of eczema. [6] Given that eczema is often the first and most prevalent manifestation of atopic diseases in early childhood [7] and given that

temporal trends in prevalence of food allergy follow tightly those of eczema [8-10], it is possible that eczema serves as a major risk factor for the development of food allergy. [88-89;119]

Of particular interest is the understanding of the role of skin barrier in the development of food allergies, as this represents a potentially modifiable risk factor. Studies report an increased risk of food allergy in children with eczema having been exposed to food allergens early in life, through their skin, suggesting that the control of eczema may reduce the risk of food sensitization and potentially allergy. [38-39] The association between eczema and food allergy has been looked at in several studies. However, it's true effect is not clear given that none of these studies controlled simultaneously for gene environment interactions. Studies report a well -documented link between the presence of early eczema in childhood and the development of food allergy, especially peanut, egg, and milk allergies. [95] Similar findings report that among children with food allergy, 50% to 70% of them had eczema in infancy [260;262] compared to 9% to 40% in the general population.[176] Further epidemiologic studies reveal that the odds ratio for developing any food allergy in those with eczema may range between 2.4 (95% CI 1.9, 3.0) [4;37] to 35.3 (95% CI 2.6,46.1)[34-35] In 2011, one study reported that food allergy and eczema often co- exist and that a higher prevalence of food allergy is observed among children with atopic disorders. [80] Similarly, a DBPCOFC, the gold standard in diagnosing food allergy, showed that food allergy is found in 35-80% of children with moderate to severe eczema. [185] In a population based case control study across Canada, it was found that eczema in the first two years of life is associated with all food allergies. [35] Furthermore, a large Canadian population based nested case-control study reported that eczema in the first two years of life was the strongest risk factor for egg, peanut, tree-nut and fish allergy. [155]

Given that the prevalence of eczema is increasing [8-10] and that eczema is highly associated with food allergy [8-10; 95], this could explain the increasing prevalence of food allergy. Thus, a deeper understanding of the skin barrier role is of particular interest, as this represents a potentially modifiable risk factor.

Genetic studies have also demonstrated a strong association between food allergy and eczema. Studies report that loss-of-function variants in the filaggrin gene, a major structural protein of the stratum corneum (outer layer of skin), essential for the composition, structure, and barrier function of the skin, are a significant risk factor for peanut allergy.[182;187]

Fillagrin mutations also contribute to the risk of eczema, but independently increase the risk of peanut allergy. [186] Null mutations in the gene are associated with increased susceptibility to eczema. Individuals with two null alleles in the filaggrin gene have been shown to be four to seven times more likely to have eczema than those without. One recent case-control study investigated

whether mutations in the filaggrin gene were also associated with peanut allergy, reporting that those with peanut allergy were around five times more likely to have filaggrin loss-of-function mutations. These results, along with previous studies suggesting that peanut sensitization may occur through damaged skin, indicate that epithelial barrier dysfunction may play a role in the pathogenesis of peanut allergy. [187]

The penetrance of filaggrin null mutations with respect to eczema is 55.6% in homozygotes and 16.3% in heterozygotes [5], and studies reveal that even in the absence of clinical eczema, filaggrin mutations result in impairment of skin barrier.[6] Furthermore, one study shows that sensitization to peanut protein may occur in children through application of peanut oil to inflamed skin, suggesting that skin barrier defects may mediate sensitization and possibly contribute to food allergy. [106]

Asthma: based on longitudinal studies of patients with atopic dermatitis and food allergies, a history of food allergy increases the risk of asthma. [227] In children with atopic dermatitis, one study reports an increased risk for the development of asthma in children with a history of egg allergy. Also, persistent asthma was significantly associated with elevated specific IgE to egg and wheat. [228] Lastly, in the study with the longest follow-up period of 22 years, egg or milk allergy in the first year of life was a major risk for adult asthma. [229] Together, these studies confirm that food allergy, and egg allergy in particular, in early infancy increases the risk for developing asthma later in life.

Epidemiologic studies thus far have been disappointing in finding conclusive non genetic factors associated with the development of food allergy, likely due to their failure to control for gene-environment interactions. This study aims to determine which non genetics factors are crucial in the development of food allergies. Given that numerous non genetic factors may contribute to the development of food allergy, a twin study design offers a clear advantage through the natural control of environmental factors in twin pairs sharing the same household, lifestyle habits and geographical location.

Table 5: Atopic co- morbidities

Factor	Author	Type of study	Results	Reference
Atopy	Liu	Cohort	Population-based serologic data on 4 foods indicate an estimated 2.5% of the US population has FA, and increased risk was found for black subjects, male subjects, and children	153
Atopy	Tsai	Case-control study	A child with a peanut allergic sibling had a fivefold increased risk of having a peanut allergy. up to the age of 4 years, the incidence of any positive food allergy test (SPT, specific IgE levels or a food challenge) was threefold higher if both parents reported having an allergic manifestation, and twofold higher if either mother or father had such a manifestation when compared with children whose parents did not report any of these conditions	89
Atopy	Kramer	Case-control	Food allergy in the index child was a significant and independent predictor of food allergy in other siblings (2.60 OR, 95 % CI, 1.20–5.60). Positive and significant associations among family members (father-offspring, mother-offspring, index-other siblings) were found for total and specific IgE to all the nine major food allergens (sesame, peanut, wheat, milk, egg white, soy, walnut, shrimp and cod fish)	114
Atopy	Du Toit	Case-control	Early introduction of peanut decreased the frequency of developing a peanut allergy in children at high risk for that allergy. Among 530 infants with negative SPT, prevalence of peanut allergy at 60 months was 13.7% in avoidance group versus 1.9% in consumption group. Among 98 infants with positive SPT, prevalence of peanut allergy at 60 months was 35.3% in avoidance group versus 10.6% in consumption group	110
Atopy	Vassallo	Cohort study	Food allergy is more common in Boston children born in the fall and winter seasons. These findings are mediated by seasonal differences in UV-B exposure. These results add support to the hypothesis that seasonal fluctuations in sunlight and perhaps vitamin D may be involved in the pathogenesis of food allergy	96
Atopy	Untersmayr	Case-control	Anti-ulcer treatment primes the development of IgE toward dietary compounds in long-term acid-suppressed patients	97
Atopy	Scholl	Case-control	Anti-ulcer drugs may lead to the induction of immediate-type food hypersensitivity toward hazelnut	98
Eczema	Ben Shoshan	Case-control	In addition to previously reported factors, eczema in the first two years of life is consistently associated with food allergies	155
Eczema	Wavrin	Case-control	Cutaneous or respiratory exposures to peanut induce Th2 priming in mice. Pre-exposures promote further sensitisation via the oral route without the use of CT; a new adjuvant-free experimental model of sensitisation to food that may reflect a realistic exposure pattern in infants. These results also suggest that non-gastrointestinal peanut exposure should be minimised in high-risk infants, even those with non-altered skin, to potentially reduce allergic sensitisation to this major food allergen.	223

Eczema	Bohme	Case-control	During the first 2 years of life there is a significant association not only between atopic dermatitis and other atopic disease manifestations, but also between atopic dermatitis and respiratory infections manifested in an increased rate of acute otitis media, pneumonia and use of antibiotics	224
Eczema	Perry	Cohort study	The major peanut allergen, Ara h 1, is relatively easily cleaned from hands and tabletops with common cleaning agents and does not appear to be widely distributed in preschools and schools. We were not able to detect airborne allergen in many simulated environments	225
Eczema	Martin	Cross sectional	Approximately, one in three infants developed eczema by 12 months of age. East Asian infants are at increased risk of eczema despite their parents having lower rates of allergy than non-Asian parents. Gene-environment interactions may explain the differential effect seen in this minority group	6
Eczema	Smidesang	Cohort study	The prevalence of atopic dermatitis among 2-year olds was high. However, more than two-thirds of the children had mild disease, which may imply that the impact of atopic dermatitis as a risk factor for future atopic disease is limited	7
Eczema	Hansen	Cross-sectional	A repeated cross-sectional survey between 1985 and 2008 documented an increasing prevalence of asthma ever and AR ever among schoolchildren (7-14 years), together with a considerably increase in current asthma, AR and eczema between 1995 and 2008	8
Eczema	Duggan	Cross-sectional	The prevalence of asthma in 6–9 year old Cork schoolchildren remained static between 2002 and 2007; however, rhino-conjunctivitis and eczema have become increasingly prevalent. Co-morbidity of allergic conditions continues to pose a considerable health burden	9
Eczema	Kolokotroni	Case-control	The prevalence of allergic diseases in Cyprus is still on the rise; recent increases appear more pronounced among children living in rural areas possibly indicating recent environmental and lifestyle changes in these communities	10
Eczema	Horimukai	Case-control	Daily application of moisturizer during the first 32 weeks of life reduces the risk of AD/eczema in infants. Allergic sensitization during this time period is associated with the presence of eczematous skin but not with moisturizer use	38
Eczema	Simpson	Case-control	Emollient therapy from birth represents a feasible, safe, and effective approach for atopic dermatitis prevention. If confirmed in larger trials, emollient therapy from birth would be a simple and low-cost intervention that could reduce the global burden of allergic diseases	39
Eczema	Gehring	Cohort study	Exposure to high concentrations of endotoxin very early in life might protect against the development of atopic eczema within the first 6 months of life, along with an increased prevalence of nonspecific respiratory diseases	184
Eczema	Shaw	Cohort study	Metropolitan living is a significant factor in predicting a higher disease prevalence with an odds ratio of 1.67 (95% confidence interval of 1.19-2.35, P=0.008). Black race (odds ratio 1.70, P=0.005) and education level in the household greater than high school (odds ratio 1.61, P=0.004) were also significantly associated with a higher prevalence of eczema. The wide range of prevalence suggests that social or environmental factors may influence disease expression	176

