Final Technical Report: TO7060

Investigation of the association of skin barrier structure and function and the development of sensitisation to food allergens: A prospective birth cohort study

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Executive Summary

Introduction

Food allergy is a growing public health issue, particularly in infants and children. Because most infants experience a food allergic reaction at first known oral ingestion of food, it is immunologically plausible that prior allergen sensitisation must have occurred by an alternative route. It has been proposed that a defective skin barrier has potential for allergen entry and priming, allowing an infant to develop sensitisation to a food allergen prior to oral ingestion. This theory has been confirmed in murine studies as systemic allergic reactions to peanut are induced by epicutaneous sensitisation across a disrupted stratum corneum. In humans, this mechanism is supported by the demonstration that application of peanut containing oils in infancy was associated retrospectively with higher odds ratios for peanut allergy later in childhood. Children who live in environments with high peanut protein levels, but who do not knowingly eat peanut, have been shown to have higher peanut allergy rates than children with low environmental exposure to peanut.

The primary hypothesis of this study was that abnormal skin barrier function (with or without eczema/ atopic dermatitis – AD) as demonstrated by measurement of transepidermal water loss (TEWL) predates and predicts food allergen sensitisation, independent of other post-natal dietary and environmental factors. The secondary hypothesis was that any relationship between skin barrier function and food allergen sensitisation is driven by loss-of-function mutations in the filaggrin (*FLG*) protein.

If a defective skin barrier is determined to be a likely route of exposure with the consequent development of sensitisation then preventative barrier augmentation or immune-modulatory strategies could be deployed topically to minimise the risk of developing food allergy.

Methods

This study exploited the availability of BASELINE, Ireland's first birth cohort study, to determine the prevalence and cumulative incidence of food allergen sensitisation and challenge-proven food allergy at 2 years, while prospectively and noninvasively measuring TEWL as an index of skin barrier function at 3 time points in the first months of life. Four scheduled study visits were offered after discharge from the maternity hospital and families were encouraged to contact the study team if food related problems arose between scheduled visits.

Screening questions for food allergy were asked at each visit, based on modified EuroPrevall criteria for assessment of suspected food allergic reactions. A detailed phone consultation took place between parent and team and if warranted, skin prick test (SPT), specific IgE (SpIgE) and oral food challenge (OFC) were arranged. All BASELINE infants were screened at 2 years for sensitisation to a panel of foods comprising cows' milk, hens' egg, peanut, wheat, cod and soya. Those with positive SPT (≥3mm) who were not safely ingesting the suspected food were offered an OFC.

Results

1903 children were recruited into the study from July 2009 to October 2011 with 1355 (71.2%) retained to 2 years. Of those who had TEWL taken in early neonatal period, mean TEWL at birth was 7.32 $g_{water}/m^2/hr$ (±3.33), rising to a mean of 10.97 $g_{water}/m^2/hr$ (±7.98) at 2 months, before plateauing to a mean of 10.71 $g_{water}/m^2/hr$ (±7.10) by 6 months.

The point prevalence of eczema/atopic dermatitis (AD) was 18.7% (299/1597) at 6 months, 15.53% (232/1494) at 12 months and 15.86% (215/1355) at 2 years.

FLG genotyping was conducted on 1300 infants with available DNA samples. The cumulative *FLG* mut rate was 10.46% (136/1300). This FLG mutation carriage rate is consistent with that of the general Irish population, in whom the association of FLG with eczema was discovered. Four infants were homozygous for *FLG* mutation, with the remainder heterozygous. The most prevalent gene mutations were GenR501X 4.23% (55/1300), Gen2282Del 3.6% (47/1300), GenS3247X 1.62% (21/1300), GenR2447X1% (13/1300).

1540 infants were retained to 12 months, 13.4% (207/1540) of participants reported a suspected adverse reaction to a food by 12 months. 96.1% (199/207) of queries were successfully followed up. There was a cumulative incidence of food allergy at 12 months of (54/1540) 3.51% (95% CI 2.59 -4.43%).

1355 infants were retained to 2 years, with 1260 of those undergoing SPT. The food sensitisation point prevalence at 2 years was 6.27% (79/1260) with a food allergy prevalence of 4.45% (56/1258)

Infants with an FLG mutation (*FLG* mut) had higher AD rates and food allergy rates than those without a FLG mutation (FLG wt). TEWL at birth was similar in *FLG* mut and *FLG* wt groups. However, by 2 months *FLG* mut infants had a significantly higher TEWL score ["*FLG* mut" 12.6 (±10.1)$g/_{water}/m^2$ compared with "*FLG* wt" 10.7 (±7.7) $g/_{water}/m^2$ p =0.04]

Using logistic regression 2 mths TEWL was found to predict food allergen sensitisation at 2 years and this effect persisted when the important variables of *FLG* status and AD diagnosed at 2 years were controlled for, as measured by total SCORing Atopic Dermatitis (SCORAD) score at 2 years, neither of which remained significant.

Conclusion

In an unselected, nationally representative and prospectively studied birth cohort of 1900 Irish children, TEWL at 2 mths predicted food allergen sensitisation and food allergy at 2 years, irrespective of the impact of the presence of AD or filaggrin gene status. TEWL at earlier (day 2) and later time points (6 months) did not show any such an association.

Implications

We have found the earliest yet detected signal of skin barrier dysfunction at 2 months and this predicts the development of food sensitisation and food allergy. This signal is not seen at birth. This suggests there is a window of skin barrier vulnerability between birth and 2 months that could be subjected to an intervention to maintain skin barrier integrity during

this critical period as a means to prevent AD and food allergy. This warrants further investigation.

Glossary

AD – Atopic Dermatitis

BASELINE- Babies After SCOPE: Evaluating Longitudinal Impact using Neurological and Nutritional Endpoints)

CUH – Cork University Hospital

CUMH – Cork University Maternity Hospital

DBPCFC – Double Blind Placebo Controlled Food Challenge

FA – Food Allergic

FBC – Full Blood Count

FS – Food Sensitised

FLG - Filaggrin

FLG mut (Filaggrin mutation carrier)

FLG wt (Filaggrin wild type – normal)

FSA – Food Standards Agency

HDM – House Dust Mite

LTFU - Lost to Follow Up

OFC - Oral Food Challenge

PA – Peanut Allergy

SCOPE study - Screening for Pregnancy Endpoints

SOP – Standard Operating Procedure

SpIgE – Specific Immunoglobulin E

SPT – Skin Prick Test

TEWL - Transepidermal Water Loss

WAO – World Allergy Association

WC – Withdrew Consent

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Introduction

Allergy & Atopy

Atopy is described as “a personal or familial tendency, usually in childhood or adolescence, to become sensitized and produce Specific Immunoglobulin E (SpIgE) antibodies in response to ordinary exposures to allergens, usually proteins”.(1) This can lead to the development of eczema, food allergy, asthma and rhinoconjunctivitis; which constitute the Atopic diseases. Atopic diseases have increased worldwide over the last decades.(2-4)Atopic diseases are one of the most common non-communicable diseases in childhood, with a recent UK study showing that up to 36% of children will have at least one of these conditions diagnosed in childhood, and 16% will have multiple allergic conditions diagnosed.(5) Evidently this places a considerable burden on both individual families and the healthcare system as a whole.(6, 7) Recently there is evidence that the pattern of atopic diseases is changing, with asthma prevalence peaking but AD and rhinoconjunctivitis continuing to rise albeit at a slower pace.(8, 9) The prevalence of food allergy also continues to rise, however, and has been described as the “Second Wave” of the allergy epidemic.(10) This increase has been seen whether food allergy is diagnosed by skin prick testing (SPT) and Oral Food Challenge (OFC), as in the Isle of Wight Study (4); by telephone interview (11) or by analysing hospital admission rates for food related anaphylaxis which represents the most severe and usually most clear cut presentation of cases of food allergy.(12)

The onset of atopic disease is usually heralded by atopic dermatitis (AD) in infancy. The classic temporal progression of atopic diseases from AD to food allergy to allergic asthma to allergic rhinitis is known as the “Atopic March” or “Allergy March”. (13, 14) This is seen as a progression from AD and food allergy in infancy to the development of asthma and allergic rhinitis in children and teenagers. There is both epidemiological and laboratory evidence to support this observation. The most significant birth cohort group to assess this was the German Multicentre Allergy Study which enrolled 1300 infants who were at both high and low risk for atopy and observed them from birth to adulthood. Children who developed AD in infancy were twice as likely as those without AD to be sensitised to food allergens at two years (OR 2.10 (1.36-3.25)). Those children with AD were also three times more likely to

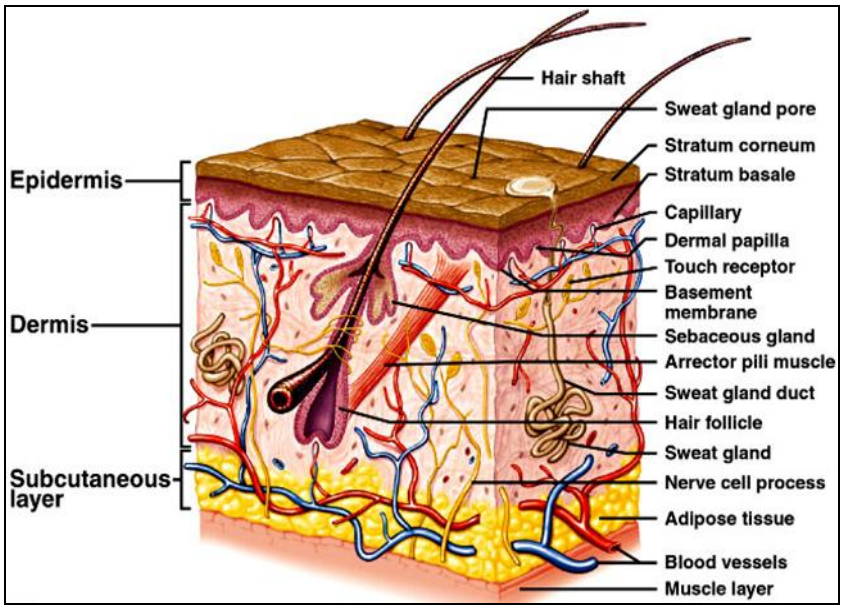
have inhalant sensitisations at two years than those without AD (OR 3.00 (1.89-4.78)). The severity of AD was seen as an important influencing factor. 21% of infants with mild AD, “AD and no scratching”, were sensitised to a food or inhalant allergen compared with 56% of those with severe AD “ AD and frequent scratching”. Those with food or inhalant sensitisation at 2 years, on longitudinal follow up, were subsequently more likely to have current wheeze and bronchial hyper-responsiveness at age 7 years.(15, 16)

These epidemiological studies provided the basis for murine studies, which looked at the role of epicutaneous sensitisation in the induction of bronchial hyperreponsiveness, as what is seen in asthma, and also in the development of food allergy. Mice that were exposed to ovalbumin through tape stripped skin, when subsequently challenged with inhaled ovalbumin had significantly increased eosinophils in BAL fluid, and had a 10 –fold greater sensitivity to metacholine challenge. Those mice with transcutaneous exposure through undamaged skin did not experience similar reactions (17) Specifically for food allergy, a murine model has shown that exposing mice to peanut antigen across a disrupted skin barrier induces systemic allergic reactions (18) This suggests that the disruption in skin barrier, as seen in atopic dermatitis, provides a route of transcutaneous sensitisation to allergens which can induce both airway hyper-responsiveness and systemic food allergic reactions.

The Skin Barrier

The skin is the largest organ of the body and provides a protective barrier to the outside environment with its associated pathogens, infections and allergens. It also provides a protective barrier from inside to outside so that protein, water and skin lipids are maintained within the body milieu. Though obviously a physical barrier, the skin barrier also has biochemical and immune barrier functions. The skin's physical barrier consists mainly of the epidermis, the outermost layer of skin. There are 4 main layers of the epidermis; from inner to outer; the stratum basale, stratum spinosum, stratum granulosom and the outermost stratum corneum.(19)

Figure 1: The Skin



(Shier, Butler, and Lewis: Chapter 6. Hole's Human Anatomy and Physiology, 11th ed. McGraw and Hill 2007)

Transepidermal Water Loss (TEWL) is a widely used method of "*in vivo"* assessment of skin barrier function (inside-outside function). A low TEWL indicates intact inside-out skin barrier function, whereas high TEWL levels indicate a non-intact barrier function. High TEWL levels can be found in disease states such as atopic dermatitis (AD) at both lesional and non lesional sites.(20) A study in 2010 by Flohr et al suggest these changes predate the onset of AD. (21) This was a study of 88 infants at higher risk of AD, who had TEWL taken at 3

months. Infants with AD, diagnosed at 3 months by U.K. diagnostic criteria-based photographic protocol of the International Study of Asthma and Allergies in Childhood (ISAAC) Phase Two, had a higher mean TEWL than those without AD. Infants with dry skin also had a higher mean TEWL than those without dry skin. This led the authors to suggest that a raised TEWL would predate the development of AD as these infants with dry skin were likely to develop AD. This may be a premature assumption, as this could only be proven once these infants were followed longitudinally for the potential definite diagnosis of AD.

TEWL can be measured by both closed and open systems. The open-chamber system, which is based on Fick's Law of Diffusion, estimates water diffusion gradient across an open chamber.(22) Because this device is open to the environment, it may be susceptible to changes in environmental airflow.(23) This influence can be negated by conducting the measurements in an environmentally controlled room. A closed chamber device is also available for measuring TEWL. In closed chamber devices TEWL is measured by the increase in relative humidity when the chamber is placed against the skin.(52) With closed chamber devices, continuous measurements cannot be taken as the probe fills with water from the increased humidity which must be expunged after each reading. In head-to-head comparison against a closed-chamber device, the open chamber device was more sensitive in detecting changes in TEWL values at lower levels (<45 $g_{water}/m^2/hr$), but the closed-chamber system was more sensitive in detecting measurements in the high-value range (>80 $g_{water}/m^2/hr$(24) However further studies suggest good correlation between both measuring devices.(25, 26)

Atopic Dermatitis

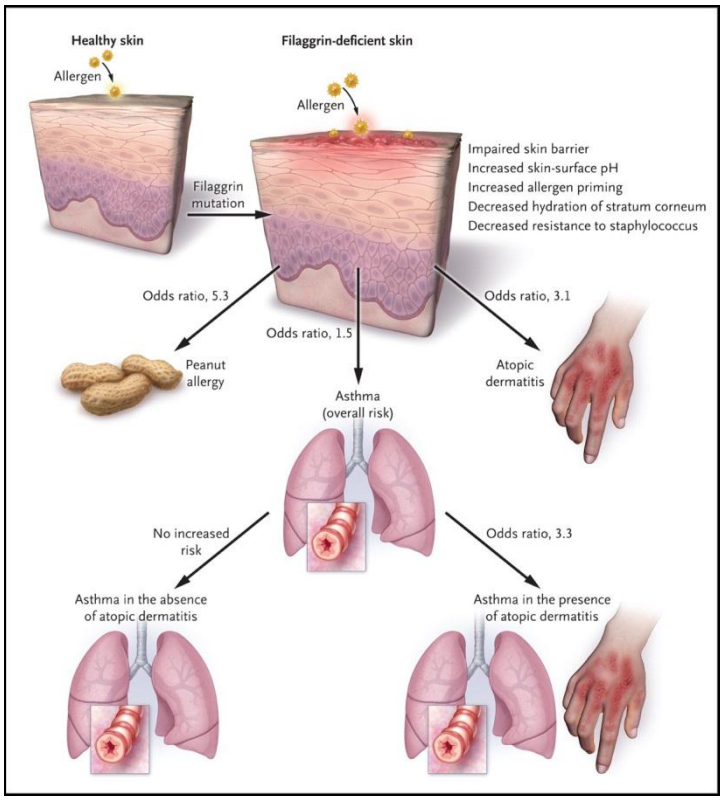
Dermatitis is often the clinical manifestation of a defective skin barrier. As described above atopic dermatitis is typically the first of the atopic diseases to present. AD mainly presents in the first year although later presentation is seen. (15) AD is a clinical condition characterised by itchy erythematous skin. The exact nomenclature used to describe atopic dermatitis is still being debated. For some time the terms “dermatitis” and “eczema” were used

interchangeably. The EAACI nomenclature task force and the World Allergy Organisation have reached agreement on the general terms.(1) Atopic eczema (or AD) refers to the presence of eczema with evidence of Specific IgE. Up to 80% of patients with “AD” have no measurable IgE therefore AD should be used only for those with documented raised SpIgE. (27)

In infants AD typically presents on the trunk and face. In childhood the flexures are more affected.(28) The exact prevalence of AD is difficult to ascertain as differing studies use various means of diagnosing and classifying AD. The natural history of AD shows an increase in prevalence in childhood with a decline to teenage years and those with more severe AD in earlier life having a decreased likelihood of resolution and also having an increasing likelihood of developing further allergic diseases. (15)

An exact genetic mechanism and inheritance pattern of Atopic disorders is as of yet unclear, it is likely interplay between genetic and environmental factors. Parental atopy is an independent risk factor for development of atopic disease and concordance for atopic dermatitis is higher among monozygotic twins than dizygotic twins.(29, 30) The most widely studied gene to influence atopic disease is Filaggrin (*FLG*). Filaggrin is a filament binding protein in the stratum corneum.(31) Mutations in *FLG* were first described in families with hereditary Ichtyosis Vulgaris.(32) Mutations were subsequently seen in AD. *FLG* loss-of-function mutations occur in 10% of Europeans. The most prevalent *FLG* mutations in Europeans being; GenR501X, Gen2282Del4, GenS3247X, GenR2447X.(33) Presence of *FLG* mut increases both the risk and the severity of development of AD, which was first described in Irish patients.(34)

Figure 2: Filaggrin insufficiency and increased risk of atopic diseases



(Irvine AD et al. N Engl J Med 2011;365:1315-1327)

Many other genes have been implicated in AD. A recent systematic review, reported on over 80 genes in 100 studies that had positive association with the development of AD. (35) The restricting factor in most of these studies, is the homogeneity of study populations with the majority of studies conducted on Caucasian populations and therefore the influence of these gene mutations in other populations is unclear

Diagnosis of AD

AD is a clinical diagnosis. There is no "Gold Standard" test with which AD can be diagnosed or accurately measured. Though clinicians may diagnose AD through taking an accurate clinical history and performing a physical exam , for research purposes it is imperative that standardised criteria for diagnosing AD are adopted.(36) The criteria most commonly used in Europe is the UK Working Party (UKWP) diagnostic criteria. They were presented in 1994,

and were a refinement of the Hannafin and Rajka criteria. They show good inter and intra observer reliability.(37-39)

Table 1. UK Working Party Diagnostic criteria for AD under 2 years

An itchy skin condition plus:
- **A history of generally dry skin**
- **A history of atopy in first degree relative**
- **A history of involvement of the flexures**
- **Visible flexural eczema (of cheeks/foreheads in under 4 years)**

Severity of AD

AD severity is an important feature. Up to 30% of children can be affected by AD with a wide spectrum of clinical severity. Those children with moderate to severe AD in infancy have a higher incidence of food allergy and sensitisation and should be distinguished from milder cases.(40, 41) Furthermore in the research setting it is imperative to have a reliable disease severity score so that changes seen in disease severity after a particular intervention are true changes in disease score rather than inter or intra-observer variation.

There are upwards of 15 severity indices for AD with Eczema Area and Severity Index (EASI) and SCORing Atopic Dermatitis (SCORAD) being most reliable.(42) The SCORAD assessment tool was developed in 1982 and compromises of three parts measuring; the extent of affected skin, the intensity of the affected areas and subjective symptoms experienced. This scoring system has been extensively studied and replicated in different populations.

The Eczema Area and Severity Index score (EASI) was proposed in 1998 as a new tool to measure severity of AD. It involves assessment of the intensity of four clinical signs at four different areas.(43) There is high correlation with SCORAD results.(44) The EASI system does not include however a symptom section, which may reduce the classification of AD if the AD is in a quiescent phase at time of review. EASI can be technically difficult and more time consuming than other scores. (42) For the above reasons we preferred SCORAD scoring system in this study.

Food Allergy & Sensitisation

There are five subtypes of immunoglobulins: IgA, IgD, IgE IgG and IgM. They form part of the innate immune system. IgE is a potent immunoglobulin which as well as its known association with allergy and hypersensitivity is responsible for the defence response to parasitic worm infections.(45) In atopic individuals with either or all of AD, Food allergy, Allergic Asthma and Allergic Rhinitis, IgE is evidently produced in excess to an antigen. In contrast Hyper-IgE syndrome is a rare immunodeficiency characterised by recurrent skin and pulmonary infections.(46)

Food sensitisation is the result of a deviant immunological response to a food protein. Immunoglobulin E is produced in response to exposure to particular food allergens. Food Allergy is a combination of both sensitisation to food and the symptoms of IgE mediated allergy elicited after exposure to the food allergen. Sensitisation can also occur without clinical allergy. This occurs when SpIgE is produced to the food allergen however on exposure to the food there are no symptoms of IgE mediated food allergy; hence the subject is considered tolerant to the food. This differentiation between food sensitisation and food allergy is imperative and therefore caution should be used when testing for SpIgE without consideration of a clinical report of whether the food in question is tolerated or not. (47, 48)

Between 1-6% of children in the developed world have IgE mediated food allergy.(49, 50) The most common food antigens are Cow's Milk, Egg, Peanut, Treenut, Fish and Wheat. Food allergy is most often seen in developed countries, but recent data has shown that even in developing countries the rate of food allergy is increasing. (51) Exact rates of food allergy differ between countries, this may reflect the different genetic, environmental and cultural influences on that population but it may also be due to the methods used to diagnosis food allergy. Rates of self-reported food allergy are far higher than physician proven allergy, with parental reports of food allergy for their offspring substantially higher than the subsequent OFC diagnosed FA.(52, 53) Self-selection of food allergic patients into food allergy research must be accounted for. Studies which use oral food challenge as an outcome for food allergy, such as ours more accurately quantify the rate of food allergy and sensitisation.