Eczema	Ben Shoshan	Case-control	In addition to previously reported factors, eczema in the first two years of life is consistently associated with food allergies	155
Atopic Dermatitis	Hon	Cross sectional	Patients with severe AD (objective SCORAD>40) were more likely to be positive for at least one of the food items (Yates corrected p=0.024 for ≥1 food-specific IgE in severe vs. moderate AD, OR 3.42 and 95% CI 1.15-10.32); and for at least seven of the food items (p=0.001 for ≥7 food-specific IgE vs. nil with OR 11.67 and 95% CI 2.29-67.77), respectively. . The Spearman coefficients between the number of positive food-specific IgE and total SCORAD, objective SCORAD, area of AD involvement, Children's Dermatology Life Quality Index (CDLQI), total IgE levels, and eosinophil counts were 0.42 (p<0.001), 0.45 (p<0.001), 0.50 (p<0.001), 0.17 (p=0.116), 0.80 (p<0.001), and 0.22 (p=0.043), respectively.	223
Atopic Dermatitis	Estrada-Reyes	Case control	There were more children with positive skin tests for food hypersensitivity among cases with atopic dermatitis than controls, OR 4.2 (95%CI 1.3 to 13.4).	224
Atopic Dermatitis	Garcia	Cross sectional	A high prevalence of food sensitization was found in infants with AD. The most frequent sensitization observed was to egg, although with little clinical relevance since this food had not been introduced into the diet. Of the 44 patients studied, sensitization to foods was detected in 27 (61 %). No changes were observed in AD during the elimination diet or when the eliminated foods were subsequently reintroduced into the diet. The results of open controlled food challenges were positive in 12 patients (27 %).	225
Atopic Dermatitis	Hill	Cohort	As atopic dermatitis severity increased so did the prevalence of IgE-mediated food allergy, defined when the SPT diameter for a specific food was greater than or equal to twice the histamine reference (Gp 0, 40/346 vs. Gp 1, 6/36 vs. Gp 2, 8/35 vs. Gp 3, 12/35 vs. Gp 4, 24/35; chi(2) = 76; p < 10(-6))	226
Asthma	Thong	Cross sectional	More than half of the patients diagnosed with food allergy had concomitant allergic rhinitis, asthma and/or eczema.	227
Asthma	Gustafssson	Cohort study	In most children both clinical allergy and sensitization to egg and milk were transient but those to peanut were persistent. Eighty per cent of the children became sensitized to airborne allergens and 75% of them noticed symptoms when exposed. Heredity for atopy and eczema, sensitization to hen's egg, and early onset of eczema entailed an increased risk of becoming sensitized. Children never sensitized had late onset of eczema and less heredity for atopic disease but did not differ in other respects from the sensitized children	228
Asthma	Rhodes	Cohort study	Prediction of adult asthma remains difficult. In this study of subjects at risk of atopy, skin sensitivity to hen's egg or cow's milk in the first year was predictive of adult asthma	229
Asthma	Annesi-Maesano	Cross sectional	In those with food allergy the OR for asthma were 1.88(1.28,2.76)	230

3.0 Study Objectives

3.1 Overall objectives

Despite best efforts to shed some light on the growing food allergy prevalence, results remain inconsistent. Given this unexplained increase in prevalence in food allergy, as well as the knowledge gaps regarding the potential genetic and environmental predictors of food allergies, this thesis aims to address the following primary and secondary research questions:

3.1.2 Primary Objective

To determine the relative contribution of genetic and environmental factors to the development of food allergies by comparing food allergy concordance in pairs of MZ and DZ twins

3.1.3 Secondary Objective

To evaluate if eczema, a modifiable risk factor, is a risk factor for the development of the major five food allergy.

4.0 Study Methodology

4.1 Study Design

To address the research questions, a twin's registry has been developed over a 2 year period. Recruitment took place from 3 sources; Multiple Births Canada (MBC) (www.multiplebirthscanada.org), Anaphylaxis Canada and the allergy clinic at the Montreal Children's Hospital. Only twins where at least one member of the twin set has peanut, tree nut, milk, egg, fish, shellfish, wheat, or soy allergy were recruited. Those (i.e. adult twins and parents of child twins) who are potentially eligible and interested in participating were invited to contact our research team by phone, mail or email. According to their preference, interested participants were mailed or emailed a consent form and a questionnaire based on previous validated food questionnaires[37] and the ISAAC (International Study of Asthma and Allergies in Childhood) questionnaire on ethnicity, clinical characteristics of most severe reaction to foods, and personal and family history of atopy.[38]

More detailed questions on symptoms pertinent to the diagnosis of eczema, based on the Williams criteria.[57] was used to validate the presence of eczema. In addition, to reduce any recall bias, participants who have eczema at the time of the questionnaire were asked to provide pictures of involved areas.[58] Participants were asked to sign a request for release of medical information, allowing us to contact the physician treating their children's allergic condition. Allergists of participants consenting to release of medical information were asked to provide the results of tests used to confirm the diagnosis of food allergy.

Participants were considered to have food allergy if he or she had a convincing history of a food-induced allergic reaction[31] in the presence of a positive skin prick test or an IgE level of at least 0.35KU/L.[37-38] as well as a physician's report of food allergy. With the help of the principal investigator and the study coordinator, I independently reviewed participants' data to ensure that recruited twins meet criteria for food allergy.

4.2 Sample Size Calculations

To determine the sample size for this study, I used the ACE model. . The ACE model refers to a genetic epidemiological model that postulates that additive genetic factors (A), common environmental factors (aspects of the environment that are common to members of the same family or people who live together and contribute to similarity between relatives = C), and specific environmental factors (factors in the environment that are not shared and hence contribute variation within family members, but not to their covariation = E) account for individual differences in a phenotype of interest.[41] I based the sample size calculations to assess the primary objective (i.e. to determine the relative contribution of genetic and environmental factors to the development of the most common food allergies by comparing concordance rates between MZ & DZ twins) on previously reported rates in a North American study on food allergy in twins; this study estimated 81.6% heritability for peanut allergy.[5]

Accordingly, I estimated the variance of the genetic component at 80% [5] and assumed the common shared and non-shared environmental variance at 10% each. [2;5] Based on these assumptions and the online tool developed by Visscher et al. for twin studies (http://genepi.qimr.edu.au/cgi-bin/twinpower.cgi) the sample size (in pairs) needed to achieve 80% power, with a Type 1 error rate of 0.05 was 12 MZ pairs and 7 DZ pairs per allergen.

To assess the primary objective, I used probandwise concordance rates between MZ and DZ pairs. There are two alternative measures of twin concordance assessment: the pairwise concordance (defined as C/(C+D) where C is the number of concordant pairs and D is the number of discordant pairs and probandwise concordance (defined as 2C/(2C+D) where C is the number of twin pairs that are both allergic to the specific food, and D is the number of pairs in which only one twin has the food allergy. It is agreed that for almost every application, the probandwise rate is preferred over the pairwise rate as the probandwise concordance serves to forecast risk at the level of the individual rather than at the level of the pair. Further pairwise concordance may underestimate the genetic effect. [182] (Table 6)

To assess objective two (whether eczema is a risk factor for the development of food allergy) a case control design was used where the cases are those with a food allergy. I will be conducting multivariate logistic regression models to evaluate the presence of an association between genetic and environmental factors to the development of food allergy. The unit of measurement will be the twin pair, and the outcome will be concordance in food allergy. The variables in my multivariate logistic regression model are: presence of eczema, presence of asthma, daycare attendance, exposure to pets before the age of 1, exposure to a farm environment before the of 1, second hand smoke history in the household, multivitamin intake of pregnant mother (for at least 3 months), breastfeeding, the age of introduction of each of the 6 major foods as well as antibiotics prescription. To assess objective 2, I will need a total of 26 concordant pairs (5 DZ, 21 MZ) and 74 non- concordant pairs (60 DZ, 14 MZ) (Table 6B).

Table 6: ACE model sample size calculation

Table 6A: Objective 1 sample size calculation:

Implied Statistics

Parameter	Formula	Value	Input Parameters
Proportion of variance due to **A**	$\mathbf{h}^2$	0.800	Variance components : **A** = 0.800 ; **C** = 0.100 ; **E** = 0.100 Type I error rate = 0.050000 Power = 0.800
Proportion of variance due to **C**	$\mathbf{c}^2$	0.100	
MZ correlation	$\mathbf{h}^2+\mathbf{c}^2$	0.900	
DZ correlation	$\mathbf{h}^2/2+\mathbf{c}^2$	0.500	
Proportion of pairs who are MZ	$\mathbf{p_{MZ}}$	0.25	

Type I error rate = 0.050000

Detection of A					**Detection of C**				
Noncentrality parameter		36.930			Noncentrality parameter		0.670		
Power		1.000			Power		0.212		
		Sample size (pairs) needed to achieve 80.0% power					Sample size (pairs) needed to achieve 80.0% power		
		All	MZ	DZ			All	MZ	DZ
User-specified $\mathbf{p_{MZ}}$	0.25	34	9	25	User-specified $\mathbf{p_{MZ}}$	0.25	1836	459	1377
Optimised $\mathbf{p_{MZ}}$	0.63	19	12	7	Optimised $\mathbf{p_{MZ}}$	0.13	1598	208	1390

Table 6B: Objective 2 sample size calculation:

	Number of concordant twin pairs (cases)	Number of non-concordant twin pairs (controls)
DZ	0.07*65=5	65-5=60
MZ	0.6*35=21	35-21=14
Total	26	74
Ratio of controls to cases	3:1	

We are planning a study of independent cases and controls with 3 controls per case. Prior data indicate that the probability of eczema among controls is 0.2. If the true odds ratio for concordant eczema in twins pairs that are concordant with food allergy relative to those that are not concordant for eczema is 5, we will need to study 19 case patients and 57 control patients to be able to reject the null hypothesis that this odds ratio equals 1 with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.04. We will use an uncorrected chi-squared statistic to evaluate this null hypothesis.