Evidence for the relationship between food allergy and the skin

A relationship between food allergy and atopic dermatitis is readily apparent clinically, however the exact mechanism of interaction or causation is not known. There is wide spectrum of severity of AD, with the majority of children having mild disease, and a minority moderate – severe. Those with moderate – severe AD have an increased risk of food allergy and sensitisation.(54) Studies of Food Allergy in patients with AD have yielded heterogeneous results, mainly related to the population studied and the methods used to diagnose allergy rather than sensitisation.

In highly atopic children, presenting to an allergist with severe AD, Food Hypersensitivity occurred in up to 56% of children.(41) When allergy testing was conducted in individuals attending a university Dermatologist, food hypersensitivity was seen in 37% of infants.(40, 55) In screening of those with Atopic dermatitis for food sensitisation in a large cohort, the severity and time of onset of AD was associated in an increased risk of concurrent Food Sensitisation. (54) It must be appreciated that sensitisation to a food allergen is the production of SpIgE in response to a food allergen. It does not equate to food allergy and there is a concern that immediate food allergy is over diagnosed in this group. There is also a risk of severe reactions on reintroduction of the food if food is inappropriately excluded in an infant who was sensitised but tolerating the food allergen in question.(56) This difficulty is reflected in current guidelines suggesting that panel testing of infants and children with atopic dermatitis is not carried out. Food allergy screening should be carried out in a controlled manner, ensuring an accurate allergy focused history and that skin is appropriately treated before allergy testing. (47, 57)

Further evidence for the association between a defective skin barrier and development of food allergy and sensitisation is seen by looking at mutations in the Filaggrin gene. *FLG* mutation carriers are known to have an increased risk of AD. In infants *FLG* mutations also significantly increase the risk of Peanut Allergy, suggesting that a defect in the skin barrier is associated with the increase risk of development of Peanut Allergy.(58) This increased risk remains significant even when controlling for coexistent AD, demonstrating that a defective skin barrier can be present even in those in whom a clinical manifestation is not evident.

Additional emerging evidence has been seen to demonstrate an association between a defective skin barrier and food allergy resulting in transcutaneous sensitisation. Mice who have primary exposure via a transcutaneous route had increased levels of peanut allergy than those who had a gastrointestinal primary exposure and when that exposure was across a disrupted stratum corneum by tape stripping a potent systemic immune response is induced. (18, 59) Specifically for peanut allergy transcutaneous exposure to peanut oil in early infancy has been associated with the development of peanut allergy. In the Avon Longitudinal Study of Parents and Children (ALSPAC) study 13971 women were enrolled from pregnancy and followed up by questionnaires over the course of the child's life. Of these, 49 children developed peanut allergy and when questioned at 5 or 7 years regarding their peanut oil use, more mothers of children with peanut allergy recalled having used peanut oil on their infant's skin than did those whose children did not subsequently have peanut allergy. This was retrospective data and the researchers attempted to eliminate bias by ensuring the interviewee was blind to parent's answers and to which creams contained peanut oil. There were no differences in rate of atopy or in other cream use between the peanut allergy group and the atopic control group. (60) This study is however subject to recall bias of parents after the development of food allergy.

Transcutaneous exposure to peanuts does not just need to occur through the topical exposure to peanut oil. Living in a household with high peanut consumption can be a risk factor for development of peanut allergy. This is shown in a study by Fox et al which assessed the peanut intake of children referred to an allergy clinic in the UK. The results showed that high household consumption of peanuts was a risk factor for development of PA when compared against low and high risk control group. Some children in the high risk control group, with high level of household peanut consumption did not develop PA however. This group were more likely to have ingested peanut orally at an earlier age. (61)

These results confirm the suggestion of previous work showing a high level of household peanut can be a risk factor for development of PA if the food is not ingested orally and early by the infant. Du Toit et al found that peanut allergy prevalence in Jewish children in Israel was low compared to the same genetic group living in London. Both groups had high levels of household peanut consumption, and had similar genetic, atopic and social demographics. One marked difference between cohorts was the timing of introduction of peanut into the

diet of infants. By 9 months of age, 69% of Israeli group were eating peanut compared with only 10% of UK infants. In the Israeli cohort this is typically in the form of Bamba, a local produced widely ingested peanut based snack. The median monthly consumption and the frequency of ingestion of peanut in Israeli infants aged 8 to 14 months was significantly higher than London group. There was a tenfold increase in PA levels between the London group and the Israeli group 1.85% v's 0.17%. (62)

If a defective skin barrier was determined to be the route of allergen exposure during infancy that leads to allergen sensitisation, then the implementation of a plan to restore or maintain the skin barrier could prevent transcutaneous exposure to allergen, perhaps limiting the prevalence of food allergy.

Project Aims

This project aimed to address the specific questions posed by the FSA in its Food Allergy & Intolerance Research Programme:

1. Investigate the causes and mechanisms of food allergy, particularly severe food allergy, in order to reduce its incidence and severity.

2. Identify risk factors (genetic, environmental, dietary or other) that are associated with the development of sensitisation to foods and clinical food allergy in early life

3. Investigate the importance of skin exposure to food and food proteins in the development of sensitisation to food

4. Characterise the conditions and mechanisms through which sensitisation via the skin may be achieved

5. Can skin exposure to allergenic foods/food proteins cause sensitisation such that subjects may subsequently display symptoms of food allergy following dietary exposure to the same foodstuff?

Study Objectives

The objectives of the study are to assess the skin barrier function of a large birth cohort group, namely the BASELINE study. This was done at three intervals in the first six months of life: early in the neonatal period preferably on day 2 of life, before leaving the maternity hospital, at two months and finally at six months. Skin barrier function was assessed by measurement of TEWL, a measurement of the water loss across the stratum corneum, representing an “inside to outside” skin barrier defect.

The prevalence of food allergy has not yet been established in an Irish population, even though there is a high preponderance of atopy in this population. The determination of the prevalence of food allergy in Ireland is a specific objective of the BASELINE study overall. The assessment of possible cases was performed using previously designed and validated means, incorporated from EuroPrevall, an EU-FP6 multinational study which recruited over

12,000 infants and established criteria for the diagnosis of food allergy in such infants, based on formal challenge (OFC).

The relationship between the BASELINE - derived food allergy and sensitisation prevalence and early-life skin barrier was then examined. If a relationship were to be shown between a defective skin barrier and the subsequent development of sensitisation or allergy to a food then an opportunity could arise to protect the skin barrier and potentially influence the development of sensitisation. This would be a novel finding in the field. Other studies have looked at this relationship, but either focussed on high risk groups or on skin barrier status and function after one year of age when food allergies are often already established.

Specific Objectives of this study:

1. Measure skin barrier function by TEWL in 2000 Irish infants at day 2 of life and at 2 months and 6 months of age (2000 infants are required in the study at the 2 year endpoint).

2. Examine the relationship of food allergen sensitisation and food allergy at 2 years with skin barrier function on day 2 of life as the primary measurement and at 2 and 6 months of age as secondary measures

3. Determine if any relationship between skin barrier function and food sensitisation/allergy is driven by FLG genotype.

Methods

This study utilised the BASELINE Birth Cohort Study (Babies After Scope Evaluating Longitudinal and Nutritional Endpoints). This was Ireland's first birth cohort group and was recruited in Cork University Maternity Hospital. This study was conceived as a paediatric follow on to the SCOPE study, which was the Irish arm of a multicentre international study examining for diseases of late pregnancy. The criteria for inclusion in the SCOPE study were healthy, primigravidous women.(63) Maternal recruitment in Cork into the SCOPE study ran from 2008 through to August 2011.

This birth cohort study was explicitly designed to assess for early markers of disease in children, focusing on nutritional and developmental outcomes. Food allergy was one of the main nutritional outcomes. The birth cohort study was originally funded by the National Children's Research Centre, Our Lady's Hospital Crumlin, Dublin, Ireland. The FSA UK funded the sub study investigating the relationship between skin barrier function and the development of food allergy and sensitisation. Infants recruited from July 2009 had skin barrier assessment completed at three time points in early infancy and had *FLG* genotyping conducted. These infants are the subject of this thesis.

i. Ethics

Ethical approval for this study was sought and approved by Cork Teaching Hospitals Medical Ethics Committee.

ii. Recruitment

Recruitment into the study began in July 2008. Recruitment was conducted by research midwives attached to the SCOPE study. Mothers on the SCOPE study were approached at 22 weeks to enrol on to the BASELINE study pending healthy live birth. After delivery cord blood samples were taken.

1903 infants were recruited to BASELINE from 1st July 2009 to October 2011. These infants had skin barrier function assessed in early infancy and these 1903 infants only will be the subject of this thesis. Recruitment from July 2009 to June 2010 was via SCOPE only. By 1st June 2010, only 452 infants had been recruited (45 infants per month over the 10 month period), representing 45% of the recruitment target. To overcome this shortfall a

supplemental, parallel recruitment stream was commenced. Prospective postnatal recruitment began on the post natal wards. The inclusion criterion for this parallel recruitment stream was a healthy singleton infant on the post natal ward. These mothers were approached by a research midwife on the post natal ward. Consent for discussion was first sought. The study was described to the parents and if permission given, consent for participation of both infant and parent was taken. A 3-month pilot phase was trialled from August-October 2010. Review of the success and viability of recruitment against agreed revised targets resulted in continuing recruitment through the secondary stream until the end of October 2011.

In total 1303 infants were recruited through the original SCOPE stream, and 600 infants were recruited via the parallel recruitment stream. From here on in if and when the antenatally and postnatally recruited infants are discussed as separate groups then antenatally recruited infants are referred to as "Stream 1" and postnatal infants are referred to as "Stream 2" recruits.

iii. Layout & Staffing

Initial recruitment was carried out by a research midwife in the antenatal clinics of Cork University Maternity Hospital for antenatally recruited infants, and the postnatal wards for postnatally recruited infants. Two month appointments were also completed in the CUMH, to facilitate use of the Peapod plethysmography to assess body fat mass composition of enrolled infants. Further appointments were conducted in the paediatric ward of the Cork University Hospital until Dec 2010 when the, Health Research Board funded, Discovery Centre was opened. The Discovery Centre availed of existing accommodation on the grounds of the Cork University Hospital, but separate from the main CUH building and was funded by the Health Research Board of Ireland. It was renovated and decorated to specifically meet the needs of research with children. This centre had three environmentally controlled appointment rooms, a waiting room and an office. From December 2010 all BASELINE appointments were conducted here, apart from medical reviews and oral food challenges.

iv. Study appointments

Newborn

Newborn appointments took place on the post natal ward of the Cork University Maternity Hospital. The appointment consisted of questionnaires for parents and measurements for infants. The infants had basic measurements including length, weight and occipital-frontal circumference conducted (see Appendix). They also had plethysmography via PeaPod. The PEA POD Infant Body Composition Tracking System is an air-displacement plethysmograph.(2) Their skin barrier was assessed by measuring Transepidermal water loss (TEWL) which is described in detail below.

2 month

Parental questionnaire was completed including a detailed allergy focused history for both parents and information on feeding, babies, health & development. The allergy focused questions were derived from the EuroPrevall. Physical measurements including plethysmography were taken. TEWL was measured.

6 month

Parental questionnaire was completed. Physical measurements conducted.
Growth parameters were checked and recorded. TEWL was measured. Skin was assessed clinically, SCORAD was completed if evidence of active AD or if diagnostic criteria fulfilled.

12month

Parental questionnaire completed. Physical measurements conducted. Skin was assessed clinically. SCORad and Nottingham Severity Score completed if evidence of Atopic Dermatitis or if diagnostic criteria fulfilled.

2 years

Parental questionnaire was completed. Physical measurements were conducted. Skin was assessed clinically. SCORad and Nottingham Severity Score completed if evidence of Atopic dermatitis or if diagnostic criteria fulfilled. TEWL was measured. All children were offered allergen sensitisation screening by Skin Prick Test (SPT) to a common food panel of cows

milk, egg, peanut, wheat, soya and cod. They also had SPT carried out to inhalant allergens; house dust mite, grass pollen and cat. Blood samples were taken for Full Blood Count (FBC), Vitamin D, Ferritin, and *FLG* mutation if infant had not had successful *FLG* typing from cord blood sample, or if no birth sample was available. SpIgE was taken if food allergen sensitisation screening was positive in the absence of safe consumption of food.

Table 2: Summary of clinic visits

	Birth	2 months	6 months	12 months	2 years
Questionnaires	√	√	√	√	√
TEWL	√	√	√	χ	√
Plethysmography	√	√	χ	χ	X
Physical Measurements	√	√	√	√	√
Biological samples	√	χ	χ	χ	√
AD assessment	X	χ	√	√	√

v. Transepidermal Water Loss (TEWL)

TEWL measurements were carried out using a widely validated open chamber system (Tewameter® TM 300; Courage+Khazaka Electronic, Cologne, Germany). (64, 65) For newborns TEWL was taken in CUMH. The subject's arm was acclimated prior to measurement by exposing the arm in a non-environmentally controlled room for 10 minutes. This was typically done in the cot beside mother's bed while an interview with the mother or parents was carried out. The infant was then brought to a windowless room where both temperature and humidity were maintained constant by an air conditioning system where TEWL was taken on the lower volar surface of the forearm. Temperature was set between 20-25 degrees Celsius by an air conditioning system. Humidity was monitored by a manometer in the room and was maintained between 30-45%. Probe was applied to exposed volar skin for approximately 15 seconds until measurement was recorded. Three readings were taken and the mean of the three readings was recorded. For TEWL readings at other time points the same procedure was carried out in the Discovery Centre. The parents were advised not to apply emollients to infant's skin for 12 hours prior to reading. At the 2 and 6 month appointment TEWL was taken in a similar manner, but with acclimatisation occurring in the room where measurements were taken. TEWL was not recorded on visibly upset infants. At the 2 and 6 month appointments, if active skin disease was already manifest, TEWL was measured at unaffected sites as previous studies have shown that TEWL readings are higher at lesional rather than non lesional sites.(66)

vi. Atopic Dermatitis

The UK Working Party diagnostic criteria for determination of AD status was used. This scoring system is based on a consensus report from experts in the field. A diagnosis of AD was made if the child has an itchy skin condition plus three or more of; history of atopic disease in a first degree relative, generally dry skin, history of flexural involvement and visible flexural dermatitis as per photographic protocol. (37-39)

On diagnosis of AD then a scoring system was used to assess disease severity by both objective and subjective means. The SCORAD system (SCORing Atopic Dermatitis) was used. This objectively measures extent of the active disease and the severity of the affected skin and also incorporates subjective symptoms experienced by the patient regarding itch and sleep disturbance. A recent systemic review recommends the SCORAD system over others assessing severity of AD for outcome in clinical trials due to its validity, internal consistency and interpretable composite score.(67)

At 12 months a Nottingham severity score was also completed. This is similar to the SCORAD with both an objective and subjective component. The objective component looks at extent of skin affected only, not severity of unaffected skin and the subjective component involves quantifying sleep disturbance and duration of symptoms.

Even though the primary design of the study was that of an observational birth cohort, in cases of severe or undertreated disease management advice was given to parents, and referral to specialist services made as required.

vii. Filaggrin genotyping

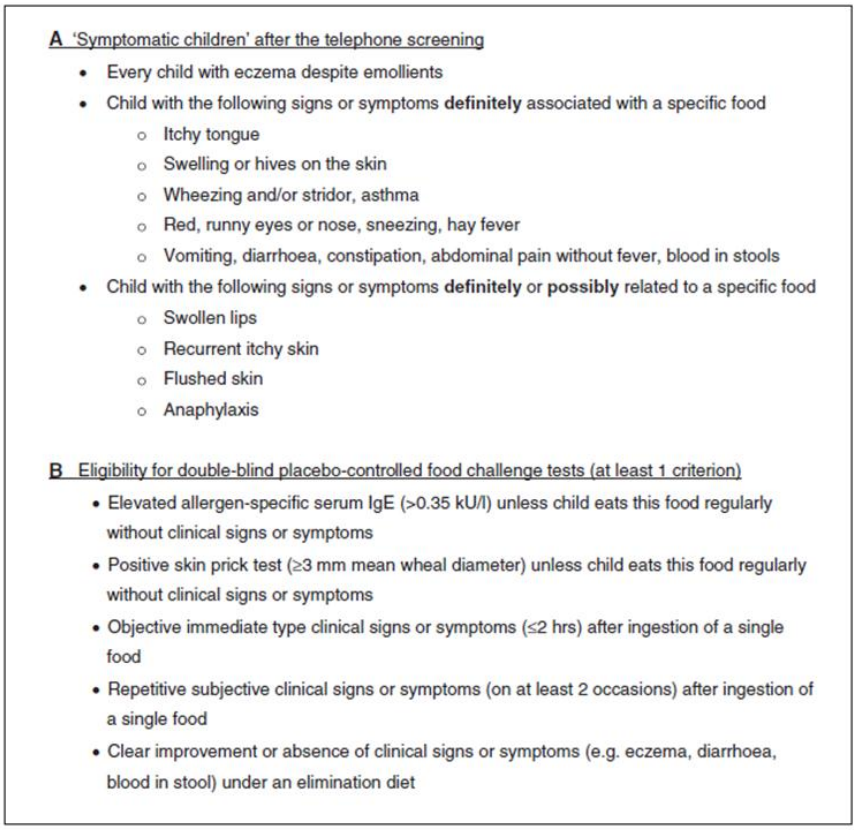
Antenatally recruited infants had Cord Blood samples stored for *FLG* genotyping. Postnatally recruited infants had Oragene Saliva samples taken at birth. All infants were also offered further blood or saliva sampling at 2 years when BASELINE bloods were being taken per BASELINE protocol. This allowed us to catch children who had not had cord blood or saliva samples or whose sample's had not yielded sufficient quality samples to allow FLG genotyping.

FLG genotyping was conducted in the McLean Laboratory, College of Life Sciences, University of Dundee, Dundee. The 4 most prevalent *FLG* mutations in Europeans were screened for; GenR501X, Gen2282Del4, GenS3247X, GenR2447X.

viii. Investigation of suspected Food allergy in infants

At each appointment parents were questioned as to the possibility of adverse symptoms related to food ingestion. These appointments occurred quite frequently in the first year of life; at birth, 2 months, 6 months and 12 months. They were next seen at 2 years. Parents were also advised to contact the study between appointments if they suspected an adverse reaction to food. The EuroPrevall criteria for assessment of suspected food allergic reaction was adopted and adapted.(68) Symptomatic children were defined as per the EuroPrevall criteria below.

Figure 3: EuroPrevall criteria for (A) defining symptomatic children after telephone screening; (B) eligibility for double-blind placebo-controlled food challenge tests.