4.0 Results

Table 7: Specific food allergies concordance and demographic characteristics

	Peanut	Almond	Pecan/walnut	Hazelnut	Cashew/Pistachio	Shellfish	Milk	Egg
Sex (% males)	25%(0.20,0.28)	22%(0.20,0.24)	77%(0.70,0.85)	21%(0.18,0.31)	73%(0.66,0.78)	15%(0.10,0.18)	16%(0.12,0.20)	78%(0.68,0.78)
Age(median,IQR)	8(IQR=8)	7(IQR=4)	7 (IQR=7.5)	7(IQR=8)	7 (IQR= 8.5)	7(IQR=7.5)	25(IQR=48)	4.5(IQR=8.5)
Number of MZ pairs	15	5	9	8	12	3	4	8
Number of DZ pairs	17	4	4	6	9	1	2	10
Concordant pairs C	12	3	6	2	8	3	5	5
Disconcordant pairs D	20	6	7	12	13	1	1	13
MZ Concordance (2C/2C+D)	0.50(0.26,0.70)	0.35(0.16,0.75)	0.52(0.20,0.80)	0.2 (0.03,0.55)	0.5 (0.27, 0.72)	0.80(0.32,0.98)	1 (0.60, 1)	0.22(0.03,0.59)
DZ Concordance (2C/2C+D)	0.59 (0.39,0.77)	0.5 (0.15,0.84)	0.55 (0.15,0.85)	0.33 (0.06,0.76)	0.57 (0.29, 0.81)	1 (0.20, 1)	0.67 (0.13,0.98)	0.51 (0.19,0.75)

Table 8: Concordance values & differences

Allergen	2C/2C+D	95% CI	Proportion 1	Proportion 2	Difference between proportions(prop1-prop2)	95%CI difference
Peanut (DZ)	0.59	(0.39, 0.77)	0.59	0.50	0.09	(0.05, 0.42)
Peanut (MZ)	0.50	(0.26, 0.70)				
Almonds (DZ)	0.5	(0.15, 0.84)	0.5	0.35	0.15	(-0.59, 0.94)
Almonds (MZ)	0.35	(0.16, 0.75)				
Pecan/walnut (DZ)	0.55	(0.15, 0.85)	0.55	0.52	0.03	(-0.47, 0.52)
Pecan/walnut (MZ)	0.52	(0.20, 0.80)				
Hazelnut (DZ)	0.33	(0.06, 0.76)	0.33	0.2	0.13	(-0.45, 0.72)
Hazelnut (MZ)	0.2	(0.03, 0.55)				
Cashew/pistachio(DZ)	0.55	(0.29, 0.81)	0.55	0.5	0.05	(0.02,0.80)
Cashew/pistachio(MZ)	0.5	(0.27, 0.72)				
Shellfish (DZ)	1	(0.20, 1)	1	0.80	0.2	(-0.25, 0.52)
Shellfish (MZ)	0.80	(0.32, 0.98)				
Milk (DZ)	0.67	(0.13, 0.98)	0.67	1	-0.33	(-1.00, 0.42)
Milk (MZ)	1	(0.60, 1)				
Eggs (DZ)	0.51	(0.19, 0.75)	0.51	0.22	0.29	(0.11, 0.78)
Eggs (MZ)	0.22	(0.03, 0.59)				

Primary objective

For peanut, cashew/ pistachio and egg allergy, the concordance among DZ pairs was higher compared to MZ pairs. (Table 7, Table 8) Among 17 DZ and 15 MZ pairs allergic to peanuts, their concordance rate was 0.59 (%95CI 0.39, 0.77) and 0.50 (%95CI 0.26, 0.70) respectively, with the difference between both concordance rates being 0.09 (%95 CI 0.05, 0.42). For almond allergy, the concordance rate in 4 DZ pairs vs. 5 MZ pairs was 0.5 (%95CI 0.15, 0.84) and 0.35 (%95 CI 0.16, 0.75) respectively, with the difference between both rates being 0.15 (%95 CI -0.59, 0.94).
For pecan/ walnut allergy, the concordance rate between 4 DZ and 9 MZ pairs was 0.55 (%95CI 0.15, 0.85) and 0.52 (%95 CI 0.20, 0.80) respectively, with the difference between both rates being 0.03 (%95 CI -0.47, 0.52). A total of 6 DZ and 8 MZ pairs were allergic to hazelnuts. The concordance rate was 0.33 (%95CI 0.06, 0.76) and 0.2 (%95 CI 0.03, 0.55) respectively, and the difference between concordance rates was 0.13 (%95 CI -0.45, 0.72). 9 DZ and 12 MZ pairs were allergic to cashews/pistachios. The concordance rate in DZ vs. MZ pairs was 0.55 (%95CI 0.29, 0.81) and 0.5 (%95 CI 0.27, 0.72) respectively, and the difference between concordance rates was 0.05 (%95 CI 0.02, 0.80). Shellfish allergy had a small sample size. The concordance rate among 1 DZ pair and 3 MZ pairs of twins was 1.00 (%95CI 0.20, 1.00) and 0.80 (%95 CI 0.32, 0.98) respectively, with the difference between both concordance rates being 0.20 (%95 CI -0.25, 0.52). Milk allergy also had a low sample size of 2 DZ and 4 MZ pairs. The concordance rate was 0.67 (%95CI 0.13, 0.98) and 1 (%95 CI 0.60, 1) respectively, with the difference between both concordance rates being -0.33 (%95 CI -1.00, 0.42). For egg allergy, the concordance rate in 10 DZ pairs vs. 8 MZ pairs was 0.51 (%95CI 0.19, 0.74) and 0.22 (%95 CI 0.03, 0.59) respectively, with the difference being 0.29 (%95 CI 0.11, 0.78).

Secondary objective

In a univariate analysis model, eczema- diagnosed in the first 2 years of life (defined hereafter as early eczema), presence of asthma, age of introduction of the specific food before the age of 1, as well as the consumption of 4 courses of antibiotics was associated with the concordance in almond, cashew/ pistachio, egg, hazelnut, and peanut allergy. (Table 9A)

For peanut and pecan/walnut allergy, early eczema was the only significant factor (OR 1.3, 95%CI 1.02, 1.81), (OR 1.96, 95%CI 1.26, 3.03) respectively. For almond allergy, both asthma and age of introduction of almonds before the age of 1 were significant factors ((OR 1.94, 95%CI 1.26, 2.98), (OR 0.6, 95%CI 0.36, 0.99)) respectively. Early eczema was the only significant factor (OR 1.67, 95%CI 1.19, 2.35) associated with cashew/ pistachio allergy. Both asthma (OR 1.39, 95%CI 1.18, 1.98) and early eczema (OR 1.94, 95%CI 1.44, 2.63) were associated with hazelnut allergy. For milk and shellfish allergy, the small sample size limited any definitive conclusion. Early eczema (OR 1.39, 95%CI 1.22, 2.09), as well as a 4 course of antibiotics factor (OR 2.35, 95%CI 1.26, 3.99) were significantly associated with egg allergy concordance.

In a multivariate analysis model, for peanut and pecan/ walnut allergy, the adjusted odds ratio for early eczema was 1.3 and 2.3 (OR 1.32, 95%CI 1.05, 1.83), (OR 2.32, 95%CI 1.58, 3.92) respectively. After controlling for age and sex, asthma was associated with almond and hazelnut allergy (OR 2.09, 95%CI 1.47, 2.97), (OR 2.11, 95%CI 1.63, 2.74) respectively. For cashew/pistachio allergy, as well as egg allergy, when age and sex were controlled for, early eczema remained significant (OR 1.81, 95%CI 1.21, 2.68), (OR 1.47, 95%CI 1.00, 2.14), (OR 2.15, 95%CI 1.96, 2.37) respectively. I observed no association between shellfish allergy and the factors investigated, possibly due to their low sample size. Food allergy concordance for all 5 foods was associated with early eczema, asthma and male gender (1.02, 95%CI 0.90, 1.08), (2.10, 95%CI 1.51, 2.32), (1.45, 95%CI 1.12, 1.68) respectively. (Table 9B)

Table 9A: Logistic regression model (secondary objective)

Univariate Analysis

	Peanut	Almond	Pecan/ walnut	Hazelnut	Cashew/ pistachio	Shellfish	Milk	Eggs
Zygosity (MZ vs DZ)	1.05;(0.75,1.46)	0.95; (0.51,1.76)	0.97;(0.54,1.76)	0.91;(0.60,1.37)	1.17;(0.78,1.77)	0.6;(0.17,2.14)	**2.50;(2.05,2.71)**	0.75;(0.49,1.15)
Age of study entry(below or above 1)	1.01;(0.99,1.02)	1.00; (0.91,1.10)	1.02;(0.98;1.07)	0.99;(0.97,1.02)	1.02;(0.98,1.05)	1.01;(0.87,1.18)	1.00(0.99,1.01)	**1.05;(1.00,1.09)**
Sex (male 1, female 0)	1.09;(0.75,1.58)	**1.62;(1.04,2.51)**	0.92;(0.57,1.48)	**1.41;(1.01,1.96)**	0.96;(0.60,1.53)	**1.30;(1.11,1.46)**	0.81;(0.31,2.13)	0.87;(0.50,1.53)
Early eczema (diagnosed in first 2years of life)	**1.3;(1.02,1.81)**	1.49; (0.86,2.57)	**1.96;(1.26,3.03)**	**1.39;(1.18,1.98)**	**1.67; (1.19,2.35)**	2.11;(0.70,6.33)	1.28;(0.61,2.67)	**1.39;(1.22,2.09)**
Asthma	1.03;(0.64,1.65)	**1.94;(1.26,2.98)**	1.69;(0.98,2.90)	**1.94;(1.44,2.63)**	1.24;(0.74,2.10)	0.47;(0.15,1.41)	1.09;(0.80,1.19)	2.14; (0.88,5.18)
Daycare attendance (in the first 2 years of life)	0.95;(0.68,1.32)	1.05; (0.56,1.95)	0.97;(0.54,1.76)	0.95;(0.64,1.43)	0.97;(0.64,1.47)	1.94;(0.83,4.52)	0.71;(0.37,1.37)	1.24; (0.80,1.93)
Exposure to pets in household(in the first 2 years of life)	0.79;(0.57,1.11)	0.75; (0.36,1.53)	0.63;(0.29,1.34)	0.83;(0.52,1.33)	0.77;(0.50,1.18)	1.13;(0.21,1.46)	1.39;(0.72,2.68)	0.96; (0.56,1.63)
Living on a farm (in the first 2 years of life)	0.67;(0.33,1.35)	0.98; (0.83,1.61)	0.80;(0.11, 1.19)	1.02;(0.79,1.52)	0.70;(0.09,1.11)	0.56;(0.11, 1.46)	0.36;(0.25,1.02)	0.74; (0.28,1.92)
Exposure to second hand smoke in household (first 2 years of life)	0.67;(0.33,1.35)	0.90; (0.83,1.61)	0.65;(0.23,1.88)	0.85;(0.39,1.10)	1.22;(0.60,2.47)	1.64;(0.46,5.84)	1.28;(0.61,2.67)	0.71; (0.40,1.26)

Vitamin intake of pregnant mother (yes or no)	0.7;(0.46, 1.06)	0.69; (0.35,1.39)	0.87;(0.39,1.92)	0.65;(0.39,1.84)	0.81;(0.40,1.65)	0.6;(0.17, 2.14)	1.15;(0.50,2.50)	0.77; (0.39,1.54)
Breastfeed practices (Exclusively, yes or no)	0.96;(0.60,1.54)	1.28; (0.49,3.36)	1.51;(0.53,4.33)	1.18;(0.67,2.06)	1.39;(0.52,3.67)	0.6;(0.17, 2.14)	0.71;(0.30,1.30)	0.75; (0.45,1.25)
Age of introduction of foods (below or above the age of 1)	0.88;(0.62,1.26)	0.6; (0.36,0.99)	0.84;(0.45,1.56)	0.71;(0.47,1.08)	0.89;(0.55,1.46)	1.94;(0.83,4.52)	1.39;(0.72,2.68)	1.01; (0.64,1.58)
4 course of Antibiotics (yes or no)	1.15;(0.69, 1.93)	1.21; (0.83, 1.61)	0.63;(0.29,1.34)	0.84;(0.48,1.47)	0.71;(0.27,1.88)	1.64;(0.46, 5.84)	0.56;(0.30,1.19)	**2.25;(1.26,3.99)**