A 'Symptomatic children' after the telephone screening

- Every child with eczema despite emollients
- Child with the following signs or symptoms **definitely** associated with a specific food
  - Itchy tongue
  - Swelling or hives on the skin
  - Wheezing and/or stridor, asthma
  - Red, runny eyes or nose, sneezing, hay fever
  - Vomiting, diarrhoea, constipation, abdominal pain without fever, blood in stools
- Child with the following signs or symptoms **definitely** or **possibly** related to a specific food
  - Swollen lips
  - Recurrent itchy skin
  - Flushed skin
  - Anaphylaxis

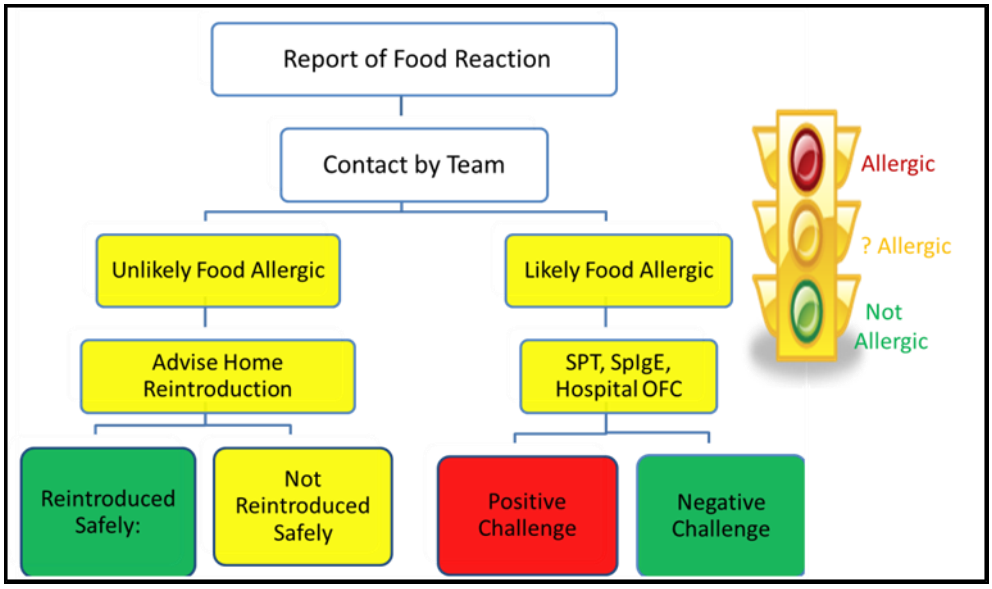
B Eligibility for double-blind placebo-controlled food challenge tests (at least 1 criterion)

- Elevated allergen-specific serum IgE (>0.35 kU/l) unless child eats this food regularly without clinical signs or symptoms
- Positive skin prick test (≥3 mm mean wheal diameter) unless child eats this food regularly without clinical signs or symptoms
- Objective immediate type clinical signs or symptoms (≤2 hrs) after ingestion of a single food
- Repetitive subjective clinical signs or symptoms (on at least 2 occasions) after ingestion of a single food
- Clear improvement or absence of clinical signs or symptoms (e.g. eczema, diarrhoea, blood in stool) under an elimination diet

The criteria was modified in that; i. OFC was only offered in the case of suspected IgE mediated or immediate reaction to food and ii. Infants under one year with severe AD were seen. Suspected non IgE mediated food allergy was not diagnosed via OFC and is discussed

below. The staff member who first dealt with the query contacted both the Clinical Research Fellow and Allergy Nurse Specialist. A detailed phone consultation took place between parent and team. Depending on the food ingested, symptoms attributed to food and the timing between ingestion and symptom appearance it was often possible to distinguish likely food allergy from unlikely food allergy by telephone contact alone.

Figure 4: Diagnostic flow of suspected food allergy queries.



If following an allergy focused history Food Allergy was deemed “unlikely” to be responsible for symptoms described then the parent was advised to reintroduce the suspected food at home. They were followed up with a phone call to ensure this had happened safely.

If the symptoms reported were “likely” or plausible for food allergy the child was seen for clinical assessment. These assessments typically took place in the CUH Seahorse Day Unit. At that assessment, baseline measurements were taken including height and weight. A focused allergy history was also elicited including; birth history, type of feeding, developmental assessment, family history of atopy, the suspected food, when it was eaten, history of ingestion, signs and symptoms seen. If food allergy was still suspected or plausible a Skin

Prick Test (SPT) for the food suspected was conducted. (see below for detailed methodology of SPT). If SPT was negative and history was not highly suggestive of food allergy, the parent was advised to reintroduce the food at home and follow up call was undertaken at a later date to ensure this had occurred safely. If SPT was negative, but history was highly suggestive of food allergy then OFC was undertaken despite initial negative testing, again parent was given advice on how to avoid the food until OFC was conducted. If SPT was positive (>3mm) then SpIgE was taken and oral food challenge was arranged. The parent was given dietary advice to avoid the food until OFC.

Identification of Latent Food Sensitisation

In the case of suspected cow's milk allergy, parents were asked about egg consumption. If egg had not been safely ingested previously then SPT to egg was conducted due to high level of egg sensitisation in cow's milk allergic infants.(15) In the same way, if egg allergy was suspected and peanut had not been previously safely ingested, infant underwent SPT to peanut as 20% of egg allergic infants are sensitised to peanut.(16) If SPT for egg or peanut was negative, parents were advised to introduce the food at home. In the event of an opportunistic positive SPT then parents were advised to avoid the food in question and OFC was arranged.

ix. Skin Prick Testing (SPT)

SPT was carried according to our local hospital protocol. (Appendix) Equipment needed for SPT included, commercial extract to be tested, positive histamine control, negative saline control. Ruler, pen, lancets, sharps box, paper towel. Child was seated on parent's knee. With their parent holding the arm in question above the elbow, and using their other hand to ensure child did not interfere with testing procedure. SPT was carried out on the forearm of the child. Cooperation was ensured by prior education of parents as to what was going to occur, and by including the child in the activity. The area on the forearm to be used was first visualised to ensure no active AD. Parents were asked about the child's use of any medications in the last 48 hours that may influence the response to SPT, mainly limited in this age group to antihistamine medication. When all drops were placed on the forearm,

then the prick was made through the solution using a lancet. A separate lancet was used for each drop to avoid cross contamination of the solutions. When each drop had been pricked through, the drops were blotted off using a paper towel. Depending on the number of antigens tested for the procedure may be repeated on the other arm with the remaining solutions. 15 minutes after testing the forearms were inspected again looking for any positive reaction. Any wheal seen was measured using a ruler. Unless a histamine control response of over 3mm was seen the test was invalidated. All other positive responses were recorded.

SPT was carried out by trained staff in the Discovery Centre. Emergency equipment and medication was available should an adverse reaction to a SPT occur and staff were trained in its use.

x. Oral Food Challenge (OFC).

During the course of the study OFC was conducted:

1. To diagnose food allergy in those with a positive SPT or food specific IgE to a food to which allergy was suspected
2. To diagnose food allergy in a child with a negative SPT but with a history highly suspicious for food allergy.
3. To differentiate between sensitisation and allergy in children with positive screening SPT at 2 years but food not safely ingested
4. To assess whether food allergy diagnosed prior to 2 years was still present at 2 years if there was no history of a recent reaction.

An OFC involves the incremental ingestion of a food to confirm or reject suspicion of food allergy.(17) Allergic reactions were expected during these challenges therefore OFC were carried out under close medical supervision. These reactions can vary from minor urticaria to severe and potentially life threatening anaphylaxis. BASELINE study OFCs were carried out in the Seahorse Day Unit of the Paediatric Ward of Cork University Hospital under the guidance of either our Allergy Nurse Specialist or Clinical Research Fellow.

We conducted either open or single blind oral food challenges rather than double blind placebo controlled challenges (DBPCFC). Our study population was infants and toddlers thus the potential for subjective symptoms was lessened, limiting the need for a placebo challenge. Also DBPCFC would necessitate two OFC for each food which would place undue pressure on our resources as many children were sensitised to more than one food.

We performed open and single blind challenges on infants from 5 months to 30 months. On morning of OFC the infant or toddler arrived onto ward. A focused allergy clinical history was taken. Baseline measurements including weight, Heart Rate, Temperature, Respiratory Rate, oxygen saturations and blood pressure were assessed. This was to ensure that the child was well prior to suspect food being administered, and also served as a baseline from which to assess other vital signs should an allergic reaction occur. Foods used in challenges included milk, egg, wheat, cod, salmon, peanut, and soya, hazelnut, walnut and cashew.

Informed consent for procedure was sought from the parent. The children in our study were too young for assent to also be sought. The food to be challenged was often masked by another food so as to ensure it was ingested by the child

Foods Used in OFC

Cow's Milk

Open; for those under 1 year of age a cow's milk formula was administered. For those over 1 year regular doorstep cow's milk was used. Increments of milk to be ingested began with 1 ml, 5ml, 10ml, 20ml, 50ml.

Egg

Single blind; for straight egg challenges, pasteurised egg protein powder was used. (Source: myprotein.co.uk) A total dose of 15 grams was given over 5 divided doses, with each dose being incrementally larger than the last. (1^{st} dose – 0.5 grams, 2^{nd} dose - 1 gram, 3^{rd} dose - 2 grams, 4^{th} dose - 4 grams and Final dose - 7.5 grams). The egg powder was mixed into a yogurt or fruit pot which the child had previously eaten safely.

Baked egg

Single blind; if the child was tolerating baked egg at home safely then they were not challenged with that food. If their reaction was to baked egg or if they had never ingested baked egg then a baked egg challenge was arranged. For this parents are given a recipe to make at home. This consisted of 2 eggs, 60 grams flour, 60 grams sugar and 60 grams butter (or dairy free alternative spread if also Cow's Milk allergic). Mixture was divided into 4 "cakes" and baked in oven for 30 minutes. The total dose needed to pass food challenge was 2 whole fairy cakes equivalent to one egg. This again was carried out in 5 divided incremental doses. (1^{st} dose -1/16 of one cake, 2^{nd} dose - 1/8 one cake, 3^{rd} dose - ¼ one cake, 4^{th} dose - ½ one cake, 5^{th} dose - 1 whole cake).

Wheat:

Open; food was administered as wheat based cereal with cow's milk (or dairy free alternative). A typical child sized portion was divided into five incremental doses with each dose administered sequentially (1/32, 1/16, 1/8, ¼, ½)

Fish:

Open; both cod and salmon challenges were administered in the form of fresh cooked cod or salmon which the parent would bring with them on the day. A typical child sized portion was divided into five incremental doses with each dose administered sequentially (1/32, 1/16, 1/8, ¼, ½)

Peanut:

Open; all children on our study were less than three years therefore peanut was administered in the form of peanut butter on a cracker or slice of bread (again ensuring no cross reactivity if child was allergic to any other foods). In the case of peanut a lip rub was conducted first to assess any reaction to mucosal contact. The whole portion of peanut butter (1 tablespoon) was then divided into 5 doses and given incrementally.

Other foods challenged on the study include Hazelnut and Kiwi, walnut, cashew.

OFC is essentially medical observation of the child eating the food (or in the case of a nut, the first contact is mucosal contact on the lip). (69, 70) Once the food portion is eaten the child is monitored and after 15 minutes they are reassessed. Their vital signs are measured and if there are no obvious signs or symptoms of an allergic reaction then the next dose is given. This sequence of "dose then review" is repeated 5 times or until all portion has been eaten. Obviously if the child becomes unwell after any ingestion, or there is evidence of an allergic reaction they are assessed and vitals taken before the 15 minute interval and treatment administered as necessary.

Food challenges are stopped when specific objective criteria are met. However there are many times when the outcome of a food challenge is less clear. In these situations the decision to proceed is a balance between seeking a correct diagnosis and thus eliminating unnecessary food avoidance and protecting the child from severe reaction if offending food is further ingested.(71) Objective clinical signs we took as suggestive of positive challenge are shown in the table below.

Table 3: Objective symptoms for stopping an OFC

System	Symptom
Skin	Urticaria, unresolving after 5 minutes
	Lip swelling
Respiratory	Wheeze, cough, hoarseness
	Rhinoconjunctivitis
	Respiratory difficulty
Gastro	Large +/- recurrent vomiting
Cardiovascular	Shock / collapse

If signs or symptoms of an allergic reaction are elicited, no further food allergen was offered to child and they was closely observed to assess the severity of the reaction. For urticaria only that was not widespread and was not troublesome to the child we did not treat the child. If there was widespread urticaria and or the child was aggravated by itch or uncomfortable we gave an oral non- sedating antihistamine at the appropriate dose for weight. If there was evidence of respiratory compromise our first line of treatment was a B-agonist via nebulised device with Oxygen. Their response to treatment was assessed by a decreased in respiratory effort, decreased wheezing and increasing oxygenation saturations. If there was no improvement in symptoms then intramuscular adrenaline was administered. If evidence of cardiovascular compromise IM adrenaline was immediately administered.
The child was observed for two hours after the last time food was ingested, or longer if not fully recovered from the allergic reaction.
If the child ate all of the doses required, they also remained under close supervision until 2 hours post the last ingestion of food. If an OFC is passed then the parents are given advice on how to introduce the food in to the diet.

Deferred Challenges

At times, OFC was deferred, either indefinitely or until a later date.

1. Intercurrent illness

In our age group infectious illness is common, and usually self-limiting.(20) Children have come for OFC with history of recent illness, most commonly upper respiratory tract infection. This may include fever, cough or wheeze. In this case if vital signs are normal and child appears well OFC may proceed. However if child was actively coughing, in any respiratory distress or appeared unwell then the OFC was postponed. This ensures that the symptoms of the intercurrent illness are not mistakenly attributed to the suspected food if these symptoms re-occur or worsen during OFC.

2. Medications

Written instructions were given all parents of a child who was to undergo OFC, detailing the instructions for the procedure. This included avoiding certain medications for specified times prior to OFC. Most importantly in our paediatric population, was to avoid antihistamine use. Despite this on occasion OFCs were deferred as children had had recent antihistamines.

3. Previous severe reaction

If child had a strongly suspicious clinical history of recent severe reaction following ingestion of food, and strongly positive testing for the food then challenge was deferred and child deemed allergic. Parents were educated on food avoidance and emergency treatment of anaphylaxis.

4. Uncooperative child

The outcome of an OFC was integrally dependent on the child ingesting the suspected food allergen. Owing to the age and developmental stage of the children in our study, occasionally they did not ingest the entire portion of food required to pass the OFC. To counter act this we encouraged parents to bring foods that they knew their child had eaten to mask the food in certain cases. As most of our children were pre – verbal their ability to communicate to us if their refusal was due to subjective symptoms or simply non co-operation was limited. Therefore we used the child's demeanour and overall activity level during the challenge as a proxy for this. Throughout the study, baked egg was shown to be the suspected food antigen with which this occurred the most.

Depending on the amount of food ingested prior to refusal then we concluded the OFC has been partially passed, and asked parents to gradually introduce the food at home with some added caution. If less than half the total dose had been ingested we repeated the OFC on another day hoping for increased cooperation, or the parent could introduce the food in very small amounts at home, at a dose lower than which the child ingested at the OFC.

5. Parents refuse challenge

This study was run on the ethical basis of informed and voluntary consent. Parents were free to opt in and out of certain facets of any study as they wish. Parents refuse consent to challenge for a number of different reasons, the most common reason seen was in the case

of a positive history of objective symptoms seen after ingestion of a suspect food along with positive testing. In this case parents did not want a diagnostic test carried out as the diagnosis was highly likely that child was food allergic. At other times parents refused challenge as the child had other medical concerns that necessitated time and appointments, and parents preferred to presume allergy to and simply avoid the food in question.

Following either a positive or negative challenge parents were given discharge advice for the next 24hours. All were followed up the next day by the team by phone.
If challenge was positive then the parents were given dietary avoidance advice along with advice on the emergency management of potential allergic reactions. They were then followed up in our paediatric allergy clinic. If challenge was negative, i.e. the child ate the food safely during challenge, the child was deemed "**sensitised, not allergic**" and parents were advised to reintroduce the suspect food into the child's diet.

xi. Screening at 2 years

The primary end point for the study was challenge proven food allergy at 2 years of age. All children were screened at 2 years for food sensitisation and allergy. This was population based screening and was undertaken whether or not the child was eating the food safely or not. The foods assessed for were cow's milk, egg, wheat, cod, soya and peanut. Other allergens tested for were the common inhaled allergens of; House Dust Mite (HDM), Grass, Cat and also insect allergens; Bee and Wasp venom.
Food sensitisation was determined by skin prick test with a positive result ≥3mm diameter. Positive SPT to food antigen at the 2 year screening appointment, where there was not a clear history of safe ingestion was reported to the Clinical Research Fellow and Allergy Nurse specialist via email to arrange subsequent follow up. Routine systematic review of the database looking for positive food SPT was also carried out to ensure no potentially allergic patients were over looked.

If the child was safely eating the food in question then they were deemed "**sensitised, not food allergic"** and parent was advised to continue to allow child to ingest the food regularly. In the case of a positive SPT (≥ 3 mm) where there was no history of ingestion, if food was not eaten safely or if parent reported symptoms suggestive of food allergy following the ingestion of the food, then the child was considered **"sensitised, possibly food allergic"**. Parents were advised to avoid the food in question until OFC was arranged. If parents gave permission for serum sampling a SpIgE for the food in question was sent also including a partial recombinant profile for peanut.

Children who presented with signs and symptoms suggestive of food allergy before the age of 2 years are discussed above. Those with OFC confirmed food allergy were included in the 2 year screening SPT, and these results were relayed back to the clinical team. This afforded us the opportunity to assess whether the food allergy diagnosed before 2 year was still present or whether tolerance had been achieved. If the allergy was still suspected then a repeat food challenge after their 2 year appointment was performed, unless there was a clear history of recent symptoms following accidental exposure to the food in question or on recent OFC. Occasionally at the 2 year appointment parents reported allergic symptoms to other food allergens for their child. If this occurred prior to appointment and the symptoms described were plausibly attributed to food allergy, then relevant additional SPT was conducted on day of 2 year appointment. If the symptoms were reported during the appointment, and the food antigen of fresh food could not be sourced on the day then the child was offered a further appointment for SPT to the food to be carried out.

xii. Management of the food allergic child

The study diagnosed more than 50 food allergic children. These children continued to be longitudinally followed in the BASELINE study from which they had been originally seen. Depending on the age of the child, the interval to next BASELINE assessment and their clinical and nutritional needs, they were also seen in the Paediatric Allergy Clinic if needed. The parents were educated on the avoidance measures needed to take, and the emergency

treatment warranted should a food allergic reaction occur. Dependent on the severity of reaction the child had, some children on the study were prescribed adrenaline auto-injectors to be carried by their parent and other carers. These Auto-injectors were prescribed in two sets of two. The food allergic children on the BASELINE also had access to a paediatric dietician to ensure their nutritional needs were being met. After their 2 year appointment they were followed up exclusively in the Paediatric Allergy Clinic as their next BASELINE appointment would not be until age 5 years.

xiii. Non IgE mediated food allergy

OFC was only carried out to confirm or rule out a suspicion of IgE mediated food allergy. Suspected Non IgE mediated food allergy was also reported by parents. Non IgE mediated food allergy was typically described as worsening of AD or gastro-intestinal (GI) disturbance hours to days after ingestion of the suspected food allergen. In this event the parent was contacted by either the Clinical Research Fellow or the Allergy Nurse Specialist to conduct an allergy focused history. For children less than 6 months with reported symptoms of milk allergy all were seen on the paediatric day unit for a physical exam and full history. If symptoms and history were suggestive of non IgE mediated food allergy then parent was advised to instigate a trial of avoidance of the food in question. Regular follow up was initiated to assess response to avoidance diet.

If symptoms did not improve with avoidance diet, non IgE mediated FA was ruled out and parents were advised to reintroduce the food. If symptoms recurred then parents were advised to continue to avoid food but to have regular scheduled trial of introduction. Occasionally parents refused reintroduction of the offending food even if there was no clinical evidence of non IgE mediated food allergy. In this case we ensured the nutritional needs of the child were being otherwise met and followed up parents at later intervals to see if attempted reintroduction of the food was amenable to them. If reintroduction of the food resulted in re-emergence of symptoms then a further cycle of trial of avoidance then reintroduction was advised.

Other studies have used OFC's when there is a suspicion of non IgE mediated food allergy.(13) This was not done in our study mainly due to resource constraint issues. However with regular phone and clinic follow up we are satisfied that these infants were appropriately followed and their nutritional needs were met.

xiv. Statistics:

An online secure database was used to record and manage data entries prospectively entered at study appointments. All data was anonymously stored.
Statistical analyses were performed using SPSS (version 21.0, SPSS Corp, Chicago, IL).
The statistical methods used are described in the relevant chapters. Statistical modelling was conducted by our trial statistician, Dr Audrey Dunn Galvin.

Statistical analyses were performed using IBM SPSS Version 21. Independent samples T test were used to compare the mean TEWL values across categorical variables. Variables with a non-normal distribution were log-transformed before inclusion. Chi- square test for Independence was used to determine the association between categorical variables. Predictive modelling was used to assess contribution of different early life variables to the diagnosis of AD at 12 months and was conducted by Dr Dunn Galvin.

Changes to original Scope of Work

As noted above antenatal recruitment was slower than anticipated and additional resources were obtained from FSA to maintain maternal engagement through increased access to antenatal ultrasound and further additional nursing time to ensure babies born to SCOPE mothers were retained in BASELINE. A further major change to the SCOPE of work involved the decision to recruit babies postnatally. This was very successful but cord blood was not available for DNA sampling. A no-cost extension of 3 months was also required to allow all the additional babies to be offered a 24m appointment.

Staff illness and personnel changes in Dundee combined with protracted inter-university contractual negotiations to significantly delay completion of FLG status analysis. FLG genotyping of all available infant DNA was completed in April 2014.

Results

Recruitment

There were two modes of recruitment into the study. Originally mothers in the SCOPE study were approached at 22 weeks to enrol into the BASELINE study pending healthy live birth from July 2008. 1303 infants were recruited by this method (Stream 1). By 1st June 2010, only 452 infants had been recruited, which fell under expected recruitment. To overcome this shortfall a supplemental, parallel recruitment stream was commenced from July 2010. Prospective postnatal recruitment began on the post natal wards. 600 infants were recruited by this method (Stream 2)

Retention

1903 infants were recruited into T07060

- By 2 month appointment – 170/1903 withdrew consent or were LTFU = 8.93%
- By 6 month appointment – 110/1733 withdrew consent or were LFTU = 6.34%
- By 12 month appointment – 83/1623 withdrew consent or were LTFU = 5.05%
- By 24 month appointment – 114/1540 withdrew consent or were LTFU = 7.46%

Cumulative drop out rate = 477/1902 = 25.06%

(1355 attended at 24 months; 1 infant died prior to 24 month appointment in RTA, 59 had telephone assessment only, 11 could not attend but wished to remain in study)

Figure 5: Retention into the study from enrolment to 2 years

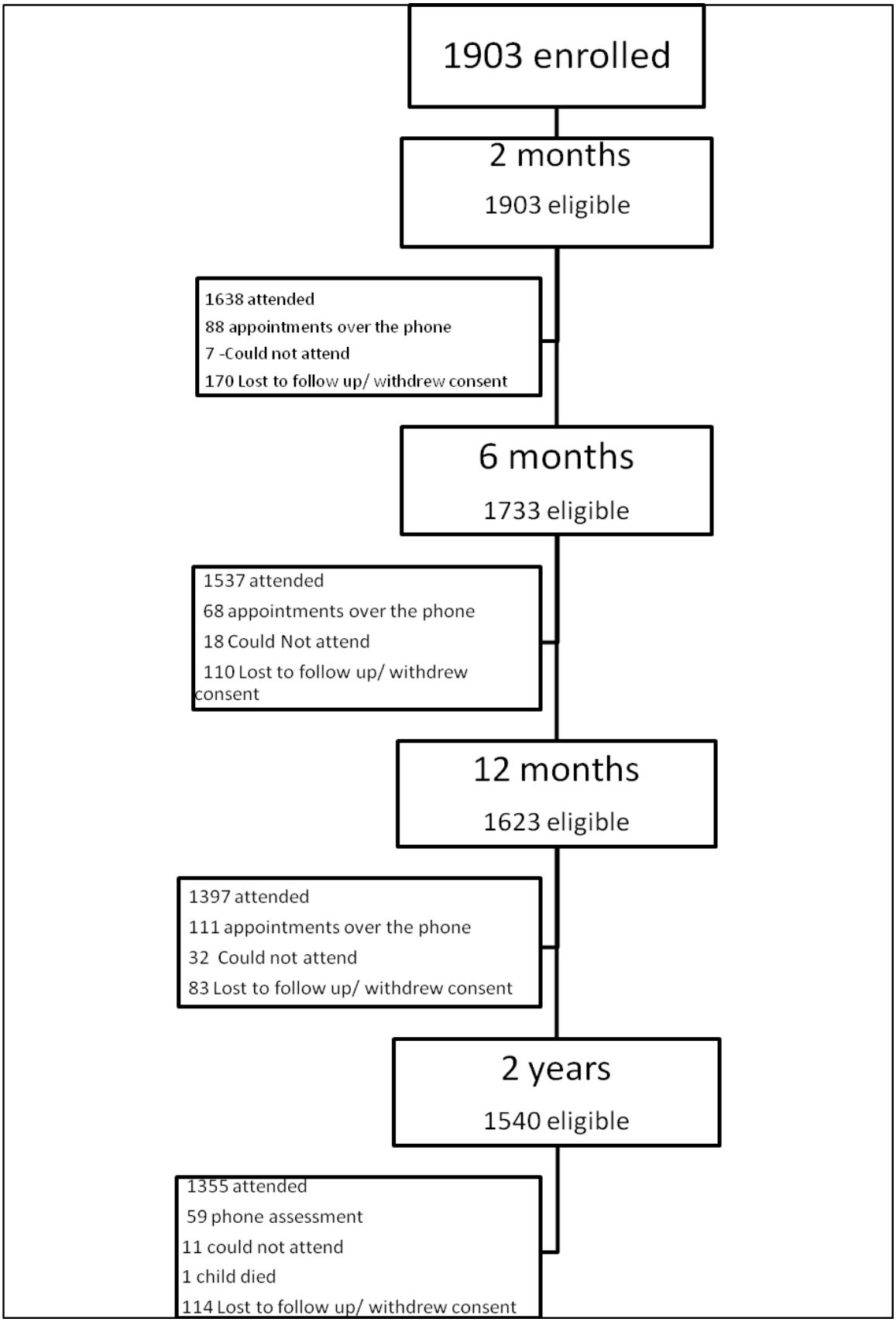


Table 3. Demographics

The baseline demographics of the recruited infants are shown below.

	N= 1903
Total enrolled	1903
Male: female %	50.4% : 49.6%
Birthweight (mean)	3489 gms (±512gms)
Gestation (mean)	279.33 days (±10.77)
Birth TEWL	7.32 $g_{water}/m^2/hr$(±3.33)
2 month TEWL	10.97 $g_{water}/m^2/hr$(±7.98)
6 month TEWL	10.71 $g_{water}/m^2/hr$(±7.10)

As per methodology all infants were offered TEWL reading shortly after birth and at 2 month and 6 month appointments. TEWL was not carried out on all infants at all appointments due to various reasons including; non calibration of machine, questionnaires over the phone or if infant was upset or uncooperative.

Table 4. Total number of infants with TEWL readings.

	Total enrolled	**Attended at 24 mth**	**SPT at 2 years**
N	1903	1355	1260
Birth TEWL	1692	1206	1119
2 Month TEWL	1614	1289	1199
6 Month TEWL	1517	1291	1199
Birth & 2 months	1437	1149	1067
Birth & 6 months	1354	1154	1070
2 & 6 months TEWL	1444	1240	1152
Full Set TEWL (Birth, 2 & 6 months)	1292	1110	**1030**

“Stream 1 infants”

1303 infants were recruited antenatally via the SCOPE study. All were first born to healthy mothers. Males: Female ratio 50.5%: 49.5%. Mean birth weight was 3.45kg (±522 grams). TEWL was measured in 84% (1094/1303) of newborn infants. Mean TEWL was 7.05 $g_{water}/m^2/hr$ (±3.35) with mean TEWL age of 46.9 hours (±38.86). The neonatal TEWL values of this group have been published previously.(12)

“Stream 2 infants”

600 infants were recruited postnatally. Male: Female ratio was 50%: 50%. Mean birth weight 3.56kg (± 482 grams). TEWL was taken in the early neonatal period in 99.5% (597/600) of infants. Mean TEWL was 7.81 $g_{water}/m^2/hr$ (±3.26) with mean TEWL age of 51.64 hours (±22.95)

Between group analysis was performed to assess for differences between the groups. Primary analysis revealed a significantly higher mean newborn TEWL reading in Stream 2 infants compared to Stream 1 with significantly increased rates of AD in Stream 2 compared to Stream 1.(see Table 3.2) At 2 and 6 months there was no significant difference between mean TEWL values.

Table 5: Differences between recruitment Streams

	Stream 1 (Antenatally Recruited)	Stream 2 (Postnatally recruited)	p value
Maternal Factors			
- Rhinitis	318/1206 (26.37%)	148/516 (28.68%)	0.32
- HDM	175/1248 (14.02%)	90/516 (17.44%)	0.123
- AD	142/1206 (11.77%)	95/516 (18.41%)	0.000**
- Food Reaction	153/1195 (12.8%)	81/505 (16.04%)	0.077
Paternal Factors			
- Rhinitis	222/1190 (18.66%)	109/510 (21.37%)	0.195
- HDM	118/1190 (9.92%)	70/510 (13.73%)	0.02*
- AD	110/1190 (10.24%)	65/510 (12.75%)	0.029*
- Food Reaction	87/1176 (7.4%)	45/503 (8.95%)	0.28
Birthweight	3.45 kg (±522 grams)	3.565 kg (±482 grams)	0.000**
Gestational Age	279.4 days (± 11.62 d)	279.24 days (±8.67 d)	0.701
Age at TEWL (in hours)	46.9 (±38.86hrs)	51.64 (±22.95hrs)	0.002**
Washed before TEWL	523/1095 (47.76%)	330/596 (55.37%)	0.003**
Newborn TEWL	7.05 (±3.35)	7.81(±3.27)	0.000**
2 month TEWL	10.98 (±7.35)	10.96 (±9.29)	0.958
6 month TEWL	10.79 (±7.2)	10.53 (±6.89)	0.498

TEWL

Newborn TEWL.

TEWL was taken in the early newborn period of 1691/1903 (88.86%) of the cohort. Mean newborn TEWL was 7.32 g $_{water}/m^2/hr$ (±3.33). (Figure 6) Univariate analysis was used to ascertain the relationship between newborn TEWL measurement and both genetic and environmental factors in early life.

Figure 6: Distribution of Newborn TEWL values

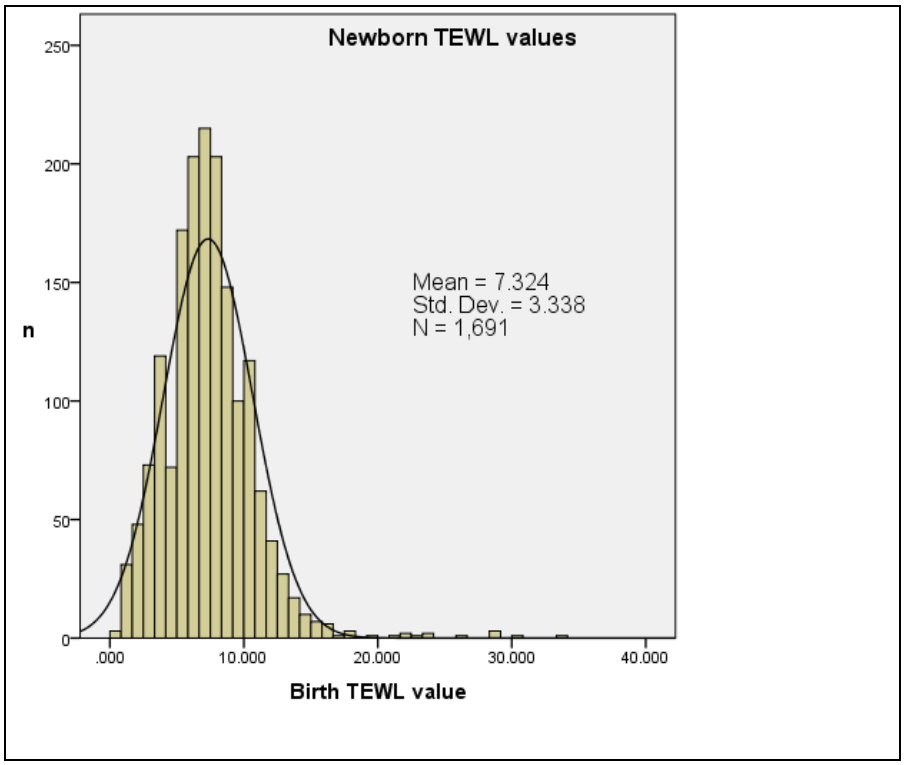


Table 6: Birth TEWL and Categorical Variables

	N	Mean Birth TEWL value	Sig
Recruitment			
- Stream 1 (Antenatal)	1094	7.05g_{water}/m^2/hr (±3.35)	
- Stream 2 (Postnatal)	597	7.81 g_{water}/m^2/hr (±3.26)	P = 0.00
Sex			
- Male	852	7.28 g_{water}/m^2/hr (±3.39)	
- Female	837	7.37 g_{water}/m^2/hr (±3.28)	p = 0.54
Gestation			
- Term (≥37/40)	1658	7.32 g_{water}/m^2/hr (±3.34)	
- Preterm (<37/40)	33	7.62 g_{water}/m^2/hr (±3.22)	p = 0.60
History of Parental Atopy			
- No parent	518	7.35 g_{water}/m^2/hr (±3.12)	
- One Parent	703	7.35 g_{water}/m^2/hr (±3.34)	Between groups
- Both Parents	253	7.30 g_{water}/m^2/hr (±3.89)	ANOVA p= 0.93
Washed Before Reading			
- Yes	852	7.21 g_{water}/m^2/hr (±3.2)	
- No	838	7.44 g_{water}/m^2/hr (±3.45)	p = 0.15

There was no significant difference in mean TEWL readings in any of absence or presence of Paternal AD, Rhinitis, FA, HDM allergy nor Maternal AD, FA, Rhinitis, HDM allergy.

Table 7. Birth TEWL and Continuous Variables

	N	Correlation
Age at measurement	1689	no correlation (r = -0.062 , sig .011)
Birthweight	1689	no correlation (r = -0.065 , sig .008)
Gestational age	1691	no correlation (r = -0.018 , sig .469)

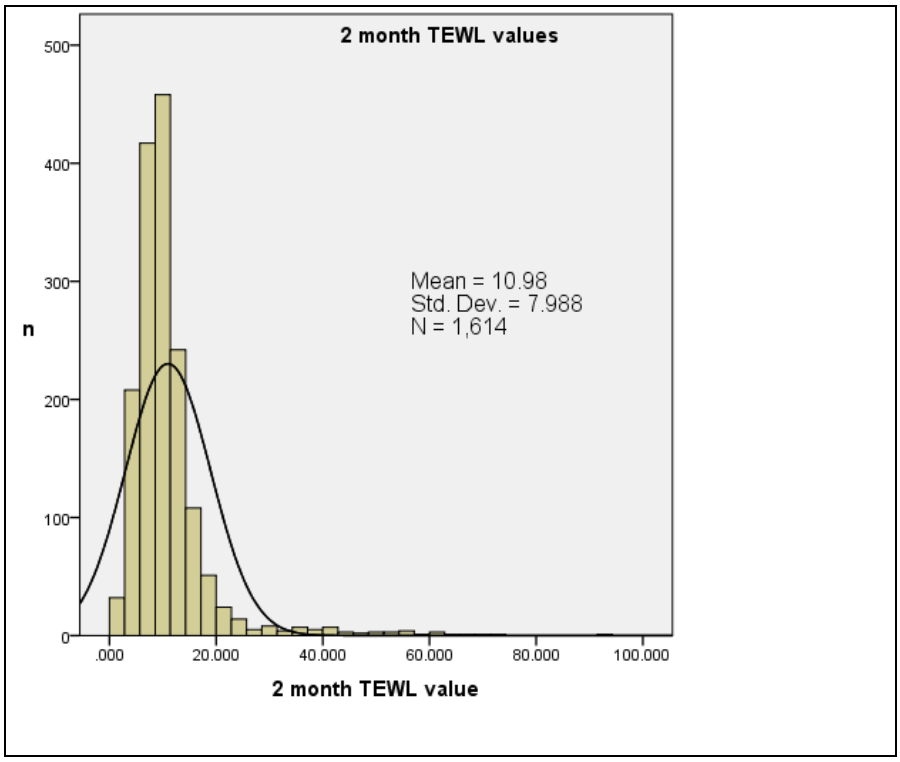
* no relationship was seen using Pearson correlation coefficient r <0.1

2 month TEWL

There was no significant difference in 2 month mean TEWL value between recruitment streams

1614/1638 infants who attended the 2 month appointment had TEWL taken (98.5%). Mean 2 month TEWL was 10.97 $g_{water}/m^2/hr$ (±7.98). (Figure 7)

Figure7: Distribution of 2 month TEWL

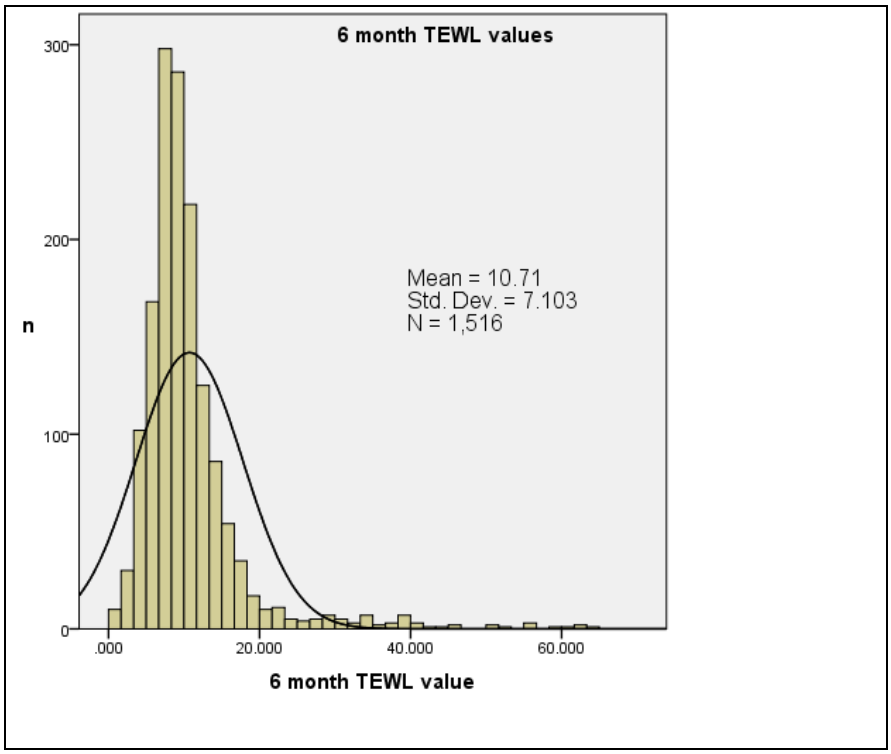


6 month TEWL

There was no significant difference in 6 month mean TEWL value between either recruitment streams.

1537 infants attended for 6 month appointment. Of those 1516 had TEWL measurement taken (98.6%). Mean 6 month TEWL 10.71 $g_{water}/m^2/hr$ (±7.10). (Figure 8)

Figure 8: Distribution of 6 month TEWL values



Atopic Dermatitis

Table 8: Overall rate of Atopic Dermatitis

	AD rate
6 months	18.7% (299/1597)
12 months	15.53% (232/1494)
2 years	15.86% (215/1355)

AD at 6 months

Of 1537 infants who attended for 6 month appointment AD screening questions were completed for 1529 infants 99.48%. Of these (292/1529) 19.1% were diagnosed AD via UK working party diagnostic criteria. 98% had a SCORAD completed. Mean score was 21.54 ±16.29 (Range 0-88). 68 infants had telephone questionnaires only, 10.29% (7/68) were diagnosed with AD, these infants did not have SCORAD completed.

AD at 12 months

Of 1397 infants who attended for 12 months appointment AD screening questions were again completed for 99% of infants (1384/1397). Of these 16.1% (223/1384) were diagnosed AD via UK working party diagnostic criteria. 98% had a SCORAD completed. Mean score was 18.56 ± 14.92 (Range 0-77). Of infants who had telephone interviews only 8.9% (9/110) had AD diagnosed. SCORADs were not completed on these infants.

AD at 2 years

Of 1355 infants who attended for 2 years appointment AD screening questions were completed for all. Of these 15.9% (215/1355) were diagnosed AD via UK working party diagnostic criteria. 95.8% had a SCORAD completed. Mean score was 19.01 ± 15.58 (Range 0-86).

SCORAD was grouped into mild <15, moderate 15 – 39, and severe ≥40. The distribution between the three severity groups remained relatively constant across the three timepoints.

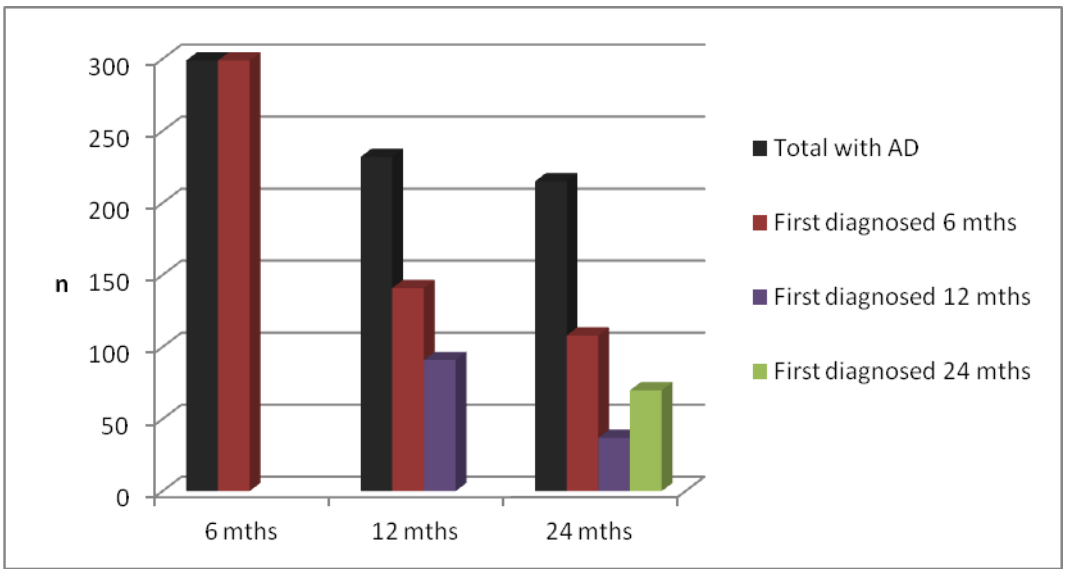
Table 9: SCORAD grouping of infants diagnosed with AD

	6 months	12 months	2 years
Mild (<15)	122/287	106/211	94/204
	42.5%	*50.2%*	*46.1%*
Mod (15-39)	127/287	84/211	90/204
	44.3%	*39.8%*	*44.1%*
Severe (≥40)	38/287	21/211	20/204
	13.2%	*10%*	*9.8%*
Total	287	211	204

The point prevalence of AD at 6 months was 18.7%, decreasing slightly to 15.86% by 2 years. At 2 years, of 215 infants with AD, 50% (108/215) were initially diagnosed at 6 months, 17.2% (37/215) had been initially diagnosed at 12 months, and 32.55% (70/215) newly presented at 2 years.