Table 9B: Multivariate regression model

Multivariate analysis (OR, 95%CI)

Food	**Eczema (diagnosed in first 2 years of life)**	**Asthma**	**4 Course of Antibiotics**	**Age of food introduction (before the age of 1)**	**Age at study entry**	**Sex (male)**
Peanut	**1.32; (1.05, 1.83)**	-	-	-	1.01;(0.99,1.03)	1.9;(0.75;1.58)
Almond	-	**2.09;(1.47,2.97)**	-	1.01;(0.96,1.07)	1.05;(0.90,1.20)	***1*.62;(1.04,2.51)**
Pecan/walnut	**2.32;(1.58,3.92)**	-	-	-	**1.04;(1.01,1.07)**	0.92;(0.57,1.48)
Hazelnut	1.14;(0.89,1.47)	**2.11;(1.63,2.74)**	-	-	1.01;(0.99,1.02)	**1.41;(1.01,1.96)**
Cashew/pistachio	**1.81;(1.21, 2.68)**	-	-	-	1.02;(0.99,1.06)	0.96;(0.60,1.53)
Egg	1.47;(1.00,2.14)	-	1.88;(0.98,3.56)	-	1.04;(0.99,1.10)	0.87;(0.50,1.53)
All foods combined (peanut, tree nuts, egg)	**1.02;(0.90,1.08)**	**2.10;(1.51,2.32)**	-	1.07;(0.90,1.11)	1.05;(0.80,1.10)	**1.45;(1.12,1.68)**

5. 0 Discussion

Only a few studies attempted to simultaneously estimate the role of genetic and environmental factors in the development of food allergies, but the results remain inconsistent and controversial. Many of those studies either assessed all food allergies in one group, or assessed food sensitivity as opposed to diagnosed food allergy. [153; 174] This study is the first to assess clinical food allergy concordance for the 5 most common food allergies in a registry of twins, and to evaluate the effect of non-genetic potentially modifiable factors on this concordance. Consistent with recent studies, [4;22;106-107;109] my findings support the hypothesis that, since the rise in prevalence of allergic diseases is occurring more rapidly than can be accounted for by population-based changes in genetic sequence, it is most likely due to non-genetic factors. Further, I identified atopic co-morbidities such as asthma and early eczema, to be associated with higher risk for food allergy concordance.

Concordance rates were significantly higher in DZ compared to MZ twins for cashew/pistachio, egg, and peanut allergy. These observations suggest, in contrast to previous studies, a role for non- genetic factors in the development of those food allergies. For almond, pecan/walnut, hazelnut, shellfish, and milk allergy, the concordance difference was not significant. Although previous evidence from twin studies suggests that genetic variation is important, it is not the only factor at play. One study of 58 twin pairs found that the concordance rate of peanut allergy among MZ twins (64.3%) was significantly higher than that among DZ twins (6.8%), and estimated the heritability of peanut allergy to be as high as 81.6%. [80] However, our results differ likely due to the fact that the previous study, using a different protocol, was conducted more than 10 years ago. It is possible that the effect of non-genetic factors operating over the last decade have been detected in our relatively younger cohort.

In line with our results, a second large twin study performed on a Chinese population assessing sensitization (i.e. presence of food-specific IgE antibodies) to common food allergens, revealed that sensitization to peanut and shellfish to be influenced by both genetic and environmental factors.[80;82]

Aside from food allergies, studies report diabetes, [254,255] irritable bowel syndrome, [256] and multiple sclerosis [257] to have a significant non- genetic component to them. A twins study revealed a greater contribution of environmental factors on the development of Type 2 diabetes. [258] Another study states the importance of non-genetic factors in metabolic diseases such as ischaemic heart disease, and hypertension. [259]

The following non- genetic factors have been reported as having an effect on the development of food allergy; timing of food introduction, feeding pattern, diet/ nutrition, exposure to environmental tobacco smoke, prematurity, low birth weight, microbial exposure and race/ethnicity. [214]

My study specifically looked at asthma, eczema (diagnosed in the first 2 years of life), daycare attendance of the twins (yes, no, duration), exposure to a farm environment or second hand smoke

(yes, no, duration), pets in the household (yes, no, quantity), vitamin intake of pregnant mother (yes, no, duration), breastfeeding practices (exclusively or not), age of food introduction (before, after 12 months of age) and whether a 4 course of antibiotics was ever taken (yes, no, duration).

After controlling for age and sex, asthma and eczema (diagnosed in the first 2 years of life) remained significant. An association was found between the concordance in almond and hazelnut allergy and asthma. Those diagnosed with asthma were 2 times more likely to have tree nut allergy concordance. This is in line with other studies. [244]

Studies report that food allergy could be a marker for a generalized atopic phenotype of asthma, rather than a direct cause for asthma attacks. [245-246] Alternatively, the relationship between food allergy and asthma could be causal, given that asthma symptoms are induced by foods in food-allergic people up to 30% of the time, although usually with other allergic symptoms. It is argued that accidental reactions to traces or occult culprit foods may contribute to food-triggered asthma episodes. [244;245]

Those with early eczema were on average 1.8 times more likely to have a tree nut, egg, or peanut allergy. Food allergy and eczema as well as other atopic diseases often co-exist and there is a higher prevalence of food allergy found in about 35–80% of children with moderate to severe eczema. [80;245246] Our results are in line with the findings of a previous cross- Canada nested case control study conducted by our group, suggesting that eczema in the first 2 years of life is consistently associated with food allergies. [155] Studies report a well -documented link between the presence of early eczema in childhood and the development of food allergy, especially peanut and egg. [95] Similar findings report that among children with food allergy, 50% to 70% of them had eczema in infancy [260;262] compared to 9% to 40% in the general population.[176] Since the prevalence of eczema is increasing, and eczema is highly associated with food allergy, it may serve as an explanation for the increasing prevalence of food allergy. It is hypothesized that exposure to food allergens through an impaired skin barrier increases the risk for food allergy. [77] Establishing a causative link between early eczema and food allergy is crucial as it may contribute to the development of primary prevention strategies. In line with this hypothesis, recent studies suggest that controlling eczema might reduce the risk of food sensitization and, potentially, food allergy. [155]

My findings suggest no significant association between concordance in food allergy and daycare attendance, exposure to pets, farm environment, exposure to second hand smoke and antibiotics prescription.

Despite a recent randomized controlled study demonstrating that early introduction of peanuts decreased the frequency of development of peanut allergy among children having eczema and at high risk for this allergy, my study failed to support this. [106]

Studies report that late introduction interacts with eczema to increase the risk of food allergy. The premise is that early cutaneous exposure to food protein through a disrupted skin barrier as occurs in eczema, leads to allergic sensitization. However, early introduction through the oral route decreases the risk of food allergy by promoting tolerance through mechanisms in the digestive system such as regulatory T-cell pathways. Similarly, an Australian study, reported that introduction of eggs at 12 months of age was associated with a higher risk of egg allergy compared with introduction at 4 to 6 months of age (OR 3.4, 95%CI 1.8,6.5). [241]

Results in the literature are inconsistent regarding the role of breast feeding and food allergy. Similarly, I did not find an association between food allergy and breastfeeding practices (exclusively or not) or vitamin intake of the pregnant mother in contrast to other studies. The inconsistent results regarding the role of breastfeeding could be explained by the presences of unaccounted confounders such as varying levels of regulatory cytokines, prebiotic factors and peptides. [242, 243]

Aside from an obvious small sample size, my study has other potential limitations. A major drawback of most studies is recall bias. Hence, it is possible that the factors identified in the regression analysis are not valid predictors of a confirmed food allergy (based on the corroboration of history with confirmatory tests). Since all the documents are mailed out, recall bias could occur due to the fact that participants are asked to recall past experiences. For example, recall bias could be present due to the inability of parents to recall the exact age at which they introduced the foods into their children's diet. Participants excluded from this study were due to either no follow-up or missing zygosity, or missing/ invalid SPT data, with regards to baseline characteristics. There was also possibility for misclassification of exposure (any environmental factor) as well as the possibility for a cohort effect. Children born more recently, to more educated and concerned parents, with high health literacy or exposed to a Western lifestyle may be characteristics unique to the group in question.

In summary, my study is the first to assess the intricate relationship between genetic and non- genetic factors in the development of the five most common food allergies by comparing MZ and DZ twin pairs. My results reveal a prominent role for atopy and mainly eczema, a modifiable risk factor in the first two years of life in the development of food allergy.

5.1 Future directions

Unfortunately, no studies to date have formally investigated the role of epigenetics in food allergy, although many are currently underway. Many believe that the disruption of appropriate allergic pathways such as Th-cell differentiation (and the corresponding cytokine expression profile) in the early postnatal period represents an important potential pathway to the development of

allergy. Several studies have demonstrated that DNA methylation and the resulting changes in chromatin structure are the primary regulators of naive Th cell differentiation.

The potential to identify distinct epigenetic biomarkers associated with food allergy has not been explored. Future food allergy studies should investigate DNA methylation status as it the most robust and readily measured epigenetic modification. [80]

Although at this point, non genetic factors were only associated with the development of cashew/pistachio, egg, and peanut allergy, data collection is under way and is continuously being performed by Dr. Ben Shoshan's research team.

Furthermore, this study provides a platform for future larger studies to explore primary prevention strategies to reduce the risk of food allergy. My results indicate that eczema is associated with the development of food allergy. Therefore, it may be interesting to conduct future randomized controlled studies to assess maintenance of barrier integrity and restoration of function when lost, through the use topical and/or systemic medication, in combination with measured oral introduction of food allergens, to see if it promotes the development of effective oral tolerance and primary prevention of food allergy.

6.0 Conclusion

In conclusion, I have shown that among a total of 59 twin pairs (28 MZ. 31 DZ), the concordance rate in food allergy was higher in DZ twins for peanut, tree nut and egg allergy, suggesting important non- genetic effects. Eczema (diagnosed in the first 2 years of life) was positively associated with the concordance rate in tree nut and peanut allergy. Asthma was also positively associated with tree nut allergy. Male gender was associated with higher odds of tree nut and egg allergy concordance. My small sample size, recall bias, cohort effect as well as loss to follow up could account for the fact that other potential associations were inconclusive. Given that the rise in the prevalence of allergic diseases has occurred more rapidly than can be accounted for by population- based changes in genetic sequence, my research highlights the importance of investigating modifiable non- genetic factors and their role in the development of food allergies. These findings may contribute to the development of policies and guidelines that will benefit those with food allergies and may serve as a platform for future research exploring the societal burden and primary prevention of food allergies.

7.0 References

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7.1 Appendix 1: Informed Consent

INFORMED CONSENT- CHILD

Title: Susceptibility To Food Allergy in a Registry of Twins (START) Investigators:

Moshe Ben-Shoshan, MD, Montreal Children's Hospital, McGill University Health Centre
Ann Clarke, MD, Professor, Faculty of Medicine University of Calgary, Calgary, Alberta, Canada, Adjunct Professor, Faculty of Medicine, McGill University, Montreal, Quebec, Canada

Research team:

Greg Shand, MSc, Study Coordinator, McGill University Health Centre
Christopher Mill, BSc, Research Assistant, McGill University Health Centre Jennifer Mill, Research Assistant, McGill University Health Centre

In the text below, "You" means "you/your child

Introduction

You are being asked to participate in this research study that will help researchers at the McGill University Health Centre learn more about the causes of food allergy. We are inviting you to participate in our research project because you have twins and indicated that at least one of them has a food allergy. We would like to determine the role of genes (shared substantially among twins) in the development of allergy. If you decide to participate in this study, we will contact you once a year for the next five years to ask about your child's food allergy and accidental reactions.

Before deciding to participate in the study, you should clearly understand its requirements, risks and benefits. This document provides information about the study, and it may contain words you do not fully understand. Please read it carefully and ask a member of the research team any questions you may have. They will discuss the study with you in detail. If you decide to participate, you will be asked to sign this form and retain a copy for your records. If your child is seven years or older, he/she will be asked to sign an assent form.

Purpose

The purpose of this study is to assemble a database of information (Registry) on food

allergies in children and adults in Canada. This database will help the research team learn more about the rate, triggers, diagnosis and management of food allergy.

Study Description and Procedures

If you agree to participate, you will be contacted by a member of our research team by mail once a year to invite you to complete questionnaires about food allergies once every year for five years. These may take up to 30 minutes to complete. These questionnaires will ask questions regarding your child's food allergy.

You will also be asked to provide salivary samples from the twins to assess the twins are identical (in case this is unknown) and to look for changes in a single skin barrier gene (Filaggrin) that is related to eczema (dry, red and itchy skin) and food allergy. In addition, to assess a special type of wheat allergy known as Celiac disease, we will send to interested families, who have twins that are at least 5 years old, a home screening blood test called Biocard Celiac Test. Celiac disease can present with no symptoms but diagnosis is important as it can prevent future complications. Early identification of Celiac disease requires a blood test. If this test is positive an assessment with a gastroenterologist is required to confirm the presence of Celiac disease. Please note that a positive blood test does not necessarily indicate the presence of Celiac disease and the final diagnosis requires assessment by a gastroenterologist.

You will find enclosed two copies of the INFORMED CONSENT requesting your permission to participate in the study. One copy is to be returned to the study investigators and the other copy is for your records.

If you wish to participate, please return the booklet marked "**Copy to Return"** which includes the completed questionnaires and the **signed INFORMED CONSENT (and ASSENT** if applicable**)** in the self-addressed, stamped envelope provided.

A member of our research team may contact you if some of the answers on the questionnaires are unclear. You may also be contacted regarding your child's food allergy and tests performed.

Once we receive your filled questionnaire and the signed informed consent (and an assent form if your child is 7 years or older), we will send you the specimen kit and a prepaid return envelope in the mail.

If you agree to participate, you will be asked to give a saliva sample of about 2ml from each twin. Your children will be asked to spit into a small container, cap it tightly, and return it in the envelope provided. The cells that come from the cheeks and gums will provide us with the DNA. There are no needles involved.

To get a good sample, your child should not eat, drink, smoke or chew gum for 30 minutes before giving the sample. A better collection will be obtained if your child runs his/her tongue around his/her cheeks and gums before spitting. Your child has up to 30 minutes to give the full sample.

If your child is unable to provide a saliva sample by spitting, an oral swab may also be taken. This involves placing a sponge on a stick into the cheek pouch, and allowing it to sit for 60 seconds. The sponge portion is then cut off into the specimen kit. This is then repeated 3-4 times, also over a maximum of 30 minutes. Please let us know if your child is unable to spit and needs the oral swabs.

After providing the specimen and capping the container tightly, put it into the plastic bag found in the envelope. Return the sample in the prepaid envelope provided.

If it is not known if twins are identical you would be asked to repeat the process above in order to analyse

the DNA to determine if the twins are identical.

For the test for Celiac disease, we will send you the Biocard test. This is a quick test in which a drop of whole blood (finger-prick) would provide results within 10 minutes. A positive test result shows two lines, while only one line appears if the test is negative. Your child should continue with his regular diet during that test. In case the test is positive a member of our research team will contact you and will provide you with a doctor's letter referring your child for an assessment by a gastroenterologist.

Then what will happen?

DNA will be isolated from the salivary specimen at the McGill University and Genome Quebec Innovation Centre, followed by examination of the DNA for genes at the same institution. Identity will be determined in a special laboratory in Alberta , by Dr Fiona Bamforth of the university of Alberta. Laboratory results will be sent back to Dr. Moshe Ben-Shoshan at the McGill University Health Centre in Montreal, Quebec, where the data will be compiled. A genetic statistician will analyze the data.

A member of our research team would contact you within a month after sending the Biocard celiac test. If the test is positive we will provide with a referral letter for a gastroenterologist and communicate these findings to a physician of your choice.

We may contact you to invite you to participate in other research studies on allergies that our research group may conduct. Although the enclosed questionnaires focus primarily on your child's food allergy, in the future, we may conduct research on other aspects of allergy. We understand that your participation in the study does not necessarily mean that you will be willing to participate in other research studies. Please check YES if you agree to be contacted, or NO if you do not agree.

□ YES □ NO

Initials _____

Risk and Inconveniences

There are no risks associated with participating in this study. There will be no inconveniences beyond the time required to complete the questionnaires.

Benefits

Your participation in this research may provide no immediate benefit to your child. However, the information collected from this study may help us better understand the causes of food allergies. It may lead to the development of more practical programs to help prevent the development of food allergies. In addition, if your child is suspected to have Celiac disease, early diagnosis will benefit him/her by preventing future complications such as anemia, poor growth, delayed puberty, and dental problems.

Voluntary Participation and/or Withdrawal

Your participation in this study is entirely voluntary. You may refuse to participate or you may discontinue your participation at any time without explanation. If you decide not to

participate or you discontinue your participation, your child will suffer no prejudice regarding his/her medical care or his/her participation in any other research studies. You will be informed of any new findings that may affect your willingness to continue your participation.

Protection of Confidentiality

The team of researchers may consult your child's medical files to take notes of data relevant to this research project, i.e., medical history, physical examinations and laboratory results**.**

All information obtained during this study will be kept strictly confidential. Your child's name will never appear in any publication of the results of this study. All of the information used for research will be collected under a code number and your child's name will never appear on the data collection sheets. The code list linking your child's name to the code number will be kept entirely confidential and locked in a filing cabinet with limited access. The research data will be available only to the research team and to persons taking part in managing and analyzing the research information.

By signing this consent form, you give us permission to obtain a copy of your child's medical records related to the diagnosis of food allergy. This information is necessary to determine the type of reaction your child had and the tests that were performed to determine that your child had an allergic reaction. Your confidentiality will be protected to the extent permitted by applicable laws and regulations.

If, after reviewing your child's medical records we find that your child does not meet our criteria to be included in this study, we will not send you further questionnaires. In this case, a letter will be sent to you if you do not qualify to be included in the study.

The information may be shared anonymously with other investigators.

Compensation

There is no monetary compensation provided for participation in this study.

Incidental Findings

Should any incidental findings develop during the course of this study that may affect your willingness to continue participating in this study, you will be informed. We will communicate these findings to a physician of your choice.

Study Records Retention Policy

For security purposes and to be able to communicate with you, your family name, first name, coordinates, the start and end date of participation in the project will be stored for fifteen

(15) years after the completion of the study in a separate registry maintained by the researcher in charge of the project or by the institution.

You have the right to consult your study file in order to verify the information gathered and to rectify it if necessary, as long as the project researcher or the institution holds this information. However, in order to protect the scientific integrity of the research project, you will have access to certain information only once this project has come to an end.

Funding of the Research Project

The research study is conducted by Dr. Moshe Ben-Shoshan and Dr. Ann Clarke. The study doctors are not being paid for including you and looking after you during participation in this study.

Control of the Ethical Aspects of the Research Project

The Research Ethics Board of the MUHC has reviewed this research project and is responsible for the oversight of the study. In addition, it will review any amendment made to the information/consent form and to the study protocol.

Quality Assurance Program

The MUHC implemented a Quality Assurance program that includes active continuing review of projects (on site visits) conducted within our establishment. Therefore, it must be noted that all human subject research conducted at the MUHC or elsewhere by its staff, is subject to MUHC Routine and Directed Quality Improvement Visits.

Contact Persons for Questions

If you have any questions about the study, you may contact Greg Shand at 514-412-4400 extension 23867 (Montreal region), by email at startmch@gmail.com or Dr. Moshe Ben-Shoshan at the Montreal Children's Hospital at 514-412-4470.

If you have any questions about your rights as a study participant, you may contact the patient representative (ombudsman) at the Montreal General Hospital, at 514-934-1934, extension 48306 or the patient representative (ombudsman) at the Montreal Children's Hospital at 514-412-4400.

TWIN 1

Title: Susceptibility To Food Allergy in a Registry of Twins (START) DECLARATION OF CONSENT

I have read the contents of this consent form, and I voluntarily agree to be a subject in this research study and to the procedures described in this form. I understand this anonymous DNA sample will be stored for research. I have had the opportunity to ask questions and any questions have been answered to my satisfaction. I have been given sufficient time to consider the above information and to seek advice if I choose to do so. I agree to complete the questionnaires and I understand that I might receive a telephone call regarding some of my answers on the questionnaires and/or regarding my child's food allergies and any testing performed.

I also agree to allow the researchers to obtain a copy of my child's medical records related to food allergy from my doctor.

I grant permission for my physician, Dr._____, telephone number_____and address_____to release the results of any tests related to food allergy.

By my signature below, I give the research group permission to use the sample of my child's saliva for genetic testing in this study and to contact my physician to obtain a copy of my child's medical records related to food allergy.