Of 299 infants diagnosed with AD at 6 months, 43% (109/251) had persistent AD at 2 years. (48 LTFU or WC). (Figure 9)

Figure 9: AD over first 2 years of life



Filaggrin

FLG genotyping was conducted on 1300 infants for whom DNA was available. The cumulative *FLG* mut rate was 10.46% (136/1300). 4 infants were homozygous for *FLG* mutation, with the remainder heterozygous for *FLG* mutation.

Table 10: *FLG* mutation rates

	Heterozygous Rate	**Homozygous Rate**	**Total**
GenR501X	4.0% (52/1300)	0.23% (3/1300)	4.23% (55/1300)
Gen2282Del4	3.62% (47/1300)	0	3.6% (47/1300)
GenS3247X	1.54% (20/1300)	0.08(1/1300)	1.62% (21/1300)
GenR2447X	1% (13/1300)	0	1% (13/1300)
***FLG* mut (total)**	10.15% (132/1300)	0.31% (4/1300)	10.46%(136/1300)

FLG and TEWL

At birth there was a no significant difference seen between *FLG* wt and *FLG* mut groups. However at 2 months a significant difference was seen between infants with *FLG* mutation compared to *FLG* wt. This difference is seen again at 6 months.

Table 11: Relationship between *FLG* status and mean TEWL values

	***FLG* wt**	***FLG* mut**	**p value**
N	*1164*	*136*	
Birth	7.3 (±3.38) $g/_{water}/m^2$	7.33 (±3.62) $g/_{water}/m^2$	0.91
2 months	10.7 (±7.7) $g/_{water}/m^2$	12.6 (±10.1)$g/_{water}/m^2$	0.04*
6 months	10.42 (±7.1) $g/_{water}/m^2$	12.25 (±6.53) $g/_{water}/m^2$	0.007
Δ Birth – 2 month	3.32 (± 8.1) $g/_{water}/m^2$	5.44 (±10.66) $g/_{water}/m^2$	0.046*
Δ Birth – 6 months	2.93 (±7.55) $g/_{water}/m^2$	4.62 (±7.11) $g/_{water}/m^2$	0.03*
Δ 2 – 6 months	-.35 (±10.11) $g/_{water}/m^2$	-.75 (±9.95) $g/_{water}/m^2$	0.685

FLG and Atopic Dermatitis

FLG mutation was associated with a significantly increased incidence of AD at each time point of 6 and 12 months and 2 years.

Table 12. Relationship between *FLG* status and AD

	***FLG* wt**	***FLG* mut**	**p value**
N	*1164*	*136*	
AD 6 months	183/1016(18%)	43/120 (35.8%)	p .000, phi .13
AD 12 months	131/939 (14%)	39/115 (33.9%)	p .000, phi .17
AD 2 years	137/938 (14.6%)	35/109 (32.1%)	p .000, phi .14

TEWL and AD

Table 13: Relationship between mean TEWL and AD at 12 months

	No AD	**AD**	**p value**
N	*1262*	*232*	
Birth	7.43 (±3.36) $g/_{water}/m^2$	7.17 (±3.34) $g/_{water}/m^2$	0.29
2 month	10.78 (±7.97) $g/_{water}/m^2$	12.00 (±8.43)$g/_{water}/m^2$	0.036*
6 month	10.27 (±6.78) $g/_{water}/m^2$	12.73(±7.86) $g/_{water}/m^2$	0.00*
Δ Birth – 2 months	3.31 (±8.49) $g/_{water}/m^2$	4.78 (±8.78) $g/_{water}/m^2$	0.024
Δ Birth – 6 months	2.71 (±7.3) $g/_{water}/m^2$	5.48 (±8.63) $g/_{water}/m^2$	0.00*
Δ 2 – 6 months	-.62 (±9.97) $g/_{water}/m^2$	0.51(±9.92) $g/_{water}/m^2$	0.128

*p <0.05

Table 14: Relationship between mean TEWL and AD at 2 years

	No AD	AD	p value
N	*1140*	*215*	
Birth	7.35(±3.30) $g/_{water}/m^2$	7.36 (±3.33) $g/_{water}/m^2$	0.97
2 month	10.86 (±8.15) $g/_{water}/m^2$	12.11(±8.52)$g/_{water}/m^2$	0.05
6 month	10.25 (±6.56) $g/_{water}/m^2$	12.76 (±8.84) $g/_{water}/m^2$	0.00*
Δ Birth – 2 months	3.50(±8.55) $g/_{water}/m^2$	4.69 (±8.87) $g/_{water}/m^2$	0.08
Δ Birth – 6 months	2.82 (±7.13) $g/_{water}/m^2$	5.14 (±9.2) $g/_{water}/m^2$	0.00*
Δ 2 – 6 months	-.65(±9.9) $g/_{water}/m^2$	.54 (±10.63) $g/_{water}/m^2$	0.13

*p<0.05

As seen from the above data, there was a direct association in univariate analysis between TEWL at 2 months, and AD at 12 months. As discussed previously AD was not screened for at 2 months in our cohort but parental report of "itchy rash" was ascertained. Therefore to ensure the signal for raised TEWL at 2 months was not just a proxy for active inflamed skin, we conducted logistic regression analysis to determine the factors at 2 months that would influence the diagnosis of AD at 12 months. The effect of *FLG* on this influence was also sought after.

"Itchy rash" at 2 months

At 2 months, 9.46% (161/1701) infants had a parental report of an "itchy rash". There was no significant difference in TEWL values at birth between "Itchy rash" at 2 months and "no itchy rash" at 2 months. Those reporting an itchy rash at 2 months did unsurprisingly, have a higher mean TEWL at 2 months that those not reporting an itchy rash.

Table 15. Relationship between "itchy rash at 2 months" and TEWL

	"No itchy rash at 2 months"	"Itchy rash at 2 months "	p value
N	*1140*	*215*	
Birth	7.37 (±3.42) $g/_{water}/m^2$	7.29 (±2.68) $g/_{water}/m^2$	0.67
2 month	10.72 (±7.9) $g/_{water}/m^2$	13.52 (±8.5)$g/_{water}/m^2$	0.00*
6 month	10.48 (±6.75) $g/_{water}/m^2$	12.93 (±9.7) $g/_{water}/m^2$	0.00*
Δ Birth – 2 months	3.34(±8.41) $g/_{water}/m^2$	6.33 (±9.43) $g/_{water}/m^2$	0.00
Δ Birth – 6 months	2.94 (±7.28) $g/_{water}/m^2$	5.87 (±10.67) $g/_{water}/m^2$	0.00*
Δ 2 – 6 months	-.33 (±9.86) $g/_{water}/m^2$	-.95 (±11.47) $g/_{water}/m^2$	0.54

Of infants with a parental report of “Itchy rash” at 2 months, 44.5% (65/1460) had AD diagnosed at 6 months; 33.09 % (46/139) had AD diagnosed at 12 months and 36.07% (44/122) had AD diagnosed at 2 years. To control for the presence of an itchy rash, without excluding the more than 55% of infants who did not go on to develop AD, logistic regression was used to estimate the factors at 2 months associated with the development of AD.

Logistic Regression Analysis

To make the TEWL values more applicable to a real life scenario they were divided into percentiles; 25th 50th and 75th respectively. ROC curves for accuracy of prediction were used.

We then used a logistic regression model to estimate the factors at 2 months that influence the diagnosis of Atopic Dermatitis (AD) at 12 months. The dependent variable which measures diagnosis at 12 months is AD 'Yes' and is equal to 1, with AD 'No' = 0.

Table 16. Odds Ratio, Chi-Square model fit, and Receiver Operating Curve (AUC) statistics for 3 models.

Logistic Regression Results Outcome Variable : Atopic Dermatitis at 12 months (Yes).			
Predictor Variable at 2 months.	Model 1	Model 2	Model 3
	Odds Ratio (Sig)	Odds Ratio (Sig)	Odds Ratio (Sig)
Parental Atopy (none) Parental Atopy; one (Yes) Parental Atopy; both (Yes)	- 1.4 (0.6) 2.7 (0.01)	- 1.7 (0.2) 7.3 (0.01)	- 1.6 (0.1) 5.8 (0.01)
FLG (No) *FLG* (Yes)	- 3.1 (0.02)	- -	- 1.7 (0.42)
Feed Type (Breast) Feed Type (Both) Feed Type (Formula)	- 0.8 (0.6) 1.1 (0.7)	- 1.7 (0.3) 2.7 (0.2)	- 3.0 (0.14) 4.9 (0.11)
TEWL Percentiles 25th (7.0) 50th (9.4) 75th(12.3)	- - -	- 1.1 (0.9) 5.4 (0.001)	- 1.04 (0.9) 7.60 (0.01)
Model Chi Square Statistic for overall model fit.	32.63 (0.04)	23.78 (0.005)	21.35 (0.01)
Area under the Receiver Operating Curve (AUC)	0.66 (0.01)	0.81 (0.01)	0.83(0.01)

Controlling for 'use of emollient prior to test reading'; 'itchy rash'; 'infant sex'; 'infant birth weight'

All 3 models were significant in terms of the Chi Square Statistic for model fit. Model 1 includes Parental Atopy, *FLG*, and Feed Type as independent variables but does not include TEWL. Parental Atopy and *FLG* significantly increases the likelihood of a positive diagnosis at 12 months by 2.7 and 3.0 times respectively, compared to infants without an atopic parent, and without an *FLG* genotype. The Receiver Operating Curve (ROC), which plots true positives (sensitivity) vs false positives (1-specificity) to evaluate the diagnostic performance of a test, is 0.66 for this model. Model 2 includes TEWL test reading scores at the 25^{th}, 50^{th} and 75^{th} percentiles (7.0; 9.4; 12.3, respectively) as an independent variable. *FLG* does not appear in Model 2. The AUC improves from 0.66 to 0.81 in this model. Infants with a TEWL reading of 12.3, or above, are 5.4 times more likely to be diagnosed with AD at 12 months than infants with a reading below this point, controlling for all other variables in the model. Finally, Model 3 includes all independent variables. The AUC improves slightly to 0.83, although it is important to note here that Model 2 demonstrates that *FLG* need not be measured in order to produce a prognosis with high accuracy.

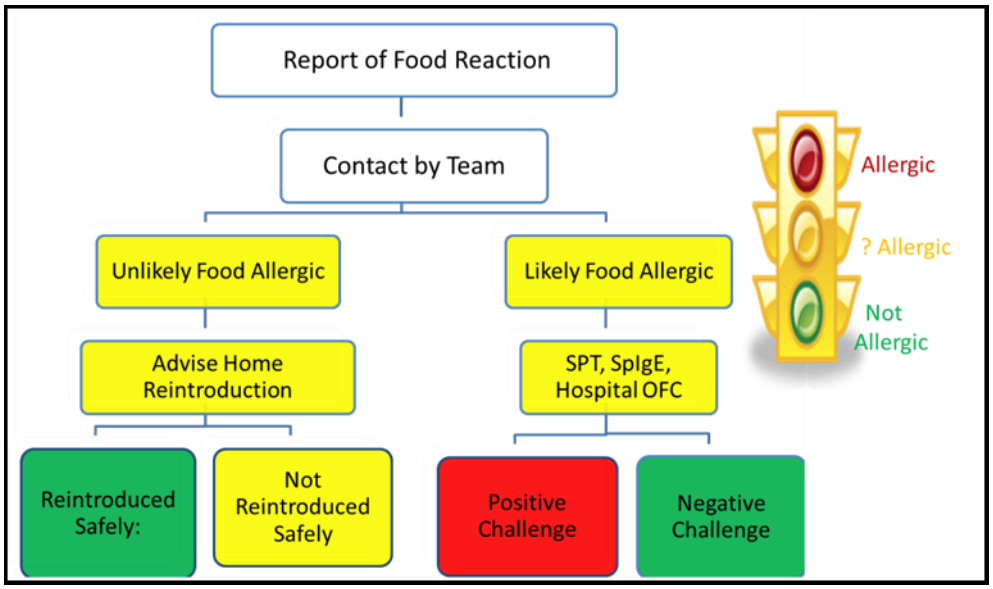
This thus shows that TEWL at 2 months is an accurate predictor of likelihood of AD at 12 months, even in the absence of *FLG* status being assessed. This has obvious real world implications for clinicians, particularly those who do not have access to *FLG* genotyping as a non invasive measurement of risk of AD development in early childhood.

Food allergy by 12 months

At 12 months 1540 infants were still participating in the study. 13.4% (207/1540) of participants reported a suspected adverse reaction to a food by 12 months. 96.1% (199/207) of queries were successfully followed up. The most commonly implicated food was Dairy 43.5% (90/207), followed by Eggs 23.7% (49/207) and Fruit 16.45 (34/207). Other foods reported included typical weaning foods; Cereal, Wheat, Meat and Fish. Less typical food ingested in this age group but implicated in suspected food allergic reactions included; Peanut, Tree Nut, Spices, Potato Crisps and Caffeine.

Adverse reactions to food were reported from a few weeks to 14 months (some infants presented later for 12 month appointment) with a mean age of 6.95 months (±3.07 months). The food queries were followed up as per Figure 10

Figure 10: Diagnostic flow of suspected food allergy queries.



Unlikely Allergic:

145 queries deemed unlikely due to food allergy. Of these 115 children safely tolerated the reintroduction of the food at home following initial consultation. In 16 cases the food was not safely reintroduced at home and following further consultation an exclusion diet deemed appropriate and initiated with team follow up. In 14 cases, parents had declined to introduce the implicated food at 12 months.

In 8 cases the infant was deemed unlikely to be allergic to the first food reported, however following an allergy focused history and appropriate investigation positive SPT was attained to a separate food. Infant then underwent OFC to differentiate between Food Allergy and asymptomatic sensitisation to that food.

Likely Allergic

54 cases were deemed likely causative. Infants had SPT and SpIgE to food implicated, and had OFC scheduled. Together with the 8 positive SPT from above, 62 children were suitable for OFC.

Table 17. First food investigated

	First Food Investigated	**Positive Challenge**	**Negative Challenge**	**Deferred**
N	**62**	**48**	**9**	**5**
Milk	22	18	3	1
Egg	37	27	6	4
Wheat	1	1	0	0
Peanut	1	1	0	0
Tree nut	1	1	0	0

Therefore 48 children had initial positive OFC, 9 had negative OFC and for 5 children parents refused OFC. This gave an 84% positive challenge rate of initially suspected food. (48/57)

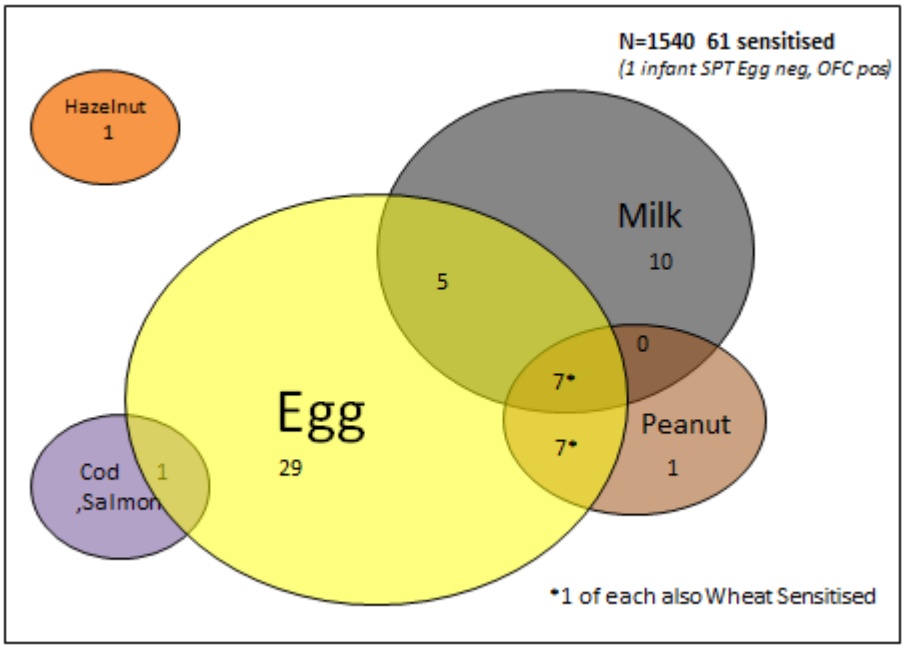
In the 5 deferred challenges, all had positive testing and documented history of symptoms on exposure to food, one to cow's milk and 4 to egg.

As stated in methods all infants with suspected cow's milk allergy were screened for egg sensitisation if not safely eating egg. Of 22 cow's milk sensitised infants, 10 infants were sensitised to milk only. 54.54% (12/22) were sensitised to egg. Of these 12, 7 were also Peanut sensitised, with 1 of these infants also wheat sensitised.

All Egg sensitised children had OFC to differentiate between egg allergy and asymptomatic Egg sensitisation. This was done as soon as practical after cow's milk OFC to limit the delay in introduction of egg into the diet.

In the same manner infants with suspected egg allergy were screened for peanut sensitisation with 18.9% (7/37) sensitised. One of these infants was also wheat sensitised.

Figure 11: Food Sensitisation "Likely Allergic" Group at 1 year.



For logistical reasons and as the study was an observational cohort study, OFC to differentiate between peanut sensitisation and peanut allergy was typically carried out after first year. Food allergy at 1 year is shown in Figure 11

Table 18: All foods investigated in first year.

	Positive OFC	Deferred (Presumed Allergic)	Negative OFC	Cumulative Incidence food allergy at 1 year.
Total Infants (some infants with > 1 OFC)	**49**	**5**	**12**	**54/1540 = 3.51%**
Milk	18	1	3	19/1540 = 1.23%
Egg	38	4	8	42/1540 = 2.72%
Wheat	1	0	1	1/1540 = 0.06%
Cod	1	0	0	1/1540 = 0.06%
Peanut	1	0	0	1/1540 = 0.06%
Tree nut	1	0	0	1/1540 = 0.06%
Salmon	1	0	0	1/1540 = 0.06%

At 12 months 76% (32/42) of infants with egg allergy tolerated Egg in baked form.

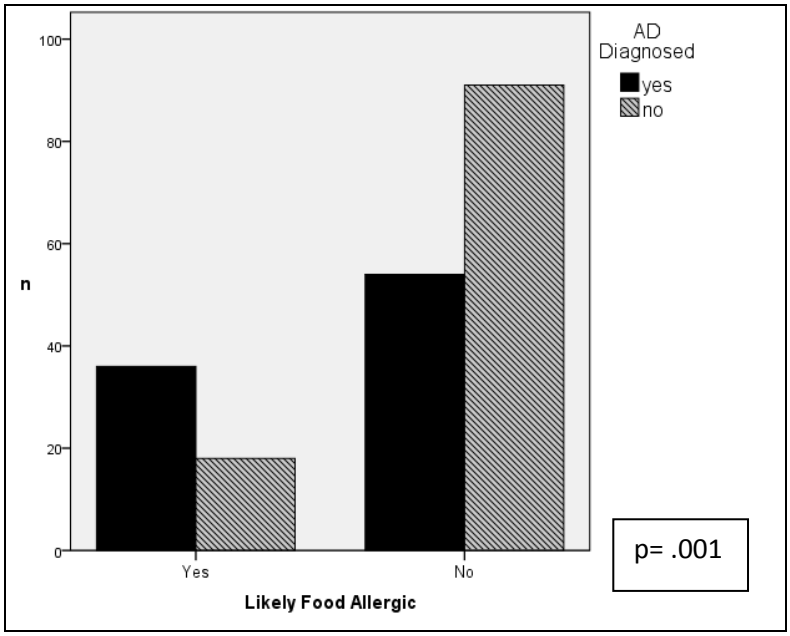
This gave a cumulative incidence of Food Allergy at 12 months of 3.51% (95% CI 2.59 - 4.43%).

Differences between" Likely Food Allergic" and "Unlikely Food Allergic" groups at 1 year

Food queries in "Likely Food Allergic" group consisted mainly of egg and cow's milk with one report each of initial suspected food allergic reaction to cod, wheat, peanut and treenut. The range of implicated foods in the "Unlikely Food Allergic" group varied more widely.

Atopic Dermatitis was diagnosed using UK Working Party Criteria. Presence of Atopic Dermatitis was significantly higher in the "Likely Food Allergic" group 66.7% (36/54) versus "Unlikely Food Allergic" group 37.2% (48/130) p= .001

Figure 12. Differences between "Likely Food Allergic" and "Unlikely Food Allergic" group.