Please sign both copies of the informed consent form **in ink** and return the booklet marked "Copy to Return" which includes the questionnaires. We request that you include your email address as, should there be any questions, our research nurse can reach you more easily.

My home telephone number is: _____

My e-mail address is: _____

By signing this consent form, I have not given up any of my legal rights.

_____ _____

Participant's name Parent/legal guardian signature

7.2 Appendix 2: Questionnaire

Project: START Susceptibility To Food Allergy in a Registry of Twins

Study ID #: ☐ ☐☐☐☐

Date: ☐☐/☐☐/☐☐☐☐

d d m m y y y y

TO BE COMPLETED BY PARENT/GUARDIAN

---

1. Age of child's mother/female guardian: _____ Age of child's father/male guardian: _____
2.
3. Education of child's mother/female guardian:

☐ High School not completed
☐ High School completed
☐ CEGEP/College
University

4. Education of child's father/male guardian:

☐ High School not completed
☐ High School completed
☐ CEGEP/College
University

5. With whom does your child live?

☐ Both parents ☐ Mother only ☐ Father only ☐ Guardian
☐ Shared custody ☐ Grandparent (s) ☐ Other: _____

6. How long has your child lived with the person(s) identified in Question 5?

_____

7. What is your child's cultural background? **Categories taken from Census Canada 2001*

☐ White ☐ Chinese ☐ Japanese ☐ Korean
☐ Black ☐ Filipino ☐ Arab ☐ Latin American
☐ South Asian (e.g. East Indian, Pakistani, Sri Lankan, etc.)
☐ Southeast Asian (e.g. Cambodian, Indonesian, Laotian, Vietnamese, etc)
☐ West Asian (Afghan, Iranian, etc)
☐ Aboriginal (North American Indian, Métis, Inuit) ☐ Other: _____

8. What is the child's mother's/female guardian's current work status?

☐ Employed full-time ☐ Employed part-time ☐ Student ☐ Retired
☐ Disabled ☐ Unemployed (please specify for how long):_____

☐ Other (please specify): _____

9. What is the child's father's/male guardian's current work status?

☐ Employed full-time ☐ Employed part-time ☐ Student ☐ Retired
☐ Disabled ☐ Unemployed (please specify for how long):_____

☐ Other (please specify): _____

Susceptibility To Food Allergy in a Registry of Twins

Initial START Questionnaire - Parent

1) Please indicate the name and gender of each twin: (Note - the names will be detached from the questionnaire.)

	First Twin (Twin 1)	**Second Twin (Twin 2)**
Name :		
Gender:	Twin 1:	Twin 2:

✂✂✂

2) What is the date of birth of the twins?_____dd/mm/yy

3) Have either of your twins **ever** been diagnosed by a physician as having any of the following conditions?

(Please check all that apply in the corresponding boxes)

	First Twin (Twin 1)	**Second Twin (Twin 2)**
Asthma		
Eczema		
Hay fever or allergic rhinitis (stuffy/runny nose or frequent sneezing)		
Hives **from an unknown cause** (itchy skin rash that comes and goes over minutes to hours) that lasted at least 6 weeks.		
Anaphylactic reaction (a severe, sudden, and life-threatening **allergic** reaction to a foreign substance that causes problems in multiple systems of the body. e.g.: wheezing or other breathing difficulties, total body hives, vomiting, loss of consciousness)		
Food allergy (list all the foods)		
List all of the above that have Resolved		
I don't have any of the above conditions		

Family History:

4) Please check if the following were **ever** diagnosed by a physician:

Asthma

Mother of twins ☐ Yes ☐ No ☐ Resolved ☐ Don't know
Father of twins ☐ Yes ☐ No ☐ Resolved ☐ Don't know
Sibling (s) (other than the twins) ☐ Yes ☐ No ☐ Resolved ☐ Don't know

Eczema

Mother of twins ☐ Yes ☐ No ☐ Resolved ☐ Don't know
Father of twins ☐ Yes ☐ No ☐ Resolved ☐ Don't know
Sibling (s) (other than the twins) ☐ Yes ☐ No ☐ Resolved ☐ Don't know

Hay fever or allergic rhinitis (stuffy/runny nose or frequent sneezing)

Mother of twins ☐ Yes ☐ No ☐ Resolved ☐ Don't know
Father of twins ☐ Yes ☐ No ☐ Resolved ☐ Don't know

Sibling (s) (other than the twins) ☐ Yes ☐ No ☐ Resolved ☐ Don't know`

Hives **from an unknown cause** (itchy skin rash that comes and goes over minutes to hours) and lasts at least 6 weeks

Mother of twins ☐ Yes ☐ No ☐ Resolved ☐ Don't know
Father of twins ☐ Yes ☐ No ☐ Resolved ☐ Don't know
Sibling (s) (other than the twins) ☐ Yes ☐ No ☐ Resolved ☐ Don't know

Anaphylaxis (a severe, sudden, and life-threatening **allergic** reaction to a foreign substance that causes problems in multiple systems of the body. e.g.: wheezing or other breathing difficulties, total body hives, vomiting, loss of consciousness)

Mother of twins ☐ Yes ☐ No ☐ Resolved ☐ Don't know
Father of twins ☐ Yes ☐ No ☐ Resolved ☐ Don't know
Sibling (s) (other than the twins) ☐ Yes ☐ No ☐ Resolved ☐ Don't know

Food allergy

Mother of twins ☐ Yes ☐ No ☐Resolved ☐ Don't know
If yes, list food (s):_____
If resolved, list food (s): _____

Father of twins ☐ Yes ☐ No ☐Resolved ☐ Don't know
If yes, list food (s):_____
If resolved, list food (s): _____

Sibling (s) (other than the twins) ☐ Yes ☐ No ☐Resolved ☐ Don't know
If yes, list food (s):_____
If resolved, list food (s): _____

5) Please indicate, in the table below, which triggers were associated with any allergic reactions in either of the twins as per the treating physician's diagnosis.

Twin 1					**Twin 2**				
Name of Trigger	Age at time of Reaction	Trigger diagnosed by general physician	Trigger diagnosed by allergist	Trigger diagnosed by other (please specify who)	Name of trigger	Age at time of reaction	Trigger diagnosed by general physician	Trigger diagnosed by allergist	Trigger diagnosed by other (please specify who)

Now we would like to learn more about food induced allergic reactions that occurred:

6) FIRST FOOD INDUCED REACTION IN EACH TWIN'S LIFE

Date of **first food induced allergic reaction** in each twin's life:

	Twin 1	**Twin 2**
Date (dd/mm/yyyy) (Approximate month/year)		

A. What food (s) triggered that reaction?

	Twin 1	**Twin 2**
Please specify which food (s):		
I don't know which food triggered the reaction		

B. Where did the reaction occur?

	Twin 1	**Twin 2**
Home		
Daycare		
School		
Restaurant		
Work		
Other (please elaborate)		
I don't know		

C. How did the reaction occur?

	Twin 1	**Twin 2**
After ingestion		
After inhalation		
After skin contact		
It was not determined		
Other (please elaborate)		
I don't know		

D. Please check the symptoms that occurred:

	Twin 1	**Twin 2**
Hives		
Redness		
Itchiness without hives		
Difficulty breathing		
Wheezing		
Runny nose and watery eyes		
Itchy throat		
Throat tightness		
Change in voice		
Vomiting		
Abdominal pain		
Swelling of the lips or face		
Passing out		
Bluish color of lips and/or fingertips		
Incontinence		
Other (please specify)		

E. How long after eating, inhaling, or coming into contact with this trigger (as specified in question A) did the reaction occur?

	Twin 1	**Twin 2**
Up to 10 minutes		
10 - 60 minutes		
1 - 2 hours		
2 - 8 hours		
If greater than 8 hours, when did the reactions occur?		
I don’t know		
Other (please specify)		

F. How long did the symptoms last?

	Twin 1	**Twin 2**
Less than 1 hour		
Between 1 to 4 hours		
Between 4 to 8 hours		
Between 8 to 24 hours		
More than 24 hours		
I don't know		

Ga. What treatments were used to treat the reaction in **twin 1**? **(Please specify for both categories) Outside Health Care Facility** (home, restaurant, school, etc…) **Health Care Facility**

☐ None
☐ Epipen®, Twinject™ (Adrenaline/Epinephrine)
☐ Antihistamines (Benadryl®, Atarax®)
☐ Ventolin® (Salbutamol)
☐ Steroids (Prednisone)
☐ Treated with medication name unknown
☐ Other_____
☐ I don't know

☐ None
☐ Epipen®, Twinject™ (Adrenaline/Epinephrine)
☐ Antihistamines (Benadryl®, Atarax®)
☐ Ventolin® (Salbutamol)
☐ Steroids (Prednisone)
☐ Treated with medication name unknown
☐ Other
☐ I don't know

If Epipen®, Twinject™ (Adrenaline/Epinephrine) was used, please indicate the name of the auto-injector: _____
☐

If more than 1 dose of epinephrine was given, please indicate the number of doses and reason for repeating the dose:
Number of doses: _____
More than 1 dose was given because:
_____
_____
☐

If Epipen®, Twinject™ (Adrenaline/Epinephrine) was used, please indicate the name of the auto-injector: _____
☐

If more than 1 dose of epinephrine was given, please indicate the number of doses and reason for repeating the dose:
Number of doses: _____
More than 1 dose was given because:
_____
_____

Gb. What treatments were used to treat the reaction in **twin 2**? **(Please specify for both categories) Outside Health Care Facility** (home, restaurant, school, etc…) **Health Care Facility**

☐ None
☐ Epipen®, Twinject™ (Adrenaline/Epinephrine)
☐ Antihistamines (Benadryl®, Atarax®)
☐ Ventolin® (Salbutamol)
☐ Steroids (Prednisone)
☐ Treated with medication name unknown
☐

☐ None
☐ Epipen®, Twinject™ (Adrenaline/Epinephrine)
☐ Antihistamines (Benadryl®, Atarax®)
☐ Ventolin® (Salbutamol)
☐ Steroids (Prednisone)
☐ Treated with medication name unknown
☐

If Epipen®, Twinject™ (Adrenaline/Epinephrine) was used, please indicate the name of the auto-injector:

I don't know

If more than 1 dose of epinephrine was given, please indicate the number of doses and reason for repeating the dose:

Number of doses: _____

More than 1 dose was given because:

I don't know

If Epipen®, Twinject™ (Adrenaline/Epinephrine) was used, please indicate the name of the auto-injector

I don't know

If more than 1 dose of epinephrine was given, please indicate the number of doses and reason for repeating the dose:

Number of doses: _____

More than 1 dose was given because:

I don't know

H. Were the twins prescribed an epinephrine auto-injector for this reaction?

	Twin 1	**Twin 2**
Yes, in the Emergency Room (ER)		
Yes, in another health care facility (please specify)		
No		
No, because the child already had an epinephrine auto-injector		
I don't know		

I. If yes, please indicate the name of the autoinjector prescribed:

	Twin 1	**Twin 2**
Epipen®		
Twinject™		
Other (please indicate which)		
No, because the child already had an epinephrine auto-injector		
I don't remember		

7) MOST SEVERE FOOD INDUCED REACTION IN EACH TWIN'S LIFE

Was the first food induced allergic reaction also the most severe one?

☐ Yes, please go to **question number 8.**

☐ No, please describe the most severe reaction below

Date of **most severe food induced allergic reaction** in each twin's life :

	Twin 1	**Twin 2**
Date (dd/mm/yyyy) (Approximate month/year)		

A. What food (s) triggered that reaction?

	Twin 1	**Twin 2**
Please specify which food (s):		
I don't know what food triggered the reaction		

B. Where did the reaction occur?

	Twin 1	**Twin 2**
Home		
Daycare		
School		
Restaurant		
Work		
Other (please elaborate)		
I don't know		

C. How did the reaction occur?

	Twin 1	**Twin 2**
After ingestion		
After inhalation		
After skin contact		
It was not determined		
Other (please elaborate)		

D. Please check the symptoms that occurred:

	Twin 1	**Twin 2**
Hives		
Redness		
Itchiness without hives		
Difficulty breathing		
Wheezing		
Runny nose and watery eyes		
Itchy throat		
Throat tightness		
Change in voice		
Vomiting		
Abdominal pain		
Swelling of the lips or face		
Passing out		
Bluish color of lips and/or fingertips		
Incontinence		
Other (please specify)		

E. How long after eating, inhaling, or coming into contact with this trigger (as specified in question A) did the reaction occur?

	Twin 1	**Twin 2**
Up to 10 minutes		
10 - 60 minutes		
1 - 2 hours		
2 - 8 hours		
If greater than 8 hours, when did the reactions occur		
I don't know		
Other (please specify)		

F. How long did the symptoms last?

	Twin 1	**Twin 2**
Less than 1 hour		
Between 1 to 4 hours		
Between 4 to 8 hours		
Between 8 to 24 hours		
More than 24 hours		
I don't know		

Ga. What treatments were used to treat the reaction in **twin 1** ? **(Please specify for both categories) Outside Health Care Facility** (home, restaurant, school, etc…) **Outside Health Care Facility**

- ☐ None
- ☐ Epipen®, Twinject™ (Adrenaline/Epinephrine)
- ☐ Antihistamines (Benadryl®, Atarax®)
- ☐ Ventolin® (Salbutamol)
- ☐ Steroids (Prednisone)
- ☐ Treated with medication name unknown
- ☐ Other_____
- ☐ I don't know

None
Epipen®, Twinject™ (Adrenaline/Epinephrine)
Antihistamines (Benadryl®, Atarax®)
Ventolin® (Salbutamol)
Steroids (Prednisone)
Treated with medication name unknown
Other
I don't know

If Epipen®, Twinject™ (Adrenaline/Epinephrine) was used, please indicate the name of the auto-injector:

If Epipen®, Twinject™ (Adrenaline/Epinephrine) was used, please indicate the name of the auto-injector:

If more than 1 dose of epinephrine was given, please indicate the number of doses and reason for repeating the dose:
Number of doses: _____
More than 1 dose was given because:

I don't know

If more than 1 dose of epinephrine was given, please indicate the number of doses and reason for repeating the dose:
Number of doses: _____
More than 1 dose was given because:

I don't know

Gb. What treatments were used to treat the reaction in **twin 2** ? **(Please specify for both categories) Outside Health Care Facility** (home, restaurant, school, etc…) **Outside Health Care Facility**

- ☐ None
- ☐ Epipen®, Twinject™ (Adrenaline/Epinephrine)
- ☐ Antihistamines (Benadryl®, Atarax®)
- ☐ Ventolin® (Salbutamol)
- ☐ Steroids (Prednisone)
- ☐ Treated with medication name unknown
- ☐ Other_____
- ☐

None
Epipen®, Twinject™ (Adrenaline/Epinephrine)
Antihistamines (Benadryl®, Atarax®)
Ventolin® (Salbutamol)
Steroids (Prednisone)
Treated with medication name unknown
Other

If Epipen®, Twinject™ (Adrenaline/Epinephrine) was used, please indicate the name of the auto-injector:

I don't know

If more than 1 dose of epinephrine was given, please indicate the number of doses and reason for repeating the dose:

Number of doses: _____

More than 1 dose was given because:

If Epipen®, Twinject™ (Adrenaline/Epinephrine) was used, please indicate the name of the auto-injector:

I don't know

If more than 1 dose of epinephrine was given, please indicate the number of doses and reason for repeating the dose:

Number of doses: _____

More than 1 dose was given because

H. Were the twins prescribed an epinephrine auto-injector for this reaction?

	Twin 1	**Twin 2**
Yes, in the Emergency Room (ER)		
Yes, in another health care facility (please specify)		
No		
No, because the child already had an epinephrine auto-injector		
I don't know		

I. If yes, please indicate the name of the autoinjector prescribed:

	Twin 1	**Twin 2**
Epipen®		
Twinject™		
Other (please indicate which)		
No, because the child already had an epinephrine auto-injector		
I don't remember		

8) Have the twins had food induced allergic reactions in the past year?

☐ No (The questionnaire ends here)
☐ Yes

9) If yes, how many food induced reactions did they have and what were the triggers?

	Twin 1	**Twin 2**
Total number of reactions in past year		
Food trigger for first reaction in last year		
Food trigger for second reaction last year		
Food trigger for third reaction last year		

Thank you for completing this questionnaire!

Susceptibility To Food Allergy in a Registry of Twins

START Environment Questionnaire – (twins under 5 years of age)

This questionnaire asks about your twins and their siblings. More specifically, we are interested in your twins and their siblings that are at least 5 years older than the twins.

1. How many siblings (brothers and sisters) do the twins have in total (including those that are less than 5 years apart in age from the twins)? _____

2. What is the birth order of the twins in the family? (Please rank the twins together) Birth order #: _____

3. Did you use any Assisted Reproductive Technology procedures when conceiving the twins (ie: in vitro fertilization (IVF), embryo transfer (ET), gamete intra-fallopian transfer (GIFT), artificial insemination (AI))

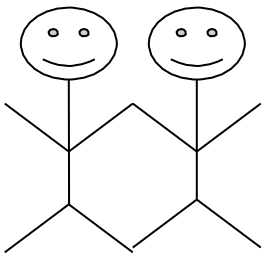
□ Yes · No · I don't know

*The following questions refer to the twins and all siblings that are **at least 5 years older** than the twins if applicable. The diagram below may be used as a guide for question 4.*

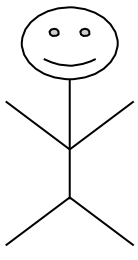
Please complete the table in question 3 and identify your twins and their siblings accordingly. If the twins have more than three older siblings, list those that are closest in age to the twins and at least five years older than the twins. For example, if the twins are five years old and they have three older siblings that are 11 years old, 13 years old and 19 years old, you would describe the 11 year-old and the 13 year-old in the table and questions below.

4. Please label the twins and siblings according to the diagram below.

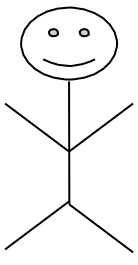
	Initials	DOB (dd-mmm-yyyy)	Sex (Male or Female)	Does this child have a diagnosed food allergy?
Twin 1				
Twin 2				
Sibling 1: At least 5 years older than the twins				
Sibling 2: At least 5 years older than the twins				



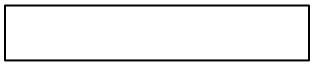
Twin 1 and Twin 2

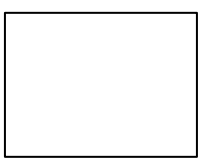


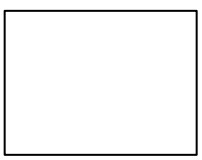
Sibling 1:*At least 5 years older than twins*



Sibling 2: *at least 5 years older than*







5. Before the children were one-year-olds, did you have a pet in your house? (For example: cat, dog, bird, rabbit)

	Yes	No	I don't know	Other
Twin 1				
Twin 2				
Sibling 1: At least 5 years older than the twins				
Sibling 2: At least 5 years older than the twins				

6. Before the children were one-year-olds, did any of them live on a farm where there were farm animals? (For example: chickens, ducks, cows or pigs?

	Yes	No	I don't know	Other
Twin 1				
Twin 2				
Sibling 1: At least 5 years older than the twins				
Sibling 2: At least 5 years older than the twins				

7. Before the children were one-year-olds, did anyone smoke regularly where they lived?

	Yes, they smoked indoors and outdoors	Yes, they smoked outdoors only	No	I don't know	Other
Twin 1					
Twin 2					
Sibling 1: At least 5 years older than the twins					
Sibling 2: At least 5 years older than the twins					

8. Please complete the following table regarding whether or not your children ever attended daycare?

	Yes, age at which first attended day care	Did not attend daycare	I don't know	Other
Twin 1				
Twin 2				
Sibling 1: At least 5 years older than the twins				
Sibling 2: At least 5 years older than the twins				

9. Now we going to ask about your children's CURRENT food intake:

A. On average, how often do your children eat fish?

	Never/ Rarely: less than once	Moderate: up to 3 times per week	Frequently: more than 3 times per week	I don't know	Other
Twin 1					
Twin 2					
Sibling 1: At least 5 years older than the twins					
Sibling 2: At least 5 years older than the twins					

B. On average, how often do your children eat shellfish? (e.g. Crab, lobster, shrimp, scallops, oyster, clams, mussels, squid, octopus, abalone)

	Never/ Rarely: less than once	Moderate: up to 3 times per week	Frequently: more than 3 times per week	I don't know	Other
Twin 1					
Twin 2					
Sibling 1: At least 5 years older than the twins					
Sibling 2: At least 5 years older than the twins					

10. The following questions refer to the mother's pregnancies with these children:

A. Did the mother avoid eating any or all of the following foods during her pregnancies?

	Milk (or dairy products)				Eggs				Wheat containing Foods				Soy			
	Yes	No	I don't	Other	Yes	No	I don't	Other	Yes	No	I don't	Other	Yes	No	I don't	Other
Twin 1																
Twin 2																
Sibling 1: At least 5 years older than the twins																
Sibling 2: At least 5 years older than the twins																

10 B. Did the mother avoid eating any or all of the following foods during her pregnancies?

	Peanut				Tree nut				Fish				Shellfish			
	Yes	No	I don't	Other	Yes	No	I don't	Other	Yes	No	I don't	Other	Yes	No	I don't	Other
Twin 1																
Twin 2																
Sibling 1: At least 5 years older than the twins																
Sibling 2: At least 5 years older than the twins																

11. Did the mother eat any raw/unpasteurized dairy products while pregnant at least once a week?
Raw/unpasteurized milk: milk that is fresh from the farm and that has not undergone pasteurization.
Raw/unpasteurized dairy products: products made from milk that has not undergone pasteurization, such as cheese.