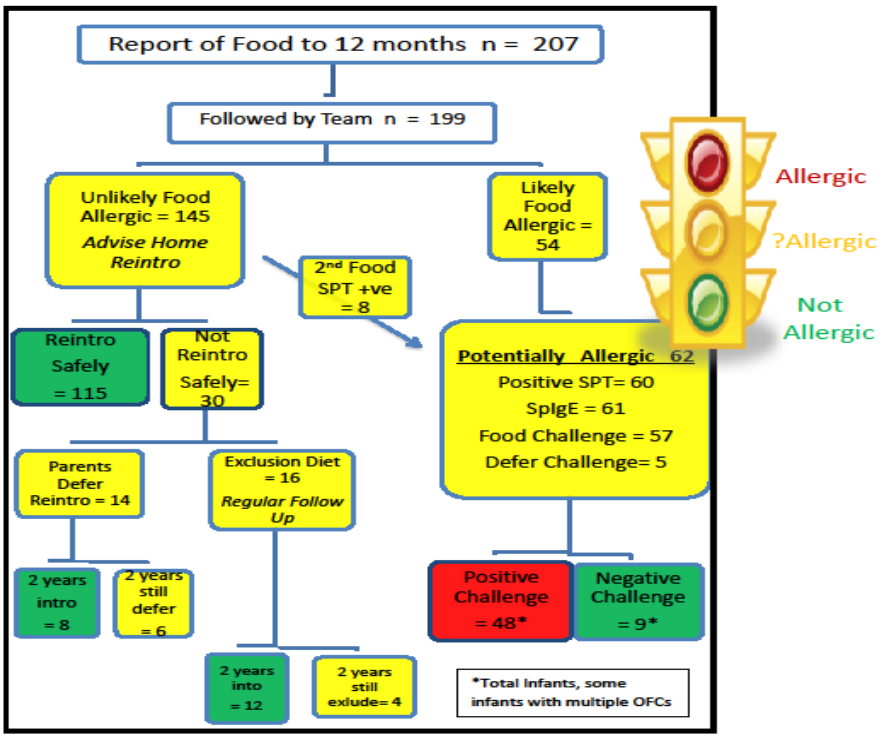
Although proportionately more boys than girls reported an adverse food reaction, 88 girls versus 119 boys; there was no significant difference in sex between the groups. 59.3% of the "Likely Food Allergic" group was male and 58.6% of the "Unlikely Food Allergic" was.

There was a no significant difference between the mean age in months at presentation of the "Likely Food Allergic" at 7.65 months (±2.41 months) versus mean age of "Unlikely Food Allergic" 6.68 months (±3.41months). (Figure 12)

Trial avoidance and reintroduction for non IgE mediated reactions

At 12 months, 16 infants reported food adverse reaction to dairy, which on allergy focused history and exam was deemed suspicious for non – IgE mediated food allergy. These 16 infants were advised to trial elimination diet for period up to 3 months, with regular trial reintroduction. By 2 years 12 of 16 infants had reintroduced the food successfully. Of the infants who did not reintroduce food successfully, two of these infants were being followed up by the Paediatric services for suspected Lactose Intolerance, the other two continue to have symptoms of suspected Non – IgE mediated Cow's Milk Allergy.

Figure 13. Final diagnostic flow for suspected food queries to 12 months



Screening for Food Allergy and Food Sensitisation at 2 years.

92.98% of children who attended 2 year appointment had SPT performed (1260/1355). 1253 had SPT to food panel, inhaled allergens, bee and wasp sting completed in full. 27 children had incomplete SPT's. This was due to either child being uncooperative during testing, or active AD that did not leave sufficient unaffected skin on forearms to accommodate the number of allergens to be tested. 7 of the 27 incomplete SPT were included in the dataset as they had positive and negative controls, and had all three main food allergens of Cow's Milk, Egg and Peanut completed. 22 parents refused consent for SPT and in 53 cases SPT was unable to be performed. Reasons for this were, recent ingestion of antihistamine medication, or child was not cooperative with testing procedure.

79 of 1260 infants had a positive skin prick test to a food. The point prevalence of sensitisation to any food at 2 years was 6.27% (95% CI 4.93 – 7.61%). The most prevalent food sensitisations at 2 years were, Egg 3.89%, Peanut 2.62% and Cow's Milk 0.95%.

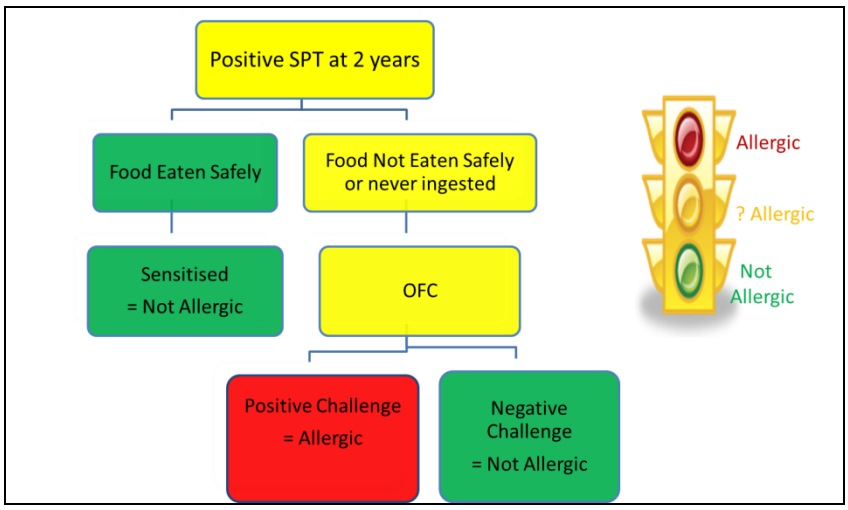
Table 19: Sensitisation rates to Food Panel Allergens at 2 years.

	Sensitised (SPT ≥ 3mm)
Total	6.19% (78/1260)
Egg	3.89% (49/1260)
Peanut	2.62% (33/1260)
Milk	0.95% (12/1260)
Cod	0.64% (8/1257)*
Wheat	0.56% (7/1259)**
Soya	0.16% (2/1260)

**3 infants did not have SPT to Cod ** 1 infant did not have SPT to wheat*

After all positive SPTs, a structured pathway of investigation was undertaken to differentiate food allergy from asymptomatic food sensitisation

Figure 14. Pathway for investigation of positive SPT at 2 years



Of 79 positive SPT, 39 children (48.7%) had been confirmed Food Allergic prior to the 2 year appointment. Of the 40 children who were not previously diagnosed Food Allergic prior to 2 year screen 22/40 (55%) were food sensitised only, 1 declined follow up and 1 declined OFC despite numerous attempts at follow up. Therefore 16/40 (37.5%) were new cases of Food allergy.

One infant sensitised at 12 month to Egg only, had negative OFC, was still sensitised to Egg at 2 years and was eating all forms of egg safely. Therefore total 23 infants were sensitised only

Figure 15: Investigation of positive SPT at 2 years.

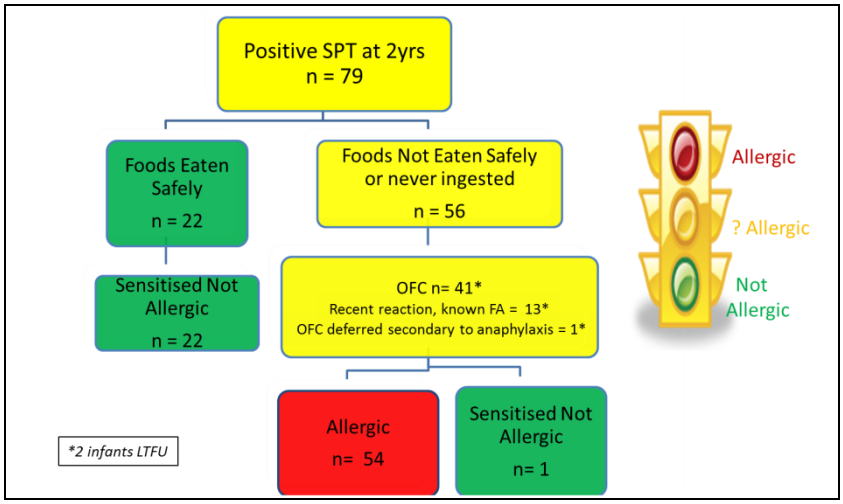


Table 20. Sensitisation to non-panel foods

Infant	Food	Allergic or Sensitised	Allergy to panel foods
1.	Hazelnut 7 mm	OFC positive	No
2.	Hazelnut 4 mm	OFC positive (at 22 mths)	No
3.	Almond 3mm	OFC negative	Peanut allergic
4.	Chickpea 10mm Sesame 11mm	Anaphylaxis to hummus	Peanut SPT 7mm, SpIgE 31.2 ara H2 45.5 - OFC refused
5.	Walnut 5mm	Refused OFC	Milk allergic
6.	Cashew 4mm	OFC positive	Straight Egg allergic

Cow's Milk Sensitisation and Allergy

Screening at 2 years

At 2 years 12 infants had positive SPT to Cow's Milk extract giving a sensitisation rate of 0.95% (12/1260). Two of these infants consumed milk safely with the other 10 Cow's Milk Allergic. Two further infants were Cow's Milk allergic at 2 years; 1 infant had negative SPT to Cow's Milk extract but positive SPT to Doorstep Milk, 1 with negative SPT to both Cow's milk extract and Doorstep milk. Both were previously diagnosed with Cow's Milk allergy, had positive SpIgE to Cow's milk and had clinical reactivity reported within 3 months of 2 year appointment. The Cow's Milk allergy rate for 2 years is therefore 0.95% (12/1260)

Resolution of Cows Milk Allergy

Seven other infants previously diagnosed with Cow's Milk allergy in the first year of life were safely consuming Cow's milk in their diet by their 2 year visit. 4 of these infants were sensitised to Cow's Milk only and were therefore discharged from the Allergy Services.

Three of these infants were still allergic to other foods despite acquiring tolerance to Cow's milk.

Cow's milk allergy rate at 1 year was 1.23% (19/1540). Resolution was seen by 2 years in 36.8% of cases (7/19).

Egg Sensitisation and Allergy

Screening at 2 years

At 2 years Egg sensitisation rate was 3.71% with 49 infants having positive SPT to Egg. Of these infants 15 infants tolerated all forms of Egg safely in their diet. 2 were allergic to all forms of Egg, whereas 31 infants were allergic to straight egg only. The remaining infant is known to tolerate baked egg but despite numerous attempts at contact and follow up it is unknown whether straight Egg is tolerated.

4 further infants had SPT <3mm to Egg at 2 years yet were still egg allergic. Three had OFC proven Egg allergy prior to 2 year screening, could tolerate baked Egg but had clinical reactivity to straight egg within 3 months of 2 year appointment. 1 had no recent history of ingestion but OFC was positive at 2 years.

Therefore total Egg allergy rate at 2 years is 2.94% (37/1259)

There was no statistical difference in SPT size between those Egg sensitised but not allergic versus those sensitised and Egg Allergic, 3.6mm (±0.63mm) versus 4.13mm (±1.23mm)

Resolution of Egg allergy

42 infants were diagnosed with Egg allergy prior to 2 year screening. They were diagnosed via interval food queries. Of these 42 infants, 38 had OFC proven food allergy, with the other 4 having positive SPT and positive clinical histories, but parents refused OFC. Egg allergy rate at 1 year was 2.72% (42/1540) with 78.04% of these infants (32/42) tolerating Baked Egg.

By 2 year visit, of these 42 Egg allergic infants; 24 were still Egg allergic (only 2 not tolerating baked egg) and 10 had fully resolved.

8 further infants have various stages of resolution. 3 infants where parents refused OFC at one year were still reacting to Egg at 2 years, one of these had positive SPT at 2 years but refused OFC again, 2 further infants refused SPT at 2 years. 5 infants were lost to follow up or moved country. All of these infants were known to tolerate at least baked egg.

Of these 42 infants 95% tolerated baked Egg at 2 years. Complete known resolution of Egg allergy occurred in 29.4% (10/34) infants.

Egg and peanut sensitisation

All infants with egg allergy were screened for peanut sensitisation. At the 2 year visit screening included Egg and Peanut as part of the food panel.

Of 42 infants diagnosed Egg allergic prior to 2 years, all were screened for peanut co-sensitisation. Of these 42 infants, 26.1% (11/42) were sensitised to peanut and 21% (9/42) were confirmed peanut allergic on OFC.

One infant had negative SPT to peanut at 1 year. Parents were advised to introduce peanut into diet regularly. This happened once without reaction, but was not continued. At 2 years SPT to peanut was 5mm to peanut and infant subsequently had OFC confirmed food allergy.

Peanut Sensitisation and Allergy

At 2 year screening 33 children had positive SPT for peanut. Sensitisation rate for peanut 2.62% (1260)

10 infants were peanut sensitised but not peanut allergic. Of these 2 had been screened due to egg allergy in first year, tolerated peanut then and continued to regularly tolerate peanut. 2 had negative SPT to peanut at 12 months, were safely tolerating peanut regularly in diet since and had SPT of 3mm and 4mm respectively to peanut at 2 year screening. 6 others

were diagnosed at screening; 5 had previously safely ingested peanut, 1 had not previously ingested peanut but underwent an OFC which was negative.

One infant who had peanut SPT 4mm and never ingested peanut was deemed lost to follow up. This infant was offered multiple appointments over a 12 month period and did not attend. They would have been advised at 2 year appointment to avoid peanut until OFC was arranged.

Peanut Allergy

22 infants are peanut allergic. Peanut allergy rate 1.75% (22/1260)

13 infants presented before 2 years. 11 of these presented with interval suspected food allergy to other foods before 12 months and underwent OFC to peanut due to positive testing. These OFC's were mainly carried out after the first year of life. Two infants presented to study between 12 month and 2 year appointment with suspected adverse reaction to peanut.

9 infants were diagnosed for first time at 2 year screening, of these only two had had a history of reacting to peanuts. The remaining 7 had never ingested peanut. One of these infants had OFC deferred as they experienced anaphylaxis to chickpea shortly before 2 year visit. Peanut had never been ingested but they were categorised allergic (SPT 7mm, SpIgE Peanut 31.2, ara h2 45.5).

There was a statistically significant difference between mean SPT for those sensitised but tolerant to peanut 3.44mm (±1mm) versus those allergic, mean SPT 7.32mm (±2.8mm) p=<0.001

Of note one infant presented at 12 months with suspected adverse reaction to peanut. SPT was 4mm with SpIgE 1.1. He underwent OFC at 14 months to peanut which was positive with urticaria on 2nd dose. At 2 year appointment Peanut SPT was negative, repeat OFC was conducted which was also negative and peanut was safely reintroduced into the diet.

Wheat Allergy and Sensitisation

At 2 year screening 7 infants had positive SPT to Wheat. (range 3-6mm, mean 4.57 ± 0.97mm) Wheat sensitisation rate at 2 years was 0.55% (7/1259)

There was no wheat allergy at 2 years

All 7 infants tolerated wheat safely in diet. 4 were mono-sensitised to wheat. 2 had comorbid food allergy. One infant initially presented with suspected wheat allergy at 6 months. Wheat SPT 5mm, SpIgE 17.3 and OFC was positive with wheeze and urticaria at 5th dose. At 14 months SPT 5mm SpIgE 3.4 , repeat OFC was negative.

Cod Allergy and Sensitisation

At 2 year screening 7 infants had positive SPT to cod. (range 3-7mm, mean 4.4, ±1.63mm)

Cod Sensitisation rate at 2 years 0.56% (7/1257)

4 infants were previously safely tolerating cod in their diet. One infant had negative OFC and 2 infants had positive OFC. Cod allergy rate at 2 years 0.16%(2/1260)

<u>Resolution of Cod Allergy</u>

One infant was diagnosed with cod allergy prior to 2 years. SPT 3mm, positive OFC. SPT negative at 2 years, safely tolerated Cod in diet.

Soya Allergy and Sensitisation

At 2 year screening 2 infants had positive SPT to Soya. (3mm and 6mm).

Soya sensitisation rate at 2 years 0.16%.(2/1260). Both infants tolerated soya and soya containing products safely in their diet.

There was no Soya allergy at 2 years.

Other foods

SPT to other foods was offered if there was a history of suspected food allergy related symptoms. Due to the increased risk of Treenut sensitisation in Peanut allergic individuals those infants with confirmed peanut allergy had SPT to any tree nut they had not previously ingested safely.(22, 23) This was carried out in the allergy clinic at their next appointment following positive peanut OFC. Those results are not included as part of the 2 year screening.

Hazelnut

One infant presented at 2 year appointment with symptoms suspicious for hazelnut. Hazelnut SPT was 7mm and OFC was positive.

One infant had presented prior to 2 years with food allergy related symptoms following ingestion of chocolate bar containing Hazelnut and Almond. He was sensitised to both, Almond OFC was negative but Hazelnut OFC was positive at 22 months. OFC was not repeated as was positive at 2 year appointment and SPT remained 4mm.

Cashew

One infant, with known egg allergy, presented at 2 years with symptoms suggestive of Cashew allergy. Cashew SPT was 5mm, OFC was positive.

Chickpea & Sesame

One infant presented with anaphylaxis to our ED department prior to their 2 year visit. There had been no report of suspicion of food allergy at previous study appointments. Anaphylaxis occurred following first ingestion of hummus. SPT carried out at clinic 6 weeks later showed Chickpea 11mm and Sesame seed 10mm. (SpIge was Sesame 23.9, Chickpea 13.5) Challenge was deferred and infant was followed in Allergy Clinic. Infant could be

allergic to chickpea or sesame or both. They were prescribed adrenaline autoinjectors and are being followed up in paediatric Allergy Clinic.

Overall Food Allergy at 2 years

Of 1260 infants who had SPT at 2 years, 79 had positive SPT to any food at 2 years. Total sensitisation rate 6.27% (79/1260) (CI 5 – 7.75%) Two further infants, with previously OFC positive food allergy remained symptomatic despite negative SPT to any food. Of these 81 children, 22 were already safely tolerating the suspected food allergen at screening time and were advised to continue to do so.

The remaining 56 infants had a positive SPT to a food they had never ingested, had symptoms suggestive of food allergy post ingestion, or had known previously diagnosed Food Allergy. Those infants were offered OFC to differentiate between the Food Sensitisation and Food Allergy, and to assess whether previously diagnosed food allergy was still present.

42 children underwent OFC with 41 positive OFC and 1 negative at 2 years. 14 children were previously diagnosed Food Allergy via OFC, they did not have repeat OFC at 2 years as there was a documented history of symptoms post ingestion in the previous 4 months.

Three infants with positive SPT did not complete OFC; one child had no previous reports of food allergy in earlier appointments, however experienced anaphylaxis to hummus shortly before 2 year appointment. Peanut OFC was subsequently refused despite no history of ingestion and child was deemed Peanut allergic. (SPT peanut 7mm, SpIgE Peanut 31.2, ara h2 45.5). Two further children were deemed lost to follow up; one infant with SPT to Egg 4mm with history suggestive of previous allergy to lightly cooked egg and another with SPT to Peanut of 4mm, with no history of ingestion were offered numerous appointments for OFC yet declined.

This resulted in a cumulative Food Allergy rate of 4.45% (56/1258) at 2 years (95% CI 3.38 – 5.74).

(41 positive OFC, 14 recent reactions to known allergic food, and 1 not completed but deemed allergic).

The most prevalent SpIgE mediated food allergens in Irish 2 year olds are; Egg 2.87%, Peanut 1.75%, Milk 0.95%, Cod 0.16%. There was no case of either Wheat or Soya allergy at 2 years.

Table 21: Individual Food Allergen Rates

	Allergic
Egg	2.94% (37/1259)*
	(95%% can tolerate well cooked egg)
Peanut	1.75% (22/1259)*
Milk	0.95% (12/1260)
Cod	0.16% (2/1260)
Wheat	0% (0/1260)
Soya	0% (0/1260)
Hazelnut	0.16% (2/1260)
Cashew	0.08% (1/1260)

(One infant with positive SPT to Egg and one infant with positive SPT to peanut lost to follow up therefore total numbers for both out of 1259)

i. AD and Food Allergy and Sensitisation

There was a significant association between Food Sensitisation and Food Allergy at 2 years and the diagnosis of AD at any timepoint 6, 12 or 2 years. When stratified for AD severity, this relationship strengthened. Of 299 infants diagnosed with AD at 6 months, 240 completed SPT at 2 years.

Table 22: Relationship between AD and Food Sensitisation

	FS at 2 year	**No FS at 2 years**	
N	*79*	*1181*	
AD at 6 months	54/79 (68.4%)	184/1138 (16.2%)	p = .000, phi = .32
AD at 12 months	48/79 (60.8%)	146/1161 (12.6%)	p = .000, phi = .32
AD at 2 years	44/79 (56.4%)	166/1181 (14.1%)	p = .000, phi =.27

Table 23: Relationship between AD and Food Allergy

	FA at 2 years	**No FA at 2 years**	
N	*56*	*1208*	*(2 LTFU)*
AD at 6 months	40/56 (71.4%)	199/1190 (17.2%)	p = .000, phi = .29
AD at 12 months	38/55 (69.1%)	156/1183 (13.2%)	p = .000, phi = .32
AD at 2 years	34/56 (60.7%)	176/1202(14.6%)	p = .000, phi =.26

Table 24: AD severity at 6 months and development of FA at 2 years

	% with FA at 2 years
Total	
AD severity 6 months	
- Mild (SCORad < 15)	8/94 (8.5%)
- Mod (SCORad 15- 39)	21/106 (19.8%)
- Severe (SCORad >40)	11/33 (33%)

ii. *FLG* mutation status

There was a significant association between *FLG* mut and both Food sensitisation and Food allergy at 2 years.