	Yes	No	I don't know	Other
Twin 1				
Twin 2				
Sibling 1: At least 5 years older than the Twins				
Sibling 2: At least 5 years older than the Twins				

12. During the mother's pregnancies, did she take a multivitamin or Vitamin D for at least 3 months?

	Yes	No	I don't know	Other
Twin 1				
Twin 2				
Sibling 1: At least 5 years older than the Twins				
Sibling 2: At least 5 years older than the Twins				

13. Did the mother breastfeed any of these children and if yes, for how long (months)?

	Yes, for how long?	No	I don't know	Other
Twin 1				
Twin 2				
Sibling 1: At least 5 years older than the twins				
Sibling 2: At least 5 years older than the twins				

14. Was a cow's milk based infant formula used and if yes, at what age did this child start with infant formula?

	Yes	For how long?	No	I don't know	Other
Twin 1					
Twin 2					
Sibling 1: At least 5 years older than the twins					
Sibling 2: At least 5 years older than the twins					

Now we would like for you to think about any illnesses these children may have had before their first birthday.

15. Before these children were one-year-olds, did they take more than 4 courses of antibiotics?

	Yes	No	I don't know	Other
Twin 1				
Twin 2				
Sibling 1: At least 5 years older than the Twins				
Sibling 2: At least 5 years older than the Twins				

16. Before these children were one-year-olds, did they have MORE THAN 10 respiratory illnesses? (e.g. common cold)

	Yes	No	I don't know	Other
Twin 1				
Twin 2				
Sibling 1: At least 5 years older than the Twins				
Sibling 2: At least 5 years older than the Twins				

17. Before these children were one-year-olds, did they have diarrhea more than 3 times?

	Yes	No	I don't know	Other
Twin 1				
Twin 2				
Sibling 1: At least 5 years older than the Twins				
Sibling 2: At least 5 years older than the Twins				

18. Before these children were one-year-olds, did they have an infection that required treatment in a hospital?

	Yes	No	I don't know	Other
Twin 1				
Twin 2				
Sibling 1: At least 5 years older than the Twins				
Sibling 2: At least 5 years older than the Twins				

Now we would like for you to think about when you first started giving these children their first foods....

19. How old were these children when you gave them their FIRST solid food?

	Age in months	Has not had any solid food	I don't know	Other
Twin 1				
Twin 2				
Sibling 1: At least 5 years older than the Twins				
Sibling 2: At least 5 years older than the Twins				

20. How old (in months) were your children when you first gave them:
(If you are unsure about the age at which your children were given the following foods, you can give your best guess):

A. How old (in months) were your children when you first gave them peanut and tree nut?

	Peanut				Tree nut (e.g. almonds, Brazil nuts, cashews, chestnuts, hazelnuts, macadamia nuts, pecans, pine nuts,			
	Age	Never	I don't	Other	Age	Never	I don't	Other
Twin 1								
Twin 2								
Sibling 1: At least 5 years older than the twins								
Sibling 2: At least 5 years older than the twins								

20B. How old (in months) were your children when you first gave them fish and shellfish?

	Fish				Shellfish (e.g. Crab, lobster, shrimp, scallops, oyster, clams, mussels, squid,			
	Age	Never introduce	I don't	Other	Age	Never introduced	I don't	Other
Twin 1								
Twin 2								
Sibling 1: At least 5 years older than the twins								
Sibling 2: At least 5 years older than the twins								

20C. How old (in months) were your children when you first gave them cow's milk or cow's milk products and cooked egg?

	Cow's milk or cow's milk products				Cooked egg			
	Age	Never	I don't	Other	Age	Never introduced	I don't	Other
Twin 1								
Twin 2								
Sibling 1: At least 5 years older than the twins								
Sibling 2: At least 5 years older than the twins								

20D. How old (in months) were your children when you first gave them wheat containing foods and soy or soy containing foods?

	Wheat containing foods				Soy or soy containing foods			
	Age	Never	I don't	Other	Age	Never	I don't	Other
Twin 1								
Twin 2								
Sibling 1: At least 5 years older than the twins								
Sibling 2: At least 5 years older than the twins								

21. Now think about what these children eat now...

A. On average, how often do your children currently eat peanut or peanut containing products and tree nut or tree nut containing products?

	Peanut or peanut containing products				Tree-nut or tree-nut containing products			
	Never/ Rarely: less than once	Moderate: up to 3 times per week	Frequently: more than 3 times per week	I don't know	Never/Rarely: less than once	Moderate: up to 3 times per week	Frequently: more than 3 times per week	I don't know
Twin 1								
Twin 2								
Sibling 1: At least 5 years older than the twins								
Sibling 2: At least 5 years older than the twins								

21B. On average, how often do your children currently eat fish and shellfish?

	Fish				Shellfish (e.g. Crab, lobster, shrimp, scallops, oyster, clams, mussels, squid, octopus, abalone)			
	Never/ Rarely: less than once	Moderate: up to 3 times per week	Frequently: more than 3 times per week	I don't know	Never/ Rarely: less than once	Moderate: up to 3 times per week	Frequently: more than 3 times per week	I don't know
Twin 1								
Twin 2								
Sibling 1: At least 5 years older than the twins								
Sibling 2: At least 5 years older than the twins								

21C. On average, how often do your children currently drink cow's milk or eat cow's milk products and egg or egg containing foods?

	Cow's milk or cow's milk products				Egg or egg containing foods			
	Never/ Rarely: less than once	Moderate: up to 3 times per week	Frequently: more than 3 times per week	I don't know	Never/ Rarely: less than once	Moderate: up to 3 times per week	Frequently: more than 3 times per week	I don't know
Twin 1								
Twin 2								
Sibling 1: At least 5 years older than the twins								
Sibling 2: At least 5 years older than the twins								

21 D. On average, how often do your children currently eat wheat containing foods and soy or soy containing foods?

	Wheat containing foods (Bread, cereal, pastries etc...)				Soy or soy containing foods			
	Never/ Rarely: less than once	Moderate: up to 3 times per week	Frequently: more than 3 times per week	I don't know	Never/ Rarely: less than once	Moderate: up to 3 times per week	Frequently: more than 3 times per week	I don't know
Twin 1								
Twin 2								
Sibling 1: At least 5 years older than the twins								
Sibling 2: At least 5 years older than the twins								

22A. Has your child ever been diagnosed with sensitivity to bread, wheat, and flour?

	Has your child ever been diagnosed with sensitivity to bread, wheat, and flour?			If **yes**, were they diagnosed with celiac disease?			If they were **diagnosed** with celiac, how were they diagnosed:		
	Yes	No	I don't know	Yes	No	I don't know	Blood Test	Endoscopy (a procedure used to visually examine the upper digestive system with a tiny camera on the end of a long, flexible tube.)	
Twin 1									
Twin 2									
Sibling 1: 5 years older than the twins									
Sibling 2: 5 years older than the twins									

22B. If they were **diagnosed** with celiac, what were their symptoms?

	Anemia	Poor weight gain	Diarrhea	No symptoms	Other (Please describe)
Twin 1					
Twin 2					
Sibling 1: 5 years older than the twins					
Sibling 2: 5 years older than the twins					

23. Please indicate if there is a history of asthma in your family.

	Asthma: A chronic condition with symptoms such as difficulty breathing, wheezing, and coughing. It may be associated with exercise. It may require medication such as ventolin® in a puffer.					
	Yes	If yes, was it diagnosed by a doctor?	Has it resolved?	At what age did it resolve?	No	I don't know
Twin 1						
Twin 2						
Sibling 1: At least 5 years older than the twins						
Sibling 2: At least 5 years older than the twins						
Mother						
Father						

24. Please indicate if there is a history of eczema in your family.

	Eczema: Chronic skin rashes, usually itchy and red, and can be swollen. It is a hereditary disorder of the skin.					
	Yes	If yes, was it diagnosed by a doctor?	Has it resolved?	At what age did it resolve?	No	I don't know
Twin 1						
Twin 2						
Sibling 1: At least 5 years older than the twins						
Sibling 2: At least 5 years older than the twins						
Mother						
Father						

25. Please indicate if there is a history of hay fever in your family.

	Hay fever or nasal allergies: Seasonal symptoms such as runny nose, itchy nose and eyes, and coughing. Symptoms appear in spring/summer months, associated with environmental allergies.					
	Yes	If yes, was it diagnosed by a doctor?	Has it resolved?	At what age did it resolve?	No	I don't know
Twin 1						
Twin 2						
Sibling 1: At least 5 years older than the twins						
Sibling 2 At least 5 years older than the twins						
Mother						
Father						

26 a. Did any of your children ever have a skin condition associated with scratching?

TWIN 1: · Yes · No · I don't know **TWIN 2**: · Yes · No · I don't know

SIBLING 1: · Yes · No · I don't know **SIBLING 2**: · Yes · No · I don't know

b. Did the skin condition involve folds of elbow behind knees and around the neck?

TWIN 1: · Yes · No · I don't **TWIN 2**: □ Yes · No · I don't

SIBLING 1: · Yes · No · I don't **SIBLING 2**: □ Yes · No · I don't

c. Did they have a history of dry skin?

TWIN 1: · Yes · No · I don't **TWIN 2**: □ Yes · No · I don't

SIBLING 1: · Yes · No · I don't **SIBLING 2**: □ Yes · No · I don't

d. Did they have atopic dermatitis(or eczema) involving cheeks and forehead in first 4 years of life?

TWIN 1: · Yes · No · I don't **TWIN 2**: □ Yes · No · I don't

SIBLING 1: · Yes · No · I don't **SIBLING 2**: □ Yes · No · I don't

e. Did the condition start before the age of 2 years?

TWIN 1: · Yes · No · I don't know **TWIN 2**: □ Yes · No · I don't know

SIBLING 1: · Yes · No · I don't know **SIBLING 2**: □ Yes · No · I don't know

If your child currently has eczema, please send us pictures of palms and any skin lesions at the following email address startmch@gmail.com excluding face or any identifying feature

Thank you for completing this questionnaire!