Table 25: *FLG* and Food Allergy and Sensitisation

	***FLG* wt**	***FLG* mut**	
N	725	78	
FS at 2 years	43/877 (4.9%)	15/104 (14.4%)	p= .001 phi = .12
FA at 2 years	31/876 (3.5%)	12/104 (11.5%)	p =.001 phi = .12

iii. TEWL and Food Sensitisation at 2 years

There was no significant difference in mean TEWL values at birth between those with or without Food Sensitisation at 2 years. Those infants who developed FS at 2 years had a non-significantly lower TEWL reading at birth than those without FS at 2 years. Mean 2 month TEWL value for infants with Food Sensitisation at 2 years was significantly higher at 12.96 $g/_{water}/m^2$ (±8.76) v's 10.92 $g/_{water}/m^2$ (±8.12) for those who did not develop FS. (p=0.037). Similarly the six month values are significantly higher in infants who had FS at 2 years compared to those who did not. (See Table 6.5 below)

Table 26. TEWL values v's Food Sensitisation at 2 years

	FS at 2 years	**Not FS at 2 years**	**p value**
N	*79*	*1181*	
Birth	6.70 (±2.87) $g/_{water}/m^2$	7.38 (±3.3) $g/_{water}/m^2$	0.088
2 months	12.88 (±8.78)$g/_{water}/m^2$	10.93 (±8.12) $g/_{water}/m^2$	0.044
6 months	15.37 (±10.84) $g/_{water}/m^2$	10.32 (±6.62) $g/_{water}/m^2$	0.00**
Δ Birth – 2 months	6.36 (±9.48) $g/_{water}/m^2$	3.52 (±8.44) $g/_{water}/m^2$	0.007**
Δ Birth – 6 months	8.13 (±10.88) $g/_{water}/m^2$	2.88 (±7.22) $g/_{water}/m^2$	0.00**
Δ 2 – 6 months	1.81 (±11.59) $g/_{water}/m^2$	-.61 (±9.92) $g/_{water}/m^2$	0.044*

**p< 0.05 **p< 0.01*

xi. TEWL and Food Allergy at 2 years

As per Food Sensitisation at 2 years, there was no significant difference in mean TEWL values at birth between those with or without Food Allergy at 2 years. As above those infants who developed Food Allergy by 2 years had a non-significantly lower TEWL reading at birth than those without FA at 2 years. Unlike food sensitisation, the mean 2 month TEWL value for infants with Food Sensitisation at 2 years was not significantly higher at 12.83 $g/_{water}/m^2$ (±9.64) than those who did not develop Food Allergy 10.98 $g/_{water}/m^2$ (±8.1). The mean change in TEWL from birth to 2 months was significantly higher infants who developed FA. The six month values similarly showed significantly higher in infants who had FA at 2 years compared to those who did not. (See Table 6.6 below)

Table 27: TEWL values v's Food Allergy at 2 years

	FA at 2 years	Not FA at 2 years	p value
N	*56*	*1202*	*(2LTFU)*
Birth	6.49 (±2.69) $g/_{water}/m^2$	7.38 (±3.3) $g/_{water}/m^2$	0.06
2 months	12.83 (±9.64)$g/_{water}/m^2$	10.98 (±8.1) $g/_{water}/m^2$	0.1
6 months	16.29 (±11.83) $g/_{water}/m^2$	10.39 (±6.65) $g/_{water}/m^2$	0.00**
Δ Birth – 2 months	6.43 (±10.47) $g/_{water}/m^2$	3.59 (±8.45) $g/_{water}/m^2$	0.02*
Δ Birth – 6 months	8.98 (±11.67) $g/_{water}/m^2$	2.96 (±7.25) $g/_{water}/m^2$	0.00**
Δ 2 – 6 months	2.65 (±12.48) $g/_{water}/m^2$	-.6 (±9.9) $g/_{water}/m^2$	0.02*

**p< 0.05 **p< 0.01*

Prediction of Food Allergy and Sensitisation.

Can TEWL at 2 months predict Allergy (yes/no) and Sensitisation (yes/no) at 2 years ?

Before we began the analysis, we ensured that all variables were significantly correlated (Spearman, two-tailed), and that assumptions were met. Variables with a non-normal distribution were log-transformed before inclusion. Non-significant variables were included in the two models as covariates (see Tables 6.7 & 6.8). We then conducted a series of hierarchical linear regressions, with 2month TEWL value acting as the dependent variable.

We predicted that food allergy at 2 years would mediate the relationship between TEWL at 2 mths and SCORAD score at 2 years, essentially suggesting that the TEWL value at 2 mths can predict food allergy at 2 years, irrespective of the impact of the presence of atopic dermatitis. Mediation is demonstrated if the partial regression coefficient for the predictor variable is reduced from significance to non-significance when the proposed mediator is added to the equation. As shown in Table 6.7, the significant effect of SCORAD Total Score at 2 years was reduced to non-significance when Food Allergy at 2 years was added to the equation (Table 6.7) and when food sensitisation was added to the model (Table 6.8). Sobel's test was significant for both models ($p<0.05$). Rsquared change = 0.39; 0.28, respectively.

Table 28: Multiple Regression Analyses for the prediction of TEWL at 2 months for food allergy at 2 years

Model *	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	9.456	3.874		2.441	.017
*FLG*mut	4.813	2.074	.247	2.320	.023
Total score	-.040	.062	-.070	-.635	.527
Birth TEWL value	.067	.221	.032	.301	.764
Sex numerical value	-.863	1.921	-.048	-.449	.655
ParentalAtopy	2.330	1.320	.194	1.765	.082
Food Allergic at 2 years	4.861	2.550	.215	1.907	.050

*Dependent Variable: 2mth TEWL value

Controlling for Baseline group, gestational length, severity, type of feed, group, and when, and with what substance, infants were washed

Table 29. Multiple Regression Analyses for the prediction of TEWL at 2 months for food sensitisation at 2 years

Model *	Unstandardized Coefficients		Standardized Coefficients	T	Sig.
	B	Std. Error	Beta		
*FLG*mut	4.930	2.067	.253	2.385	.020
Total score	-.033	.061	-.058	-.535	.594
Birth TEWL value	.092	.223	.044	.411	.683
Sex numerical value	-.476	1.953	-.027	-.244	.808
ParentalAtopy	2.312	1.320	.192	1.751	.084
Food sensitised at 2 years	4.435	2.297	.220	1.931	.050

*Dependent Variable: 2mth TEWL value

Controlling for Baseline group, gestational length, AD severity at 2 years, type of feed, group, and when, and with what substance, infants were washed

Main findings

This study is part of a large, prospective, perinatally recruited and heavily phenotyped birth cohort study. It is the first study of this scale to assess skin barrier function in the newborn period and early infancy under a controlled environment. Outcomes of AD and FA were diagnosed via internationally validated criteria in a renowned research centre.

Our study establishes a normal dataset for TEWL values in a large group of neonates, whom we then followed longitudinally through early infancy and childhood. Multiple smaller studies of heterogeneous risk profiles and age groups have reported varying results for TEWL values in infants. From our data we know now that TEWL is lowest at birth, and increases over the first 2 months when it reaches a plateau. This reflects the time period where the skin is dynamically adapting to the non-aqueous extra-uterine environment. This increase in TEWL values from birth to 2 months was the opposite of what we had suspected; that newborn infants had an impaired skin barrier. However our, mainly term gestation cohort, showed a functioning skin barrier with mean TEWL value of 7.32 $g_{water}/m^2/hr$ rising to a mean of 10.97 $g_{water}/m^2/hr$ at 2 months. Whether this intact skin barrier function at birth is due to or in response to an aquatic environment surrounded by amniotic fluid in utero is as of yet unknown. No individual factor such as parental atopy or *FLG* status, which we had hypothesised, would affect skin barrier function at birth, significantly influenced mean birth TEWL.

By 2 months however, a signal for impaired skin barrier function was seen, even in those without clinical evidence of atopic dermatitis. Firstly, infants with Filaggrin mutation had a significantly higher mean TEWL by 2 months than those without; "*FLG* mut" 12.6 (±10.1)$g/_{water}/m^2$ versus "*FLG* wt" 10.7 (±7.7) $g/_{water}/m^2$. At this 2 month timepoint, TEWL values were also higher in those infants who went on to develop AD by 12 months than those who did not; "AD at 12 months" 12.00 (±8.43)$g/_{water}/m^2$ versus "no AD 12 months" 10.78 (±7.97) $g/_{water}/m^2$. As discussed in Chapter 4, we did not screen for AD in infants at 2 months of age. However parental report of an "itchy rash" was sought. Evidently infants with AD can present prior to 6 months and "itchy rash" at 2 months may be a subjective marker. In our cohort just over one third of infants who had a parental report of an itchy rash at 2 months had AD diagnosed by 12 months. To remove those infants with an itchy

rash would erroneously exclude 70% of infants who did not go on to develop AD therefore this potential contributing factor was controlled for using logistic regression. The signal for impaired skin barrier function at 2 months, in infants without clinical evidence of disrupted skin barrier remained.

From our data we have shown that changes in skin barrier function predate clinical atopic dermatitis, representing a signal for barrier impairment in asymptomatic infants. These changes are seen in both high risk and low risk infants, are independent of *FLG* status and are not present at birth. The fact that these changes are not seen until 2 months postnatally likely reflect the interplay between genetics, heritability and external environmental factors that influence the development of atopic diseases.

With regards food allergy, this is the first unselected Birth Cohort since EuroPrevall, to use the same methodology for diagnosing food allergy. A two pronged approach to diagnosing food allergy was adopted with interval symptomatic screening prior to 2 years, and population screening of all participants at 2 years. The cumulative incidence of Food Allergy in Irish one year olds is 3.51% (54/1540) with prevalence of food allergy at 2 years of 4.45% (56/1258).

In the first year, the main foods implicated in SpIgE mediated food allergy are the typical weaning foods of milk and egg. Wheat allergy was rather lower than seen in other cohorts, with only one confirmed case and one asymptomatic sensitisation to 1 year of age. Peanut and Treenut are typically not introduced in an Irish infants diet, until after at least one year and again were only reported once each as the initial implicated food. This figure would be expected to rise by 2 years.

At 2 years, the prevalence of Food Allergy for participants on the BASELINE study is 4.45% (95% CI 3.38 – 5.74%) The most prevalent food allergens are; Egg, Peanut and then Milk. There was no Wheat or Soya allergy in our cohort at 2 years.

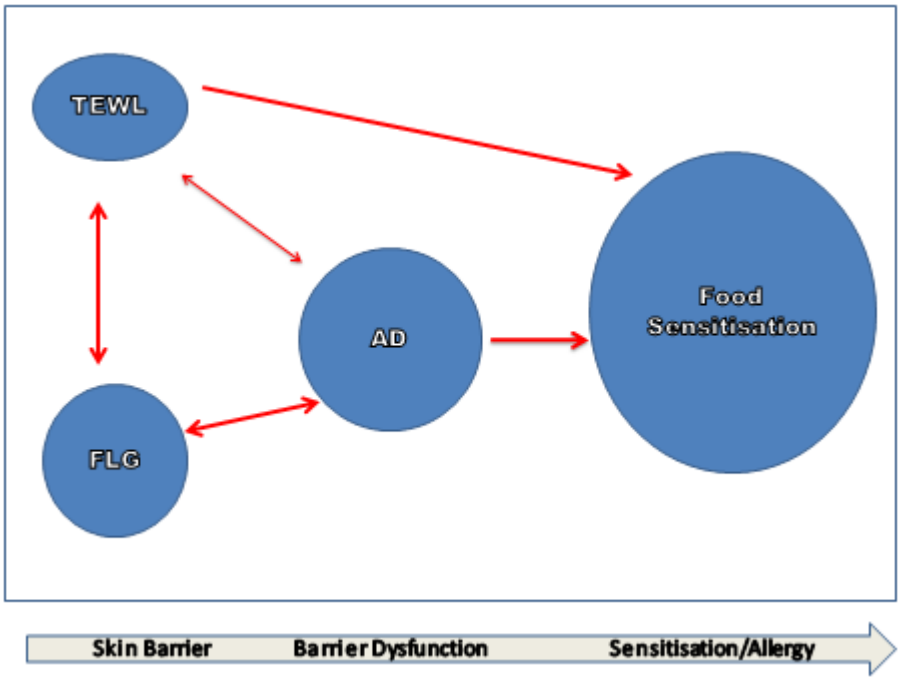
Regarding the relationship between the skin barrier and food sensitisation and allergy, our study firstly confirmed in a large unselected cohort that infants with AD were significantly more likely to have FA and FS at 2 years. In our cohort, 71% of infants with FA at 2 years had a diagnosis of AD at some or all time-points of 6 & 12 months and 2 years. When looking at

early onset severe AD, 33% of infants with severe AD at 6 months had confirmed food allergy at 2 years. *FLG* mut was also significantly associated with both FA and FS at 2 years.

A signal for FA was seen by 2 months in infants with Food Allergy at 2 years. Compared to children who did not develop food allergy, food allergic infants had a higher mean change in TEWL from birth to 2 months, reflecting a mal-adaption of skin barrier in early infancy ("FA at 2 years" 6.43 (±10.47) $g/_{water}/m^2$ versus "no FA at 2 years" 3.59 (±8.45) $g/_{water}/m^2$ p= 0.02).

To ensure this signal was not reflective of only of infants with clinical AD, multiple regression was conducted. This demonstrated a signal for raised TEWL at 2 months even in infants without AD. This gives objective and reliable supportive evidence for the initiation of food allergen sensitisation across a skin barrier defect. The fact this effect is present irrespective of the presence of atopic dermatitis shows that although the skin barrier may not be clinically deficient or abnormal, functional deficiency of the skin barrier may lead to sensitisation and possible allergy to food. It provides prospective epidemiological data to support the basic science observations of such an effect in murine models of sensitisation across a disrupted skin barrier.

Figure 16: Skin barrier function and structure and Food Sensitisation



Skin barrier dysfunction as expressed by active AD has long been associated with development of food allergy and sensitisation. However, this study is the first to demonstrate that this impaired barrier does not need to be clinically evident to be implicated. Raised TEWL, reflecting an impaired skin barrier, even in the absence of AD is associated with food allergy sensitisation. Demonstrating the ability of the skin barrier to bypass the clinically apparent dysfunctional stage yet still be causative in the development of food allergy and sensitisation through transcutaneous sensitisation.

The effect of Filaggrin mutation on the development of Food Sensitisation appears to be via its direct impact on a clinically disrupted skin barrier with 93% of food sensitised infants with *FLG* mutation having clinically diagnosed AD at 6 months.

Our study shows the earliest signal of impaired TEWL in asymptomatic infants. We cannot say more definitively when this signal appears but in BASELINE infants we know it occurred between birth and two months, the first two fixed time points of assessment in our study.

Interventions targeted at this time interval may be able to preserve or restore skin barrier function and perhaps prevent aeroallergen and food allergen sensitisation by the transcutaneous route.

Tolerance Acquisition

Although we have described a signal for impaired skin barrier function and demonstrated a route sensitisation to food allergens, this arm of the BASELINE study has not revealed why some infants with this impaired barrier develop food sensitisation but have acquired tolerance and yet others develop food sensitisation and subsequent food allergy. There are many underlying factors that potentially influence the development of tolerance compared to allergy.

Weaning practices are considered to have a significant influence on the acquisition of food tolerance. Most recent evidence suggests that the development of tolerance to food allergens is enhanced by the early introduction of allergenic food. The results of a randomised controlled trial comparing the late versus early introduction of such foods are

eagerly awaited. The mothers of infants in the BASELINE study completed weaning diaries on introduction of solid foods, so we have the potential to address this question in an observational birth cohort. The role of vitamin D in the development of food allergy has also recently been discussed. Lower vitamin D levels have been associated with the development of food allergy however there has been some debate whether low Vitamin D levels precede food allergy or whether Vitamin D deficiency is as a result of a restrictive diet imposed on children due to their food allergy. Again, the BASELINE study constitutes a unique opportunity to demonstrate prospectively whether low vitamin D levels predispose to the development of food allergy as our infants had vitamin D levels measured in Cord blood samples. As our mothers were closely monitored during pregnancy, we will demonstrate whether maternal Vitamin D levels impact on allergy development in their offspring.

As discussed in previous chapters, the rates of breastfeeding in Ireland are among the lowest in Europe. Complementary breast feeding is thought to promote the tolerance of allergenic foods during weaning. The BASELINE study collected prospective data on all infants with regards manner of feeding from birth.

In time, by amalgamating all arms of the BASELINE study we hope to inform the discussion regarding how some food sensitised infants develop food tolerance while others develop food allergy will near resolution.

Limitations

This study was a population based study from one large maternity hospital, Cork University Maternity Hospital, Cork, Ireland. This maternity hospital is the sole hospital providing maternity services to Cork and the surrounding areas. Despite this, women recruited to the SCOPE study and thus the infants recruited to BASELINE were mainly Caucasian of Irish or Eastern European descent. The ethnicity of our group is therefore quite homogenous and limits the applicability of our findings to other ethnic groups and skin types.

Two thirds (1303/1903) of our mother- infant dyads were recruited antenatally via the SCOPE study. These mothers were consented to the BASELINE study at 22 weeks gestation and were recruited at birth of live infant. These infants were first born infants, and had cord blood sampling at birth. The postnatally recruited infants (600/1903) were recruited on the postnatal floors. This supplementary recruitment strategy was employed as actual recruitment lagged significantly behind projected recruitment in the first year of this arm of the study. 35.8% of these women were primigravidous, as per inclusion criteria of Stream 1, however the remainder were multiparous. When examining TEWL values in early infancy, one of our major outputs from this study, birth TEWL values were significantly higher in Stream 2 infants. However by 2 months, and again at 6 months, there was no significant difference between values.

Although ideally, all infants in the study would be recruited in the same manner, this more heterogeneous Stream 2 group may make our findings more relevant to non-first born infants, whom we know from previous studies may have lower rates of atopic disease.

Implications and Future work

This prospectively conducted study in unselected infants from a representative sample of Irish children supports former experimental and epidemiological research that suggests food allergen sensitisation occurs across the skin prior to the acquisition of oral tolerance. It is a particular strength of this study that it was carried out in a homogeneous population that was not selected as being high risk for atopy. Therefore the methodology should be usable by other groups if the study were to be repeated.

Atopic diseases including food allergy have increased exponentially over the last few decades, particularly in the developed world. This brings significant cost and morbidity to both the individual and the health care system. As of yet, treatment of this group of diseases has been symptomatic rather than preventative, or disease modifying. With this research, which is new and novel, we may have found the first evidence on which to base a strategy to prevent atopic dermatitis, and perhaps then limit the progression of the atopic diseases including food allergy. The findings of this study now demand an intervention trial to seek and treat those with impaired skin barrier function in early infancy.

Outputs

Publications

Kelleher MM, O'Carroll M, Gallagher A, Murray DM, Dunn Galvin A, Irvine AD, et al. Newborn Transepidermal Water Loss Values: A Reference Dataset. Ped Derm. 2013;30(6):712-6.

Awards

Best Investigator Scholarship,

- Irish Paediatric Association Meeting, Dublin, November 2013

Travel Award Recipient

- European Academy of Allergy & Clinical Immunology ,Paediatric Asthma & Allergy Meeting, Athens, October 2013.

Fellow- in –Training Travel Award Recipient

- American Academy of Asthma, Allergy & Clinical Immunology, Annual Meeting, Texas, February 2013.

Oral Abstract Presentation Prize Winner

- EAACI 2014: Best Poster Prize Notification
- European Academy of Allergy & Clinical Immunology, Paediatric Asthma & Allergy Meeting, Barcelona, October 2011.

European & International Presentations

Early Life Transepidermal Water Loss (TEWL) Values Can Predate Atopic Dermatitis at Six and Twelve Months in Asymptomatic Infants: Results from the BASELINE Study.

- American Academy of Asthma Allergy Clinical Immunology, Texas, Feb 2013

Early Life Transepidermal Water Loss (TEWL) Values as Predictors of Atopic Dermatitis

- EAACI, Paediatric Asthma and Allergy Meeting, Athens, October 2013

Establishing Normal Values for Newborn Transepidermal Water Loss Using the Cork BASELINE Birth Cohort Study

The EuroPrevall Criteria for Identifying Cases of Food Allergy are Effective in an Unrelated Irish Birth Cohort Study: BASELINE

- EAACI Congress, Geneva, June 2012

Assessing the Effect of Filaggrin Mutation on Skin Barrier Function and Eczema Status

- EAACI, Paediatric Asthma and Allergy Meeting, Barcelona, October 2011

Appendix

Appendix 1: 2, 6 & 12 months measurement

BASELINE No.					D.O.B.			
Fitzpatrick Score			Mother's Score			Father'sScore		
2 Month Visit			Weight kgs		Length cms		Head Circumference	
Date:								
Skinfold Thickness			Mid Arm		Triceps		Subscapular	
	Temp	Humidity	First	Second	Third	Average	Emollient	TEWL area
TEWL							**Y / N**	**Y / N**
6 Month Visit			Weight kgs		Length cms		Head Circumference	
Date:								
Weaning Diary Returned	Y / N							
Skinfold Thickness			Mid Arm		Triceps		Subscapular	
	Temp	Humidity	First	Second	Third	Average	Emollient	TEWL area
TEWL							**Y / N**	**Y / N**
12 Month Visit			Weight kgs		Length cms		Head Circumference	
Date:								
Skinfold Thickness			Mid Arm		Triceps		Subscapular	
	Temp	Humidity	First	Second	Third	Average	Emollient	TEWL area
TEWL							**Y / N**	**Y / N**
24 Month Visit			Weight kgs		Length cms		Head Circumference	
Date:								
Skinfold Thickness			Mid Arm		Triceps		Subscapular	
	Temp	Humidity	First	Second	Third	Average	Emollient	TEWL area
TEWL							**Y / N**	**Y / N**

sheet

Appendix 2: 2 year measurement sheet

BASELINE No.				D.O.B.			
Fitzpatrick Score			Mother's Score		Father'sScore		
24 Month Visit			Weight kgs	Height cms		Head Circumference	
Date:							
Waist Circumference			Mid Arm	Triceps		Subscapular	
BP			Hip Circumference	Wrist Circumference		Knee-Ankle Length	
Bone Desnity	SOS		Z-Score	Capol Diary returned			Y/N
				First Steps card returned			Y/N
				Food Diary given			Y/N
	Temp	Humidity	First	Second	Third	Average	Emollient	TEWL area
TEWL							**Y / N**	**Y / N**
Off anti-histamines for 7 days			Yes/ No	Blood samples taken				
Test Reagent:		Result		Last time ate/ drank:				
Negative Control								
House Dust Mite								
Cat Hair								
Grass Pollen				**Was baby ever been stung by a bee or wasp:**				
Cow Milk				YES/ NO				
Soya Bean Flour								
Egg White								
Wheat Flour								
Peanut								
Cod								
Bee								
Wasp								
Positive (histamin)								

Appendix 3: Standard Operating Procedure for TEWL measurement

SOP FOR TAKING TEWL MEASURMENTS USING THE TEWAMETER TW 300

EQUIPMENT REQUIRED: Tewameter device with ambient condition sensor
1 measuring probe
Power cable
Plastic protection cap for the probe
Check calibration cap
Adhesive fixing strips for the probe

ROOM CONDITIONS: 20 degrees Celsius
40-60% air humidity
Check that mum has avoided emollients on morning of assessment: if not record.

TEWL measurement:

1. Turn on the machine
2. Calibrate machine – for this you will need the check calibration cap, seal the probe head (open chamber) with the cap. Place the plastic ring over the probe head first. Note that the ring can only go over the head in one way. Do not use any force on the probe head while trying to put the ring on it. After that push the probe head in the check calibration cap and push it down until it is completely sealed by the ring. Place the probe head on the table and let it acclimatise for 10 min. Do not move it during the check calibration process.
3. *Record wash history and number of hours since birth for the newborns*.
4. 2 day baby measurement – expose forearm for 10 min. prior to measurement. 8 week and 6 mth baby measurement expose forearm at start of visit and do tewl as first baby measurement (i.e. before weight and length)
5. Hold probe gently between your fingers at the end of the grip (handle).
6. Optimal results are achieved if the aperture of the probe id held in a vertical position.
7. Place the short end of the probe head on the skin.
8. Use forearm, take 3 measurements in one general area. Avoid active areas.
9. To avoid skin entering the probe, do not press probe too tightly against the skin. Hold the probe absolutely still during the measurement.
10. Press the button at the side of the handle to start the measurement.
11. Record three measurements in hardcopy. Calculate average and enter average reading only into database.
12. Calculate Fitzpatrick skin type using table below and enter on hardcopy and database.

Appendix 4. Standard Operating Procedure for SPT

SOP FOR SKIN PRICK TESTING (SPT) IN BASELINE

Equipment required: Allergy extract solutions for skin prick testing (Soluprick solutions)

Lancets

Ruler

Pen

Sharps bin

Storage:

1. Soluprick solutions are to be kept refrigerated at 2-8^{0}C, when not in use. Soluprick solutions are to avoid light and are not to be frozen.
2. The lancets, ruler and pen can also be stored with the Soluprick solutions, when not in use.
3. Once a solution of Soluprick has been opened it has a self live of six months and it is important that you write the expiry date on the bottle.

Preparation:

1. Ensure that the participant has not taken any anti-histamines for seven days prior to having a SPT.
2. Use of creams (i.e. steroid) will not affect the results of the SPT but you may need to wipe the arm, before undertaking the SPT, if it is greasy.

BASELINE babies for SPT

1. Professor Hourihane decides on what SPTs are to be performed.

24 month BASELINE appointment

1. All BASELINE babies attending for their 24 month appointment are to have SPT, unless otherwise instructed by Professor Hourihane.
2. If a BASELINE baby has had a SPT that is included in the 24 month appointment in the previous three months, that particular SPT is to be excluded at the 24 month

appointment. This is only applicable if the SPT was undertaken by Professor Hourihane or a member of his team.

3. The SPTs included in the 24 month assessment are as follows:
   Negative Control (this should read 0mm)
   House Dust Mite/ HDM
   Cat hair
   Grass Pollen
   Cow's milk (boiled)
   Soya bean flour
   Egg white/ Hen's egg
   Wheat flour
   Peanut
   Cod
   Positive/ Histamine (this needs to read ≥ 3mm for the test to be valid)

Performing a SPT

1. Get everything ready first: bottles all lined up and lancets opened.
2. Do the SPT on the inner aspect of the arm
3. DO NOT perform the SPT on skin that has active eczema, rash or broken skin. If unable to perform the SPT, then another appointment should be arranged.
4. Get the child to sit on a parent/ guardian's lap. The parent/ guardian can then assist in the SPT by holding the child's arm for the SPT. It is important that the arm not being tested on is held away (so not to interfere with the operator), while also keeping the arm that is being tested on straight.
5. Use the pen, to write on the outer aspect of the arm, where the Soluprick solutions are to be tested. It is the individual choice of the operator how they wish to write on the arm, but they must be able to distinguish the readings for each SPT.
6. A space of 2cms should be left between each Soluprick solution. This is to ensure that should a reaction occur that the operator can differentiate and measure which Soluprick solution caused what reaction.
7. The order of SPT should always follow the direction given by the BASELINE database. SPT results are shared between HSE and BASELINE, and it is important that record keeping is kept the same to avoid any transcription errors.

8. Each Soluprick solution drop should be pricked by separate lancets, to avoid contamination. After each lancet is used it should be placed in the sharp bin, to avoid any accidents.
9. When all Soluprick solutions have been pricked, soak the solution off (not rub).
10. It is important to remind the parents not to allow the child to scratch their arm while waiting to see the results.
11. Wait 12 to 15 minutes before measuring the results. To measure the size of reaction, if any, examine the skin and determine if a hive is visible. If a hive is present use the pen to mark around the edge of the hive and with the ruler measure the size (in mm).

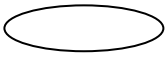
<u>To measure a hive (circular):</u>

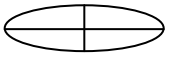
Example 1:

- Measure the internal length and width of the shape 
- Give your reading in mm as an average of the two readings

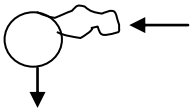
<u>To measure different shaped hives (not circular):</u>

Example 2:



- Measure the internal length and width of the shape 
- Get the average of the two measurements for your final reading in mm

Example 3:

- Section the shape off  This is called a pseudopod

This is the circle to measure, as in example one.

- Give the measurement in mm and the size (small, medium or large) pseudopod. For example, 5mm + large pseudopod.

Interpreting SPT results:

1. SPTs are only 40-60% positively predictive, so positive tests must be interpreted carefully in light of the history.
2. SPTs are > 95% negatively predictive for milk, egg, peanut and fish so if the test is negative the food is usually safe for consumption.
3. A negative result is no hive or a hive that measures ≤ 2 mm.
4. A positive result is a hive that measures ≥ 3 mm.
5. Should a negative result be achieved but the history is positive, SpIgE bloods should be taken in case of false negative.
6. Should a positive result be achieved but the history is that the food is safely consumed, then there should be no change to the diet and SpIgE bloods are not required.

Appendix 5: Letter to parent prior to 2 year appointment

What to expect at your child's 2 year BASELINE appointment

- We will have either emailed or posted questionnaires relating to nutrition and allergies which you should fill out at home before your appointment. We will clarify any unclear questions on the day. You may also have been given questionnaires about your child's development by post which you should fill in at home before you come, if possible.
- Your child's weight (standing on scales) and height (against a height chart) will be done.
- Measurements will be taken of different parts of the body with a measuring tape e.g. waist, wrist, head, upper arm, lower leg length.
- Skin-fold thickness measurement will be done on arm and back (this was done at 6 and 12mth appointments also)
- A simple skin water loss test will be done on the forearm (the same test which we did at 2 and 6 months).
- An allergy test will be done. This is a skin prick test. It involves placing a drop of the allergen onto the arm. The skin is then pricked through the drop. The result is available in 15mins. A positive result looks like a hive or nettle sting. We look for the common inhaled and food allergens, and also bee and wasp. This test is not painful or uncomfortable.
- You will be asked if you are willing for your child to have a blood test. Numbing cream is used for this test to limit any discomfort to the child. A full blood count will be one of the tests done. A blood pressure measurement will also be carried out.
- We will be carrying out a dietary assessment at the appointment. This will take approximately 10 minutes.

We would like to meet some of the BASELINE children for another appointment where our Research Psychologist will look at how your child's thinking, talking and movement skills are developing. We would like to see the children who had poor growth in the womb to see how they are developing around 2 years of age. Even if your baby did not have poor growth we might give you the opportunity to come for this assessment. This assessment will give us important information about how Irish children develop in the important first few years of life.

Preparing for the appointment

At 2 years of age your child has become much more independent. They are curious about what is happening around them on a day to day basis. This curiosity can be positively influenced by preparing them for their appointment using some of the tips we outline.

- Practice measuring some body parts with a measuring tape e.g. head, upper arm, and waist.
- Practice standing on the weighing scales with all their clothes off.

- You can explain the other measurements and tests to your child if you feel it would benefit them to be prepared for what will happen at the visit.
- We would ask you to not use any moisturisers or emollients on your child skin for 12 hours before the appointment as this may affect some of the test results. If you have to use them you can let us know on the day and we can make adjustments.
- Any anti-histamine taken in the 7 days before to the allergy test may affect the results. If your child has to take them, don't worry, but please inform us on the day of your appointment.
- Please make sure that your child has had plenty to drink on the day of the appointment as this will make the blood test easier for them.

After the appointment:

The questionnaires on your child's development will be reviewed by our Research Psychologist. If there are concerns regarding any areas of their development then you may be invited to come to the Discovery Centre for further assessment.

If any of the allergy screening tests are positive we will discuss them with you on the day and depending on the results you will then be followed up by the allergy team with regards the need for further testing.

Your child's Full Blood Count result will be reviewed by the Paediatrician on the study and you will be contacted if there are any concerns with it.

We would also like to inform you that we have recently received funding from the National Children's Research Centre in Crumlin to follow up the BASELINE children at 5 years of age. This will be the next appointment with us, in the meantime we will be circulating a newsletter to all parents by email, informing them of interesting findings and published research from the Cork BASELINE Birth Cohort.

Finally we would like to say a huge thank you to you and your family for continuing to support the study, without your commitment and support the study would not be as successful as it is.

Kind Regards,

Cork BASELINE Birth Cohort Study team

Appendix 6: OFC Policy & Procedure

Feidhmeannacht na Seirbhíse Sláinte
Health Service Executive

CUH
Ospidéal Ollscoile Chorcaí
Cork University Hospital

POLICY AND PROCEDURE ON THE

MANAGEMENT OF FOOD CHALLENGES IN CHILDREN

IN CORK UNIVERSITY HOSPITAL GROUP

Reference Number: PPG-CUH-PED-7	**Revision No:** 02	**Review Cycle:** 2 years
Author (Lead): Deirdre Daly Paediatric Allergy Nurse	**Owner:** Marie Watson A/CNM3	
Approver (Lead): Mary Mills Clinical Governance Lead	**Approval Date:** 7th December 2012	

Policy Statement

The management of a food challenge in children is to be performed in accordance with the procedure outlined in this policy

Purpose

The purpose of this policy is to outline a standard using an evidenced based approach to the management of food challenges in children attending the CUH allergy services.

Scope

This policy applies to all nursing and medical staff involved in food challenges in CUH group children's unit

Target population
All children (0-16 years) who require a food challenge.

Legislation/Related Policies

- Policy and procedure on child identification in Cork University Hospital Children's unit: PPG-CUH-PED-25
- Policy and procedure on the administration of oral medication to children in Cork University Hospital: PPG-CUH-PED-17
- Policy and Procedure on the admission of children to the Cork University Hospital: PPG-CUH-PED-18
- Infection prevention and control policies, procedures and guidelines Cork University Hospital Group: PPG-CUH-PAT-871
- Policy and Procedure on Skin Prick Testing in children in Cork University Hospital Group: PPG–CUH–PED-8

Glossary of Terms and Definitions

Food challenge

An oral food challenge is a procedure where the child is given incremental doses of the food being tested until a normal portion of the food has been consumed.
Food challenges are performed:

- To see if the child has outgrown the allergy.
- If unsure what food the child has reacted to.
- Educational – older children may not remember the reaction and this can alert them to the type of symptoms they may feel while having a reaction.
- To determine tolerance.

Allergen

A substance that is capable of inducing allergy or specific hypersensitivity.

Skin prick test

A skin prick test is a simple procedure where a drop of the allergen is placed on the skin and introduced to the mast cells of the skin by using a small lancet

Adrenaline Auto Injector

A preloaded self injectable adrenaline dose

Specific Immunoglobulin E (IgE)

An antibody which plays a key function in the immune response. When a person is allergic to a particular substance, such as a food, the immune system can mistakenly believe that this usually harmless substance is actually harmful to the body. In an attempt to protect the body, IgE is produced by the immune system to fight that particular substance. This starts a chain of events leading to allergy symptoms.

Exposure level of learning

The stage at which a student is introduced to a clinical experience by observing a competent Registered Nurse

Roles and Responsibilities

Responsibility for complying with the policy

The CNS and medical personnel are responsible for:

- Having the necessary evidence-based knowledge and skills to ensure the delivery of safe care.
- Ensuring that he/ she is aware of and adheres to the contents of this policy.

Responsibility for ensuring compliance with the policy

The CNS, Unit Manager (CNM3) and Consultant are responsible for:

- Implementing this policy.
- Reviewing and updating this policy on a regular basis to ensure that the procedures are in line with evidence-based best practice.
- Facilitating attendance at appropriate education and training.
- Ensuring that all necessary equipment is accessible to all staff.

Procedure

Procedure preparation

The nurse must:

- Ensure resuscitation equipment and rescue medication are available, working and ready for immediate use.
- Admit the child to the Day Unit using the children's medical day case documentation.
- Apply an identification bracelet as per Hospitals identification policy (2010).
- Assess that the child is fit for procedure i.e. afebrile, free of cough and cold symptoms. Children with allergic rhinitis or seasonal asthma need to have their challenges outside their inhalant allergen season. If the child has any of the above symptoms discuss with the medical team and postpone and reschedule the challenge for a later date.
- Check that the child is off Antihistamines for 1 week pre procedure as advised by doctor (Appendix II). If the child has been given antihistamines after the stipulated time, the challenge must be postponed and rescheduled for a later date.
- Record the child's baseline observations:
  - Weight
  - Temperature
  - Pulse
  - Blood pressure
  - Peak flow as appropriate
  - Oxygen saturations
- Check the child's Skin Prick Test results (if performed longer than 6 months before the challenge the test must be repeated), and specific Immunoglobulin E (IgE) level and record these in the child's food challenge form (Appendix III).
- Inform the child/parent/guardian in appropriate language about the procedure on carrying out a food challenge and the potential risk and benefits of same.
- Ensure the parent/guardian is adequately informed of procedure and that the written consent form is completed by medical doctor / clinical nurse specialist.

Check that the medical doctor has prescribed the rescue medication on the child's medication record sheet. Medication doses are in accordance with the current edition of BNF for children. The medical doctor must give first doses of intravenous drugs.

Rescue Medication includes:

- Oral Ceterizine
- Oxygen therapy
- Adrenaline 1:1,000,
- Salbutamol nebuliser
- Chlorpheniramine I.V,
- Hydrocortisone I.V,
- Adrenaline 1:1,000 nebuliser

- The admitting doctor may decide to insert an intravenous cannula if child has a history of a serious food reaction as per Hospital policy and as per Consultant's instructions.
- The Nurse performing the food challenge must:
  - Prepare the food in a clean safe environment
  - Wear a fresh pair of clean latex free gloves for each contact with the food and only the nurse performing challenge should handle the food.
  - Ensure that gloves do not touch the child.

Food Challenge

The nurse must:

- Use the appropriate food challenge technique: as directed by the relevant Consultant/Allergy Nurse Specialist
  - **0pen** technique
    The child, family and staff know what's being given
  - **Single blind** technique
    The child does not know what they are eating – usually babies and toddlers.
  - **Double Blind Placebo Controlled Food Challenge** technique**.**
    This is performed in 2 parts. This is the gold standard and is used when anxiety is high and is also used for research purposes. Neither the child, parent nor medical team know which part has the food. This is revealed at the end of the procedure and all reactions are dealt with appropriately.
- Give the child food in incremental doses every 15-20 minutes and closely monitor for any reaction (Appendix IV).
- Permit the child to play and ensure he/she is not over active. Ideally they will remain in bed.
- Administer all food at bedside.
- Record in the food challenge information sheet the following observations after each incremental dose of food:
  - Pulse
  - Respiratory rate
  - Blood pressure
  - Peak flow (when obtainable)
  - Oxygen saturations
- Monitor and record any changes in the child's general appearance and behavior at each stage in food challenge sheet.
- Allow the child to drink plain fluids e.g. water or fruit juice that have been previously tolerated throughout the challenge in small amounts. The child may be permitted to eat food 30 minutes after the challenge has been completed.
- Ensure the child remains on the ward throughout whole procedure including two hours observation post completion of the challenge.

Reaction to food challenge (Positive Result)

The nurse must:

- Stop the challenge.
- Reassure the child/parent/guardian.
- Observe the airway, breathing, and circulation and monitor the child's vital signs. Record findings on food challenge information sheet and promptly report abnormal findings to the medical team.
- Administer the appropriate medication liaising with the medical team/ Consultant.
- Ensure the child remains on bed rest post initial recovery from reaction. Observe the child for 2-4 hours or admit for overnight observation as per Consultants instructions.
- Document all care in child's nursing notes.
- Record reaction in the relevant section of food challenge information sheet.

Preparation for Discharge following positive result

The nurse must:

- Ensure the child is assessed by the doctor prior to discharge.
- Give the child/parent/guardian written information following a positive challenge prior to discharge. Ensure they understand that there is a risk of delayed reaction and they are aware of the appropriate action to take in the event of a delayed reaction (Appendix V).
- Give the child/parent/guardian the appropriate contact details to access help/advice if required and ensure appropriate follow up arrangements are organised.
- If adrenaline auto injectors have been prescribed ensure adequate training has been given.
- Ensure dietary exclusion understood by child/parent/guardian.
- Make a referral for the child/parent/guardian with the dietician if appropriate
- Ensure GP letter is sent.
- Ensure appropriate follow up appointment has been arranged in the Allergy Clinic

No reaction to food challenge (Negative Result)

The nurse must:

- Observe the child for 2 hours after the last dose of…..
- Ensure the child/parent/guardian–understands the risk of delayed reaction and the appropriate action to take in the event of a delayed reaction.
- Ensure parents have appropriate contact details to access help/advice and ensure appropriate follow up arrangements in place. Provide parent with discharge advice information leaflet (Appendix V).
- Advise parents to slowly introduce the food at home in incremental doses starting in the next few days after the challenge.
- Ensure GP letter is sent.
- Ensure appropriate follow up appointment has been arranged in the Allergy clinic

Next day follow up

The nurse must:

- Phone the parent/guardian and check if there have been any delayed reactions. Allow time to ask questions.
- Advise parent/guardian if child has passed the challenge to slowly introduce the food at home in incremental doses. Advise parent/guardian if child has a positive reaction to avoid the food.
- Document phone call and any information given in the medical notes.

Documentation and recording of information

The nurse must:

- Record nursing interventions and communication in line with An Bord Altranais (2002) recommendations in recording Clinical Practice and Policy and Procedures on the completion of the Patient Profile document, nursing care plans and related documents by nurses in Cork University Hospital (2009).
- Complete the children's medical day case documentation on admission
- Record observations using the food challenge record form.

Implementation Plan

The policy and supporting evidence will be made available to all wards/units through Q-PULSE system.

Revision and Audit

Revision

This policy will be reviewed on a 2 yearly basis or earlier if indicated.

Audit

An audit will be carried out when this policy is up for revision by the Clinical Nurse Specialist

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